A SUPPRESSOR OF *SNF1* MUTATIONS CAUSES CONSTITUTIVE HIGH-LEVEL INVERTASE SYNTHESIS IN YEAST

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ABSTRACT

The *SNF1* gene product of *Saccharomyces cerevisiae* is required to derepress expression of many glucose-repressible genes, including the *SUC2* structural gene for invertase. Strains carrying a recessive *snf1* mutation are unable to ferment sucrose. We have isolated 30 partial phenotypic revertants of a *snf1* mutant that were able to ferment sucrose. Genetic characterization of these revertants showed that the suppressor mutations were all recessive and defined eight complementation groups, designated *ssn1* through *ssn8* (suppressor of *snf1*). The revertants were assayed for secreted invertase activity, and although activity was detected in members of each complementation group, only the *ssn6* strains contained wild-type levels. Synthesis of secreted invertase in *ssn6* strains was found to be constitutive, that is, insensitive to glucose repression; moreover, the *ssn6* mutations also conferred constitutivity in a wild-type (*SNF1*) genetic background and are, therefore, not merely suppressors of *snf1*. Pleiotropic defects were observed in *ssn6* mutants. Genetic analysis suggested that the *ssn6* mutations are allelic to the *cyc8* mutation isolated by R. J. Rothstein and F. Sherman, which causes increased production of iso-2-cytochrome *c*. The data suggest a regulatory function for *SSN6*.

THE *SNF1* gene product of *Saccharomyces cerevisiae* is required to derepress expression of many glucose-repressible genes (CARLSON, OSMOND and BOTSTEIN 1981). The *SNF1* gene was first identified as a gene essential for sucrose fermentation; *snf1* mutants fail to derepress synthesis of the secreted form of invertase and do not ferment sucrose or raffinose, another substrate of invertase. The *snf1* mutants were found to have pleiotropic defects, including an inability to grow on a variety of carbon sources (galactose, maltose, melibiose, glycerol, ethanol) that are utilized via pathways including glucose-repressible enzymes. These mutants also grow more slowly than wild type on glucose, but sufficiently high concentrations of glucose remedy this defect. Diploids homozygous for a *snf1* mutation do not sporulate. Ten recessive *snf1* alleles have been isolated and all are similarly pleiotropic (CARLSON, OSMOND and BOTSTEIN 1981; L. NEIGEBORN and M. CARLSON, unpublished results).

The defect in expression of the structural gene for invertase (the *SUC2* gene in strain S288C) is probably at the transcriptional level; the 1.9-kb *SUC2* mRNA encoding the secreted invertase is not produced by *snf1* mutants (CARLSON and BOTSTEIN 1982; CARLSON *et al.* 1983). In wild-type strains, synthesis of this mRNA is regulated by glucose repression. The cytoplasmic invertase, which plays no known role in sucrose fermentation, is encoded by a 1.8-kb mRNA transcribed constitutively from the *SUC2* gene. The cytoplasmic invertase and the 1.8-kb mRNA are produced normally by *snf1* mutants. Thus, the *snf1* mutation only affects the glucose-regulated expression of the *SUC2* gene. The phenotype of the mutants suggests that the *SNF1* gene product acts positively to derepress gene expression in response to low glucose concentrations in the environment.

The *SNF1* gene has been cloned by complementation of a *snf1* mutation in yeast and genetically mapped to a position distal to *rna3* on chromosome *IV* (CELENZA and CARLSON 1984a). Null mutations were constructed at the chromosomal *SNF1* locus using the cloned DNA; the resulting null mutants were viable and displayed 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). The protein product of the *SNF1* gene has not yet been identified.

We report here the isolation of revertants of *snf1* mutants on the basis of ability to utilize different carbon sources (sucrose, raffinose, galactose and glycerol). We expected to recover true *SNF1* revertants, unlinked suppressor mutations and perhaps mutations in the regulatory regions of particular glucose-repressible genes (such as *SUC2*, when revertants were selected for sucrose fermentation). By isolating unlinked suppressor mutations we hoped to identify other genes involved in regulating expression of glucose-repressible genes, including genes encoding products that interact physically with the *SNF1* product (JARVIK and BOTSTEIN 1975).

MATERIALS AND METHODS

Yeast strains: All strains used in this study were isogenic or congenic to S288C (*Matα SUC2 gal2*), with the exception of D518-4D and W218-4B. The origins of *snf1*, *suc2*, *ade2*, *his4*, *lys2*, *GAL2* and *MAT***a** alleles have been described previously (CARLSON, OSMOND and BOTSTEIN 1981); *his4-539* and *lys2-801* are amber mutations, and *ade2-101* is an ochre mutation. The *ura3-52* allele, which was isolated in the FL100 genetic background (LACROUTE 1968), was crossed into the S288C genetic background by a series of nine backcrosses to strains isogenic to S288C and was then introduced into the strains described here. The genotypes and sources of strains 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 and galactose fermentation was carried out under anaerobic conditions in a GasPak Disposable Anaerobic System (BBL).

Isolation of revertants: Single colonies of the *snf1* strain were resuspended in water and plated on rich medium (YEP) containing sucrose, raffinose, galactose or glycerol to select for revertants able to utilize the carbon source. (Raffinose is a substrate for invertase.) Spontaneous revertants were selected by incubating the plates at 26° for 7–12 days; anaerobic conditions were used except when glycerol was the carbon source. Four mutations (*ssn1-7*, *ssn1-8*, *ssn1-9*, *ssn4-2*) were induced

TABLE 1

List of yeast strains

Strain	Genotype[a]	Source or reference
DBY640	*MATa ade2-101 SUC2 gal2*	This laboratory
DBY782	*MATα ade2-101 SUC2 gal2*	This laboratory
DBY934	*MATa snf1-28 his4-619 SUC2 gal2*	CARLSON and BOTSTEIN (1982)
DBY947	*MATα ade2-101 ura3-52 SUC2 gal2*	This work
DBY992	*MATα snf1-28 ade2-101 SUC2 gal2*	This work
DBY1052	*MATα snf1-28 his4-539 lys2-801 SUC2 GAL2*	This work
DBY1053	*MATa snf1-28 his4-539 lys2-801 SUC2 GAL2*	This work
MCY254	*MATα ade2-101 lys2-801 SUC2 GAL2*	This work
MCY318	*MATα ade2-101 lys2-801 suc2-202 GAL2*	This work
MCY321	*MATa snf1-28 ade2-101 his4-539 suc2-202 gal2*	This work
MCY369	*MATα ssn1-6 snf1-28 ade2-101 SUC2*	This work
MCY394	*MATa ssn3-2 snf1-28 his4-539 lys2-801 SUC2 GAL2*	This work
MCY397	*MATα ssn2-5 snf1-28 his4-539 lys2-801 SUC2 GAL2*	This work
MCY406	*MATα ade2-101 ura3-52 suc2-432 gal2*	This work
MCY409	*MATa ade2-101 ura3-52 his4-539 SUC2 gal2*	This work
MCY413	*MATα ade2-101 ura3-52 his4-539 SUC2 gal2*	This work
MCY438	*MATα ssn5-4 snf1-28 his4-539 ura3-52 SUC2*	This work
MCY442	*MATα ssn4-1 snf1-28 lys2-801 ade2-101 SUC2*	This work
MCY451	*MATα ssn6-3 snf1-28 lys2-801 ura3-52 SUC2*	This work
MCY456	*MATα ssn7-1 snf1-28 his4-539 ade2-101 SUC2 gal2*	This work
MCY459	*MATα ssn6-2 snf1-28 lys2-801 ura3-52 SUC2*	This work
MCY472	*MATa ssn6-1 his4-539 lys2-801 ura3-52 SUC2 GAL2*	This work
MCY475	*MATa ssn6-1 snf1-28 his4-539 lys2-801 ura3-52 SUC2*	This work
MCY477	*MATα ssn6-1 snf1-28 his4-539 lys2-801 SUC2*	This work
MCY479	*MATα ssn8-1 snf1-28 lys2-801 ade2-101 SUC2*	This work
D518-4D	*MATa cyc8-1 cyc1-13 trp1 his1 SUC*	R. ROTHSTEIN
W218-4B	*MATα cyc1 cyc2-12 lys2-1 his1 trp1 trp5 leu1-12 ura3-1*	R. ROTHSTEIN

[a] The genotype at the *GAL2* locus is included where known.

by exposing plates to 23 Jm^{-2} of ultraviolet radiation prior to incubation. Revertant colonies were purified by single-colony isolation and tested. Spontaneous revertants were considered to be independent only if derived from different single colonies of the parent strain. Revertants *ssn1-7* and *ssn1-8* were derived from one single colony but are likely to be independent because of the stimulation of reversion frequency by ultraviolet radiation.

Complementation analysis: Strains with markers allowing prototrophic selection of diploids carrying the suppressor mutations were constructed by crossing some of the pseudorevertants to wild-type *SNF1* strains. Appropriately marked segregants carrying *snf1* and the suppressor mutation were recovered. A few pseudorevertants (carrying *ssn1-1*, *ssn2-1* and *ssn4-1*) were also crossed to *snf1* strains, but the diploids failed to sporulate, as was anticipated because homozygous *snf1/snf1* diploids do not sporulate (CARLSON, OSMOND and BOTSTEIN 1981).

To test pairs of mutations for complementation, heterozygous diploids were constructed and isolated by prototrophic selection. The diploids were then tested for ability to utilize the indicated carbon source. Complementing *ssn* alleles confer a sucrose-nonfermenting phenotype to a diploid homozygous for *snf1*, and noncomplementing *ssn* alleles confer a sucrose-fermenting phenotype. Because the haploid parents (*snf1 ssn*) of each diploid were sucrose fermenters, all sucrose-fermenting diploids were single colony purified, and several colonies were tested to confirm that the sucrose-fermenting phenotype was truly a property of the diploid and not due to contamination by parental haploids.

Invertase assays: For assays of secreted invertase, cells were prepared as described before (CELENZA and CARLSON 1984a). Samples of glucose-repressed cells were taken from cultures growing exponentially in YEP-2% glucose liquid medium (repressing conditions), and derepressed cells were obtained by shifting such cells to YEP-0.05% glucose medium (derepressing conditions) for 2.5 hr. Whole cells were assayed for secreted invertase by the method of GOLDSTEIN and LAMPEN (1975), as described by CELENZA and CARLSON (1984a). The dry weight of cells per milliliter was determined for each culture by filtering 25 ml of cells onto a glass fiber filter (Whatman GF/C). The filter was then washed with 25 ml of water, dried under a heat lamp, weighed, exposed to heat again, and weighed again. For the gel assay, cell extracts were prepared and the two forms of invertase were separated by electrophoresis on a 5% polyacrylamide gel as described (CARLSON, OSMOND and BOTSTEIN 1981), except that on occasion derepressed cells were prepared by growing the culture in medium containing sucrose or raffinose. Invertase activity was detected *in situ* by the staining procedure of GABRIEL and WANG (1969).

Analysis of mating ability: Mating type was routinely determined by testing the ability of a given strain to conjugate with *MAT***a** and *MAT*α tester strains carrying complementing auxotrophic markers and thereby to produce prototrophic diploids. Cells were mixed and allowed to conjugate on rich medium and then replicated to selective medium. When testing the mating type of *MAT*α *ssn6* strains, we noted that prototrophic colonies appeared as papillae on the replicated mixture of *MAT*α *ssn6* cells and *MAT*α tester cells, which otherwise showed no growth on selective medium. No papillae were observed when *MAT***a** *ssn6* and *MAT***a** tester cells were similarly tested for mating. *MAT*α *ssn6* and *MAT***a** *ssn6* cells appeared to mate normally with cells of opposite mating type. To determine whether the diploids resulting from the apparent mating of *MAT*α *ssn6* and *MAT*α tester cells had a *MAT*α/*MAT*α genotype, experiments were carried out in which the resulting diploids were homozygous for an auxotrophic marker (but were selectable by virtue of other complementing mutations) and could, therefore, easily be tested for ability to mate with *MAT***a** or *MAT*α haploids. MCY451 (*MAT*α *ssn6-3 lys2 ura3*) and MYC459 (*MAT*α *ssn6-2 lys2 ura3*) were mated to MCY406 (*MAT*α *ade2 ura3*). Diploids were selected for growth on minimal medium supplemented with uracil and tested for ability to mate with DBY640 (*MAT***a** *ade2*) or DBY782 (*MAT*α *ade2*).

RESULTS

Isolation of revertants: Partial phenotypic revertants of *snf1-28* mutant strains (DBY934, DBY1052 and DBY1053) were selected by their ability to form colonies anaerobically on media containing sucrose, raffinose or glycerol as carbon source. Sucrose- and raffinose-fermenting revertants were recovered with a spontaneous frequency of approximately 10^{-6} to 10^{-5}. Colonies appeared on glycerol-containing media at a similar frequency, but the colonies were very small and upon retesting grew on glycerol only slightly better than the *snf1* parent. Selections for growth on galactose yielded no revertants among 3×10^7 cells plated.

The 30 revertants listed in Table 2 were purified and tested for growth on a spectrum of carbon sources. All revertants, including those isolated on glycerol medium, were able to grow on sucrose and raffinose, although none grew as well as the *SNF1* control strain. None showed substantially better growth on glycerol than the *snf1* parent. Revertants of DBY1052 and and DBY1053 failed to grow on galactose; revertants of DBY934 (*gal2*) could not be tested directly on galactose due to the *gal2* mutation. In addition, many revertants had acquired a flocculent phenotype. Thus, all of the revertants showed only partial phenotypic reversal of the *snf1* defect.

To test for dominance, each revertant was crossed to a *snf1* (*SSN*) strain (DBY992 or MCY321). In each case the resulting diploid was unable to ferment sucrose, indicating that all of the suppressors are recessive.

TABLE 2

Origin and complementation analysis of SSN *mutations*

Mutant allele	Parent strain	Selection	Complementation[a]							
			ssn1-1[b]	*ssn2-2*	*ssn3-1*	*ssn4-1*	*ssn5-4*	*ssn6-2*[c]	*ssn7-1*	*ssn8-1*
ssn1-1	DBY934	Sucrose	−							
ssn1-2	DBY934	Sucrose	−	+						
ssn1-3	DBY934	Sucrose	−	+						
ssn1-4	DBY934	Sucrose	−	+						
ssn1-5	DBY934	Sucrose	−	+						
ssn1-6	DBY934	Sucrose	−	+	+			+		+
ssn1-7[d]	DBY934	Sucrose	−	+						
ssn1-8[d]	DBY934	Sucrose	−	+						
ssn1-9[d]	DBY934	Sucrose	−	+						
ssn2-1	DBY934	Sucrose	+	−	+					
ssn2-2	DBY934	Sucrose	+	−						
ssn2-3	DBY1052	Raffinose	+	−			+	+	+	
ssn2-4	DBY1053	Raffinose	+	−	+	+	+	+	+	
ssn2-5	DBY1052	Glycerol	+	−			+	+	+	
ssn3-1	DBY934	Sucrose	+	+	−					
ssn3-2	DBY1053	Raffinose	+	+	−	+	+	+	+	
ssn4-1	DBY934	Sucrose	+	+	+	−				
ssn4-2[d]	DBY934	Sucrose	+	+	+	−				
ssn4-3	DBY1052	Glycerol	+	+	+	−	+	+	+	+
ssn5-1	DBY1052	Raffinose	+	+			−	+	+	
ssn5-2	DBY1053	Glycerol	+	+	+	+	−	+	+	
ssn5-3	DBY1053	Glycerol	+	+	+	+	−	+	+	
ssn5-4	DBY1052	Glycerol	+	+		+	−			
ssn6-1	DBY1052	Raffinose	+	+	+	+	+	−	+	
ssn6-2	DBY1053	Raffinose	+	+	+	+	+	−		
ssn6-3	DBY1053	Raffinose	+	+	+	+	+	−	+	+
ssn7-1	DBY1052	Glycerol	+	+			+	+	−	
ssn8-1	DBY1052	Raffinose	+	+	+	+	+	+	+	−
ssn8-2	DBY1052	Raffinose	+	+	+	+	+	+	+	−
ssn8-3	DBY1053	Raffinose	+	+	+	+	+	+	+	−

[a] The result of each complementation test is reported only once; due to asymmetry in the table, some data appear at positions above the diagonal.
[b] Mutations isolated in DBY1053 were tested for complementation with *ssn1-6*.
[c] The *ssn6-1* allele was identified by a complementation test with *ssn6-3*.
[d] Induced by ultraviolet radiation. All other mutations arose spontaneously.

These results suggest that most, if not all, of the revertants are pseudorevertants carrying an extragenic mutation that partially suppresses the *snf1* mutation. Our experiments support this conclusion, so for convenience we will henceforth refer to these suppressors as *ssn* mutations, for suppressor of *snf1*.

Complementation analysis: Complementation tests were carried out as described in MATERIALS AND METHODS. Those *snf1/snf1* diploids that were heterozygous for two complementing *ssn* mutations exhibited the Snf$^-$ phenotype and failed to ferment sucrose. Those *snf1/snf1* diploids that carried noncomplementing suppressor alleles were sucrose fermenters. Each of the 30 suppressor mutations was assigned to one of eight complementation groups (*ssn1* through *ssn8*) on the basis of the results shown in Table 2.

Synthesis of invertase by ssn *mutants*: A *snf1* mutant produces the cytoplasmic form of invertase but is defective in derepressing synthesis of secreted invertase (CARLSON, OSMOND and BOTSTEIN 1981). The *ssn* mutations restore the ability of a *snf1* strain to grow on sucrose and raffinose, and the simplest explanation for this phenomenon is that the *ssn* mutations suppress the defect in synthesis of secreted invertase. To test this idea, we assayed a representative *ssn snf1* strain from each complementation group for production of invertase during growth under conditions of glucose repression and derepression. Secreted invertase activity was assayed quantitatively in whole cells (Table 3). The *ssn2*, *ssn3*, *ssn4*, *ssn5* and *ssn8* mutants produced only very low levels of secreted invertase upon derepression, although these levels were all higher than those of the *snf1* parents. Derepressed *ssn1* and *ssn7* strains produced moderate amounts of secreted invertase but considerably less (fivefold and 14-fold, respectively) than a wild-type *SNF1* strain (DBY782). In contrast, the derepressed *snf1* strain carrying an *ssn6* mutation produced even higher levels of secreted invertase than wild type; moreover, assays of glucose-repressed cells showed that synthesis of secreted invertase by the *snf1 ssn6* strain was constitutive, that is, insensitive to glucose repression.

To determine whether an *ssn6* allele conferred constitutivity in a *SNF1* genetic background, an *ssn6-1 SNF1* segregant was recovered from cross 64 (Table 4) and assayed for secreted invertase following growth in glucose-repressing and -derepressing conditions (Table 3). Synthesis of secreted invertase was constitutive, and the activity was greater than that detected in derepressed wild-type strains. Growth in conditions of glucose limitation resulted in higher secreted invertase activity than growth in excess glucose, as was observed for the *ssn6 snf1* strain and for diploid strains (see Table 5).

To assay for the presence of the nonglycosylated, cytoplasmic form of invertase in *snf1 ssn* mutants, cell extracts were prepared and the two forms of invertase were separated by electrophoresis in a polyacrylamide gel (CARLSON, OSMOND and BOTSTEIN 1981). Invertase activity was detected *in situ* (GABRIEL and WANG 1969). At least one representative from each of the eight groups was assayed following growth under repressing and derepressing conditions, and in all cases the nonglycosylated form of invertase was detected at approximately normal levels (data not shown). Derepressed mutant strains were found also to contain glycosylated invertase, indicating that the activity detected in whole cell assays most likely represents the glycosylated species that is normally secreted. Although the gel assay is not quantitative, the amount of the glycosylated invertase in each strain approximately corresponded to the level of secreted invertase activity detected by assay. These gel assays also confirmed that all three *ssn6* mutants synthesize constitutively the glycosylated invertase.

TABLE 3

Secreted invertase activity in ssn *mutants*

Strain	Relevant genotype	Invertase activity[a]	
		Repressed	Derepressed
DBY782	*SSN SNF1*	<1	210
DBY1052	*SSN snf1-28*	<1	<1
MCY369	*ssn1-6 snf1-28*	5	40
MCY397	*ssn2-5 snf1-28*	<1	3
MCY394	*ssn3-2 snf1-28*	<1	2
MCY442	*ssn4-1 snf1-28*	<1	3
MCY438	*ssn5-4 snf1-28*	<1	4
MCY477	*ssn6-1 snf1-28*	200	270
MCY472	*ssn6-1 SNF1*	300	380
MCY456	*ssn7-1 snf1-28*	<1	15
MCY479	*ssn8-1 snf1-28*	5	2

[a] Micromoles of glucose released per minute per 100 mg of dry weight of cells.

ssn2 *through* ssn8 *mutations confer a clumpy phenotype*: As mentioned earlier, many of the pseudorevertants showed a clumpy or flocculent phenotype. None of the *ssn1* mutants were clumpy, and most of the pseudorevertants in the other complementation groups were clumpy. Furthermore, we observed 2:2 segregations for this clumpy phenotype in tetrads from diploids heterozygous for the following suppressor mutations: *ssn2-2*, *ssn2-4*, *ssn3-1*, *ssn4-1*, *ssn4-3*, *ssn6-1*, *ssn6-2*, *ssn7-1*, *ssn8-1* and *ssn8-2*. Although segregation of the *ssn* mutation could not easily be followed because the diploids were not homozygous for the *snf1* mutation, the *snf1* spore clones carrying the suppressor could be identified, and in each cross these spore clones were clumpy. These data suggest the association of a clumpy phenotype with these suppressor mutations. In addition, it is likely that the *ssn5* mutations also confer clumpiness. Among the four pseudorevertants carrying *ssn5* mutations, only the *ssn5-4* strain was not clumpy and clumpy segregants were recovered from a cross of the *ssn5-4* revertant to a wild-type strain (cross 52; Table 4); moreover, diploids heteroallelic for *ssn5-4* and other *ssn5* mutations were clumpy, although clumpiness was recessive in heterozygous diploids. These data suggest that the *ssn5-4* pseudorevertant carries an additional suppressor of the clumpy phenotype and that *ssn5* mutations confer clumpiness in addition to suppressing *snf1*. Similar data suggest the presence of a suppressor of clumpiness in the *ssn6-3* pseudorevertant.

ssn *mutations are not tightly linked to* snf1: In the course of constructing strains for the complementation analysis, at least one psuedorevertant from each group, *ssn1* through *ssn8*, was crossed to a wild-type *SNF1 SSN* strain. The resulting diploids were induced to sporulate and were subjected to tetrad analysis. Spore clones were tested for growth on sucrose and glycerol. Ability to utilize glycerol segregated 2+:2− in these crosses and this phenotype was used to follow segregation of the *snf1* marker; the *snf1* genotype of key strains was confirmed by complementation tests. For unknown reasons it was difficult to

TABLE 4

ssn *loci are not tightly linked to* snf1

Diploid[c]	Relevant genotype	Segregation of sucrose-fermenting phenotype in tetrads			Total no. of spores[a]	
		PD[b] 4+:0−	T[b] 3+:1−	NPD[b] 2+:2−	Suc$^+$	Suc$^-$
40	*ssn1-1* *snf1-28* / + +	1	6	0	22	6
44	*ssn1-6* *snf1-28* / + +	1	3	0	22	7
41	*ssn2-2* *snf1-28* / + +	5	3	0	39	5
56	*ssn2-4* *snf1-28* / + +	4	2	0	22	2
43	*ssn3-1* *snf1-28* / + +	0	3	1	16	7
42	*ssn4-1* *snf1-28* / + +	0	3	1	17	8
63	*ssn4-3* *snf1-28* / + +	1	4	2	20	8
52	*ssn5-4* *snf1-28* / + +	0	5	1	20	8
64	*ssn6-1* *snf1-28* / + +	0	7	1	23	9
60	*ssn6-2* *snf1-28* / + +	2	5	0	23	5
58	*ssn6-3* *snf1-28* / + +	1	2	0	20	5
55	*ssn7-1* *snf1-28* / + +	2	4	2	24	8
59	*ssn7-1* *snf1-28* / + +	0	5	2	19	9
65	*ssn8-1* *snf1-28* / + +	1	5	0	19	5
66	*ssn8-2* *snf1-28* / + +	1	1	2	18	7

[a] Including spores from incomplete tetrads.

[b] PD, parental ditype; T, tetratype; NPD, nonparental ditype.

[c] Diploids were constructed by crossing the pseudorevertant carrying the indicated *ssn* allele to a wild-type strain, as follows: the wild-type parent of diploids 40 through 44 was MCY254; the parent of diploids 52, 55, 57, 63 and 64 was MCY409; the parent of diploid 56 was MCY413; the parent of diploids 58 and 60 was DBY947; and the parent of diploids 65 and 66 was DBY640. Diploid 59 was constructed by crossing a segregant from cross 55 to DBY947.

score glycerol utilization in crosses including an *ssn6* allele, and for these crosses we relied on complementation to identify *snf1* spore clones. Sucrose-fermenting *snf1* segregants were presumed to carry the *ssn* mutation; in many cases the clumpy phenotype of these segregants provided supporting evidence for the presence of an *ssn* mutation. In each cross segregations of 4+:0−,

TABLE 5

Constitutivity of invertase synthesis in cyc8/ssn6 *diploids*

Strain	Relevant genotype	Invertase activity[a]	
		Repressed	Derepressed
D518-4D	*cyc8-1* *SNF1*	20	200
D518-4D × MCY477	*cyc8-1* / *ssn6-1* + / *snf1-28*	100	320
D518-4D × DBY1052	*cyc8-1* / *SSN6* + / *snf1-28*	8	210
W218-4B × MCY475	*CYC8* / *ssn6-1* + / *snf1-28*	<1	260
MCY472 × MCY477	*ssn6-1* / *ssn6-1* + / *snf1-28*	320	480

[a] Micromoles of glucose released per minute per 100 mg of dry weight of cells.

3+:1− and 2+:2− were observed for sucrose fermentation (Table 4). The sucrose-nonfermenting spore progeny were all unable to utilize glycerol, and we infer that these sucrose nonfermenters are *snf1 SSN* recombinants. The recovery of such recombinants at high frequency indicates that none of the *ssn1* through *ssn8* loci are tightly linked to *snf1*. The *ssn2* locus, however, might be weakly linked to *snf1*. Among 21 tetrads from crosses heterozygous for *snf1* and *ssn2*, 15 were parental ditype for these markers, and six were tetratype. These data suggest a linkage between *ssn2* and *snf1* of about 15 cM.

ssn *is not tightly linked to* SUC2: One possible class of suppressors would be mutations in the regulatory region of the *SUC2* gene that render gene expression independent of *SNF1*. We would expect such mutations to be dominant; however, it seemed possible that weak expression of a single *SUC2* gene might suffice to confer a sucrose-fermenting phenotype to a haploid strain but have no detectable effect on the growth properties of a heterozygous diploid. The only likely candidates for this kind of mutation seemed to be the *ssn1* group because the others were associated with clumpiness, a phenotype unlikely to result from a mutation in the *SUC2* regulatory sequences. Also, *ssn6* has been shown to map elsewhere. To determine whether *ssn1* is tightly linked to *SUC2*, an *ssn1* pseudorevertant (*ssn1-1 snf1 SUC2*) was crossed to MCY318 (*SSN1 SNF1 suc2*) and tetrad analysis was carried out on the diploid. If *ssn1* were tightly linked to *SUC2*, all tetrads would show 2:2 segregations for sucrose fermentation, and the phenotype would be determined by the *SUC2* genotype. Because the *SUC2* allele would be linked to a suppressor of *snf1*, segregation of *snf1* would have no effect on the sucrose-fermenting phenotype. If *ssn1* were not tightly linked to *SUC2*, segregations of 0+:4−, 1+:3− and 2+:2− would be expected in the relative ratio of 1:16:19. Among 14 tetrads from this cross, four showed 1+:3− segregation and ten showed 2+:2− segregation. The occurrence of 1+:3− tetrads indicates that *ssn1* and *SUC2* are not tightly linked.

Linkage of ssn6 *to* lys2: We noted that all *snf1 ssn6* spore clones derived from crosses 58, 60 and 64 (Table 4) also carried the *lys2* marker, which was contributed to the diploid by the parent pseudorevertant. Crosses 60 and 64 were then examined for cosegregation of *lys2* and the clumpy phenotype associated with *ssn6*. All tetrads that showed 2+:2− segregations for both markers were parental ditype (seven tetrads from cross 60 and five tetrads from cross 64). Thus, the *ssn6* locus appears linked to *lys2*.

Effect of ssn6 *mutations on mating specificity*: It was found that *MATα ssn6* cells not only mated with *MAT***a** cells but also mated, although at very low efficiency, with *MATα* cells; *ssn6* had no apparent effect on the mating phenotype of *MAT***a** cells (see MATERIALS AND METHODS). This property was observed in *MATα* strains carrying any of the three *ssn6* alleles and was independent of the genotype at the *SNF1* locus. To determine whether the diploids resulting from the apparent mating of *MATα ssn6* and *MATα* cells had a *MATα/MATα* genotype, their ability to mate with *MAT***a** or *MATα* haploids was tested (see MATERIALS AND METHODS). In each case the diploids mated readily with the *MAT***a** haploid but not with the *MATα* haploid. Control *MAT***a**/*MATα* strains mated with neither haploid tester. These studies indicate that *MATα ssn6* cells mate with *MATα* cells, generating *MATα/MATα* diploids.

ssn6 *mutations prevent sporulation*: To test the effects of *ssn6* mutations on sporulation, we constructed diploids of genotype *MAT***a**/*MATα snf1/SNF1 ssn6-1/ssn6-1* and *MAT***a**/*MATα snf1/SNF1 ssn6-1/ssn6-2*. Neither diploid sporulated, and after a week on sporulation plates most cells appeared to be very unhealthy. These diploids grew on glycerol but much more slowly than an *SSN6/SSN6 snf1/SNF1* strain. A similar defect in growth on glycerol was observed in *ssn6 SNF1* haploids.

ssn6 *is allelic to* cyc8: The linkage of *ssn6* to *lys2* prompted us to examine the yeast genetic map in the vicinity of *lys2* for previously mapped mutations that might be allelic to *ssn6*. The *cyc8* mutation isolated by ROTHSTEIN and SHERMAN (1980) maps 4.5 cM from *lys2* and confers clumpiness; the *cyc8* mutant was selected as an overproducer of iso-2-cytochrome *c*. A sucrose-fermenting *cyc8* strain, D518-4D, was examined for regulation of secreted invertase synthesis by glucose repression, and significant invertase activity was detected in glucose-repressed cells, although the level was tenfold lower than that of derepressed cells (Table 5). The interpretation of this finding is complicated by the fact that the genetic background of D518-4D is different from that of S288C and that the *SUC* gene in D518-4D is not *SUC2* (B. OSMOND and M. CARLSON, unpublished data).

To assess the possibility of allelism of *ssn6* and *cyc8*, a complementation test was carried out. The *cyc8* strain D518-4D was crossed to an *ssn6* mutant. If *ssn6* and *cyc8* were complementing mutations, the diploid would be expected to exhibit normal regulation of secreted invertase synthesis in response to external glucose concentration. If, however, *ssn6* and *cyc8* were allelic, the diploid would most likely synthesize secreted invertase constitutively. The diploid was grown in glucose-repressing and -derepressing conditions and assayed for secreted invertase activity. The results, shown in Table 5, demonstrate that

cyc8-1 and *ssn6-1* fail to complement for the constitutive expression of invertase. Furthermore, the *cyc8* mutation failed to complement the *ssn6* mutation for its clumpy phenotype or its sporulation defect; control strains (*cyc8/SSN6* and *CYC8/ssn6*) did not aggregate extensively and sporulated normally. Therefore, we conclude that these two mutations are allelic.

DISCUSSION

We have isolated 30 partial phenotypic revertants of a *snf1* mutant by selecting for growth on sucrose, raffinose or glycerol. These revertants ferment sucrose and raffinose and perhaps grow slightly better on glycerol than the *snf1* parent. The 30 revertants were found to carry recessive mutations that fell into eight complementation groups, *ssn1* through *ssn8*. It seems likely that additional groups would be identified by isolating more revertants, and the fact that we recovered no reverse mutation at the *snf1* locus can probably be attributed to the relatively large number of genes that can yield suppressors.

Strains representing each complementation group were assayed for synthesis of the two forms of invertase. All strains produced the constitutive, cytoplasmic enzyme at normal levels, as does the *snf1* parent. When grown under conditions of glucose limitation, all strains also derepressed synthesis of the secreted invertase, although the activity detected in *ssn2*, *ssn3*, *ssn4* and *ssn5* mutants was very low and the activity in *ssn1* and *ssn7* strains was well below that of wild type (*SSN SNF1*). The *ssn6* strains contained high levels of secreted invertase, even when grown in excess glucose; however, some additional derepression occurred when cells were shifted to glucose-deficient medium. This observation may have implications regarding the mechanism by which *SUC2* gene expression is regulated in response to glucose. The constitutive synthesis of secreted invertase was also observed in *ssn6 SNF1* strains and was, thus, independent of the genotype at the *SNF1* locus.

The *ssn* suppressors appeared to suppress specifically the sucrose- and raffinose-nonfermenting phenotypes of the *snf1* mutation. However, the detection of only low levels of invertase in many of these sucrose-fermenting strains may mean simply that growth on sucrose (and raffinose) requires very little enzyme and not that the inability of *snf1* mutants to synthesize invertase is specifically suppressed. It is also possible that other mutations could be isolated in some of these same complementation groups that would more effectively suppress the *snf1* defect and restore capacity for growth on glycerol and galactose. In the case of *ssn6*, a better argument can be made for the specificity of suppression. The *ssn6* mutations cause synthesis of invertase at high levels and yet produce no detectable reversal of the other *snf1* phenotypes.

Mutations in all groups except *ssn1* were pleiotropic. In addition to their effects on the synthesis of secreted invertase, they conferred a phenotype of clumpy growth. This tendency to aggregate probably results from an alteration in cell surface properties, but we have no explanation for its association with suppressors of *snf1*.

We have not absolutely ruled out the possibility tht some of the *ssn* mutations are informational suppressors. All were isolated as suppressors of the *snf1-28*

allele, which is probably a point mutation and is not an amber mutation (B. OSMOND and M. CARLSON, unpublished data). No amber or ochre suppressors were recovered in this study, as judged by the phenotypic expression of the *ade2-101* (ochre), *his4-539* (amber) and *lys2-801* (amber) alleles. The *ssn6* mutations clearly are not informational suppressors because they confer the identical constitutive phenotype in a *SNF1* background.

The allele specificity of the suppressors, other than *ssn6*, has not been investigated. We have, however, isolated suppressors of the *snf1-20* mutation that failed to complement *ssn1* or *ssn4* (B. OSMOND and M. CARLSON, unpublished results), suggesting that these two groups are not allele specific.

In general one can imagine two mechanisms by which suppression of the *snf1* defect could occur, other than informational suppression. First, if the product of an *SSN* gene interacted physically with the *SNF1* gene product, the suppressor mutation could compensate for a structural alteration caused by the *snf1* mutation, thereby restoring partial function. Second, the *ssn* mutation could allow the cell to bypass its requirement for a functional *SNF1* gene product in order to derepress expression of the structural gene for invertase (*SUC2*). We prefer the latter possibility because of the surprisingly large number of genes yielding recessive suppressor alleles and because the recessiveness suggests loss, not gain, of function. Further studies will be required to determine the mechanisms by which the *ssn* mutations result in suppression.

The *ssn6* mutations were unusual among the *ssn* mutations in that they restored high-level synthesis of secreted invertase and also abolished repression by glucose. Moreover, the *ssn6* mutations conferred constitutivity to invertase synthesis in a wild-type (*SNF1*) genetic background and are, therefore, not merely suppressors of *snf1*. Preliminary results suggest that the effect of *ssn6* on *SUC2* gene expression occurs at the RNA level. In wild-type cells producton of the 1.9-kb mRNA-encoding secreted invertase is sensitive to glucose repression; however, this mRNA is present in *ssn6* cells grown in medium containing excess glucose (L. NEIGEBORN and M. CARLSON, unpublished data).

Although the effect of the *ssn6* mutations in suppressing *snf1* appears to be specific to *SUC2* gene expression, there being no detectable suppression of other *snf1* phenotypes, the *ssn6* alleles are clearly pleiotropic. The *ssn6* mutants were clumpy and *MATα ssn6* strains mated with other *MATα* strains with unusual frequency. Diploids homozygous for *ssn6* failed to sporulate. In addition, *ssn6* appears allelic to *cyc8*, which confers clumpiness and causes overproduction of iso-2-cytochrome *c* (ROTHSTEIN and SHERMAN 1980). It is perhaps significant that mutations at another locus, called variously *tup1*, *cyc9*, *flk1* and *umr7*, are similarly pleiotropic; these mutations cause utilization of exogenous deoxythymidine monophosphate, constitutive synthesis of invertase and other glucose-repressible enzymes, clumpiness, increased levels of iso-2-cytochrome *c*, α-specific mating defects, inability of homozygous diploids to sporulate and other phenotypes (WICKNER 1974; SCHAMHART, TEN BERGE and VAN DE POLL 1975; ROTHSTEIN and SHERMAN 1980; LEMONTT, FUGIT and MACKAY 1980; STARK, FUGIT and MOWSHOWITZ 1980).

The properties of the *ssn6* mutants suggest *SSN6* as a likely candidate for a

regulatory gene. The simplest model consistent with these data is that the *SSN6* gene product acts as a repressor of expression of *SUC2*, *CYC7* (the structural gene for iso-2-cytochrome *c*) and other genes affecting cell surface properties and mating. The *SNF1* gene product would then regulate repressor activity in response to external glucose concentration. The loss of repressor activity in *ssn6* mutants would account for both the suppression of the *snf1* sucrose-nonfermenting phenotype and the effects on gene expression in *SNF1* strains. However, the data are also consistent with other models, and further study is needed to determine the function of *SSN6*.

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