MOLECULAR AND CELLULAR BIOLOGY, Feb. 1987, p. 672–678
0270-7306/87/020672-07$02.00/0

Vol. 7, No. 2

SSN20 Is an Essential Gene with Mutant Alleles That Suppress Defects in *SUC2* Transcription in *Saccharomyces cerevisiae*

LENORE NEIGEBORN, JOHN L. CELENZA, AND MARIAN CARLSON*

Department of Genetics and Development and Institute for Cancer Research, Columbia University, College of Physicians and Surgeons, New York, New York 10032

Received 18 July 1986/Accepted 7 November 1986

Dominant and recessive mutations at the *SSN20* locus were previously isolated as extragenic suppressors of mutations in three genes (*SNF2*, *SNF5*, and *SNF6*) that are required in *trans* to derepress invertase expression. All *ssn20* alleles cause recessive, temperature-sensitive lethality. In this study we cloned the *SSN20* gene, identified a 4.6-kilobase poly(A)-containing RNA, and showed that disruption of the gene is lethal in a haploid cell. Genetic mapping of *SSN20* to a locus on chromosome VII 10 centimorgans distal to *cly8* led to the finding that *SSN20* is the same gene as *SPT6*, which affects expression of δ insertions in the 5′ noncoding region of *HIS4* (F. Winston, D. T. Chaleff, B. Valent, and G. R. Fink, Genetics 107:179–197, 1984). We also showed that an *ssn20* mutation restored expression of secreted invertase from deletions of the *SUC2* upstream regulatory region; *ssn20* restored derepression of *SUC2* mRNA in strains with a *SUC2* upstream region deletion or a *snf2* mutation. Increased or decreased gene dosage of *SSN20* also suppressed defects that are suppressed by *ssn20* missense mutations. These findings suggest that *SSN20* plays a role in general transcriptional processes.

The *SSN20* gene was originally identified during our genetic analysis of glucose (carbon catabolite) repression in *Saccharomyces cerevisiae*. We have analyzed the control of expression of a particular glucose-repressible gene, *SUC2*, the structural gene for invertase. The *SUC2* gene encodes two forms of invertase: a secreted invertase that is responsible for the extracellular hydrolysis of sucrose and raffinose and an intracellular invertase with no obvious physiological function. The *SUC2* gene encodes these two forms via two mRNAs: secreted invertase is translated from a glucose-repressible 1.9-kilobase (kb) mRNA and intracellular invertase from a constitutive 1.8-kb mRNA (4, 7, 20). Analysis of the *cis*-acting regulatory elements at *SUC2* has shown that an upstream regulatory region is required for derepression of the 1.9-kb mRNA (23) and is sufficient to confer glucose-repressible expression to a heterologous promoter (24).

Previously, mutations have been isolated in six *trans*-acting genes required for derepression of *SUC2* in response to glucose deprivation, designated *SNF1* to *SNF6* (sucrose nonfermenting) (6, 17). Mutations in the *SSN20* gene were isolated as extragenic suppressors of *snf2* and *snf5*, which are pleiotropic mutations causing defects in the utilization of many carbon sources that are subject to glucose repression (raffinose, galactose, maltose, ethanol, glycerol) and also a defect in derepression of acid phosphatase (1). The defect in raffinose utilization results from failure to derepress the 1.9-kb *SUC2* mRNA under conditions of glucose deprivation (1).

The *ssn20* mutations (suppressors of *snf2*) were isolated as suppressors of the defect in secreted invertase synthesis caused by *snf2* or *snf5* mutations (18). Selection for raffinose-fermenting revertants of *snf2* and *snf5* mutants yielded pseudorevertants carrying mutations at the *SSN20* locus. Alleles with dominant, partially dominant, and recessive suppressor phenotypes were recovered. All were only partial suppressors of *snf2* or *snf5*, reversing the defect in utilization of raffinose, but not other carbon sources. The raffinose-nonfermenting phenotype of *snf6* was also suppressed by *ssn20*. An *ssn20* mutation had no significant effect on *SUC2* expression in a wild-type (*SNF*) strain. All *ssn20* alleles conferred a recessive, temperature-sensitive lethal phenotype regardless of the *SNF* genotype.

In this study, we cloned the *SSN20* gene and showed by gene disruption that it is an essential gene. Genetic mapping led to the finding that *SSN20* is the same gene as *SPT6*, which was previously identified by the isolation of extragenic suppressors of δ insertions in the 5′ noncoding region of *HIS4* (34). We examined the effects of *ssn20* mutations on expression of a series of deletions of the upstream regulatory region at the *SUC2* chromosomal locus. Suppression by altered *SSN20* gene dosage was also examined.

MATERIALS AND METHODS

Strains and genetic methods. The strains of *S. cerevisiae* used in this work are listed in Table 1. Standard methods were used for genetic analysis (28) and transformation (14). Segregants carrying *ssn20* were identified by their temperature sensitivity. Segregants carrying *SUC2* upstream deletions were identified by phenotype or by gel-transfer hybridization analysis (30).

Chromosomal mapping of *SSN20* was done by gel-transfer hybridization analysis of separated chromosome-sized DNAs (27) with a blot provided by C. R. Cantor.

Subclones. pCE200 and pCE201 (see Fig. 1) were derived from pLN206. All other plasmids of the pCE series are subclones in pCGS40 (11). pCE205 differs from pCE204 in the orientation of the insert. The pCN plasmids are subclones in YIp5 (2). pCN212 was constructed by inserting the *HIS3* *Bam*HI fragment (31) in pCN204. pCC211 is a subclone in pBR322.

Preparation and analysis of DNA and RNA. DNAs were prepared as described previously (23). Preparation and gel-transfer hybridization analysis of poly(A)-containing RNAs

* Corresponding author.

TABLE 1. Strains of *S. cerevisiae*

Strain	Genotype	Source or reference[a]
MCY501	*MATα ade2-101 gal2 SUC2*	
MCY847	*MATα cly8 ade6 leu2-3*	9
MCY863	*MATα ssn20-6 snf2-50 his4-539 ura3-52 SUC2*	
MCY935	*MATα snf2-50 lys2-801 Δtrp1 SUC2*	
MCY939	*MATa ssn20-1 his4-539 ura3-52 SUC2*	
MCY1094	*MATα ade2-101 ura3-52 SUC2*	
MCY1145	*MATa snf2-50 his4-539 ade2-101 SUC2*	
MCY1146	*MATα suc2-Δ−1900/−390 ssn20-6 his4-539 lys2-801*	
MCY1147	*MATa suc2-Δ−1900/−390 ade2-101 his4-539*	
MCY1257	*MATa/MATα Δhis3/Δhis3 lys2-801/lys2-801 ade2-101/+ +/ura3-52 SUC2/SUC2*	
MCY1258	*MATa/MATα +/Δhis3 +/lys2-801 ade2-101/+ ura3-52/ura3-52 SUC2/SUC2*	
MCY1318	*MATa snf2-50 ssn20-1 his4-539 ade2-101 SUC2*	
MCY1529	*MATa/MATα ssn20-14/SSN20 his4-539/+ ade2-101/+ +/lys2-801 ura3-52/ura3-52 SUC2/SUC2*	
CC7	*MATa spt6-140 his4-917δ ura3-52 SUC2*	C. Clark-Adams and F. Winston
FW147	*MATα spt6-167 his4-912δ ura3-52 SUC2*	F. Winston
BH9-4	*MATa cdc62-1 his3 lys11 met4 aro7 trp1 (ura)*	P. Hanic-Joyce

[a] All strains for which no source or reference is listed are from this laboratory and are isogenic or congenic to S288C.

were as described previously (25), except when noted. ^{32}P-labeled probes were prepared by nick translation (21).

Assays for invertase. Cultures were prepared as described previously (8). Glucose-repressed cultures were grown to the mid-log phase in rich medium (yeast extract peptone) containing 2% glucose. To prepare derepressed cells, we shifted such cultures to YEP containing 0.05% glucose for 2.5 h. For strains carrying episomal plasmids or integrated pCN213, cultures were grown in synthetic complete medium (28) lacking uracil, and derepression was for 3.5 h. Secreted invertase activity was determined by assaying (12) whole cells as previously described (8).

RESULTS

Cloning the *SSN20* gene. A plasmid carrying the *SSN20* gene was isolated from a library by complementation of the temperature-sensitive growth defect caused by an *ssn20* mutation. The library (4), which contained DNA from a strain isogenic to S288C cloned into the episomal vector YEp24, was used to transform an *ssn20 ura3* mutant strain (MCY939) with selection for both the plasmid *URA3* marker and growth at nonpermissive temperature (37°C). Plasmid pLN206 (Fig. 1) was recovered from five transformants by passage through bacteria (8). To confirm its ability to complement *ssn20* mutations, we used pLN206 DNA to trans-

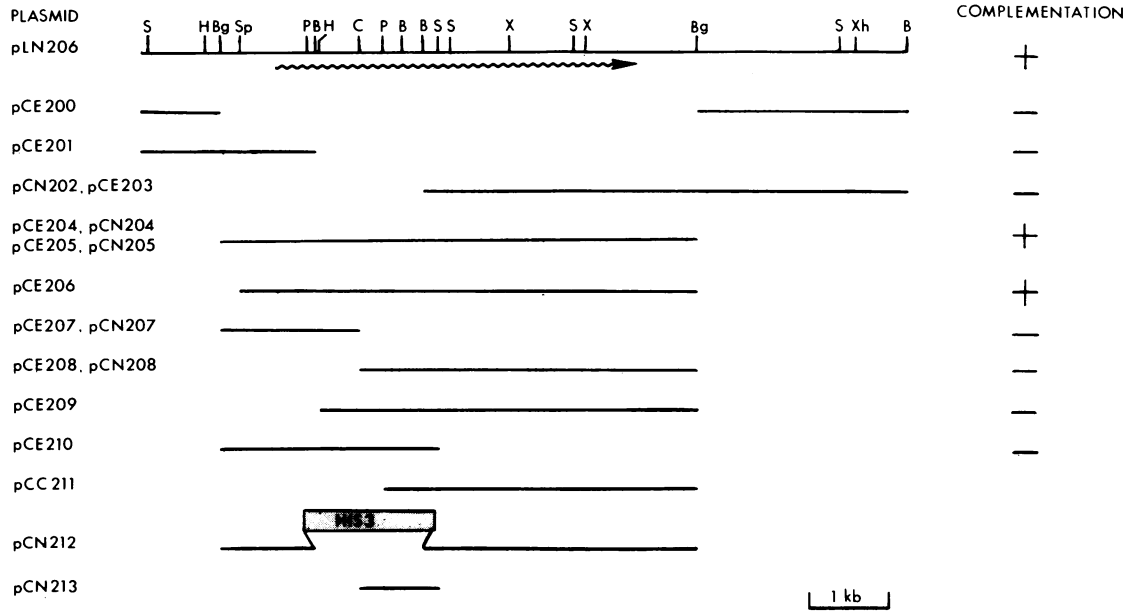


FIG. 1. Restriction maps of clones containing *SSN20* sequences. Plasmids are described in Materials and Methods. Only the cloned DNA segment is indicated. Restriction sites within the *HIS3* fragment (shaded box) are not shown. pCN212 has an *Sph*I site in the vector sequence near the right edge of the cloned segment. The ability of the episomal plasmids to complement the temperature-sensitive lethal phenotype of an *ssn20* mutant is indicated. The wavy arrow indicates the approximate position and direction of transcription of the *SSN20* RNA. Restriction sites: B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; H, *Hin*dIII; P, *Pvu*II; S, *Sal*I; Sp, *Sph*I; X, *Xba*I; Xh, *Xho*I. Not all *Sph*I, *Xba*I, or *Cla*I sites are shown.

form MCY939 (*ssn20-1*) and MCY863 (*ssn20-6 snf2*) to uracil prototrophy. pLN206 complemented the temperature-sensitive lethality of both *ssn20* alleles. In addition, pLN206 complemented *ssn20-6* for suppression of *snf2*: MCY863 transformants were unable to grow on minimal medium containing raffinose as the carbon source and showed decreased levels of secreted invertase activity relative to control cultures of MCY863 carrying YEp24 (Table 2).

Evidence that pLN206 carried the *SSN20* gene was obtained by demonstrating genetic linkage of the cloned DNA to the *SSN20* locus. Strain MCY1094 (*SSN20 ura3*) was transformed with pCN202 DNA (Fig. 1) that had been linearized by cleavage with *Bgl*II to target integration to the homologous genomic locus. Four Ura$^+$ transformants were crossed to MCY939 (*ssn20 ura3*), and the resulting diploids were subjected to tetrad analysis. In the 16 tetrads examined, the Ura$^+$ phenotype and the ability to grow at 37°C cosegregated 2:2. These results indicated that pCN202 integrated at a locus tightly linked to *SSN20*.

Localization of *SSN20* gene. To delimit the *SSN20* gene within pLN206, we constructed subclones in episomal vectors (pCE series; Fig. 1). These subclones were used to transform MCY939, and in each case four Ura$^+$ transformants were tested for growth at 37°C. Sequences within the 5.7-kb region between the *Sph*I site and the *Bgl*II site in pCE206 were required for complementation of the temperature-sensitive growth defect of *ssn20* (Fig. 1).

***SSN20* is a unique gene.** Total genomic DNA was digested with *Eco*RI and analyzed by gel-transfer hybridization (30). Only the expected restriction fragments were detected with a probe prepared from pCN205, which complements for *SSN20* function, indicating that pCN205 contains no repeated sequence (data not shown).

Identification of *SSN20* RNA. To identify the RNA encoded by *SSN20*, we examined poly(A)-containing RNAs from both glucose-repressed and -derepressed cultures by Northern blot hybridization (Fig. 2). A 4.6-kb RNA homologous to pCN207 and pCN208 was detected. In addition, a 1.4-kb RNA hybridized to pCN207, and a 1.7-kb species hybridized to pCN208; these two RNAS are presumably encoded by genes adjacent to *SSN20*. These findings, in conjunction with the complementation data, suggest that *SSN20* encodes a 4.6-kb poly(A)-containing RNA and that the *SSN20* gene lies completely within the 6-kb *Bgl*II fragment. The approximate position of this RNA is indicated in Fig. 1; the direction of transcription was determined by sequence analysis (M. Swanson and M. Carlson, unpublished data). Comparison of the relative abundance of the *SSN20* RNA in glucose-repressed and -derepressed cells indicated that the level of this RNA is not regulated by glucose repression (Fig. 2).

TABLE 2. Effects of *SSN20* gene dosage on invertase expression

Relevant genotype	Secreted invertase activity[a]	
	Repressed	Derepressed
Wild type(YEp24)	<1	195
Wild type(pLN206)	<1	240
snf2-50 ssn20-6(YEp24)	3	180
snf2-50 ssn20-6(pLN206)	3	20
snf2-50(YEp24)	<1	9
snf2-50(pLN206)	2	52
suc2-Δ−456/−223 *ssn20-6*	7[b]	190[b]
suc2-Δ−456/−223(YEp24)	2	12
suc2-Δ−456/−223(pLN206)	6	56
suc2-Δ−1900/−436 *ssn20-6*	25[b]	100[b]
suc2-Δ−1900/−436(YEp24)	3	4
suc2-Δ−1900/−436(pLN206)	4	15
suc2-Δ−1900/−436 *ura3*::pCN213 / *suc2*-Δ−1900/−436 *ura3*	2	6
suc2-Δ−1900/−436 *ssn20*::pCN213 / *suc2*-Δ−1900/−436 *SSN20*	4	15

[a] Micromoles of glucose released per minute per 100 mg (dry weight) of cells. Values are the average of assays of two transformants. Standard errors were <10%. Cultures were grown in synthetic complete medium lacking uracil. The presence of YEp24 had no significant effect on invertase activity (data not shown).

[b] Values taken from Fig. 3.

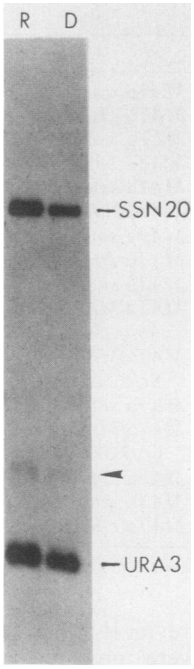


FIG. 2. Northern blot analysis of the *SSN20* RNA. Poly(A)-containing RNAs from cells of the wild-type strain MCY501 grown under repressing (R) or derepressing (D) conditions were separated by electrophoresis on a 0.9% agarose gel containing formaldehyde (15) and transferred to nitrocellulose (33). RNAs homologous to radiolabeled pCN208 DNA were detected by hybridization and autoradiography. The arrowhead points to the position of a 1.7-kb RNA that was detected after longer exposure.

Disruption of *SSN20* is lethal. To ascertain the phenotype of an *ssn20* null mutation, we used the cloned DNA to disrupt the *SSN20* gene at its chromosomal locus. We first used the integrative plasmid pCN213, which contains the 1-kb *Cla*I-*Sal*I fragment internal to the *SSN20* gene (Fig. 1), to transform the diploid strain MCY1258 to uracil independence. Integration of the plasmid at the homologous chromosomal locus results in the creation of two partial copies of the gene, one with a C-terminal deletion and the other with an N-terminal deletion (29). A diploid was used because the temperature-sensitive lethality of all 42 *ssn20* alleles suggested that *SSN20* is an essential gene. Four transformants were examined by gel-transfer hybridization analysis of genomic DNA to verify that the *SSN20* locus of one chromosome homolog was disrupted. The four transformants were subjected to tetrad analysis, and spores were allowed

to germinate on rich medium (YEP-glucose) at 25°C. In each case only two viable spore clones were recovered from each of seven four-spored asci, and all were Ura$^-$. Thus, the lethality cosegregated with the *URA3* marker on the integrated plasmid. Examination of 41 spores that failed to form colonies showed that 21 failed to bud, 13 budded once, and the remaining 7 had two or three buds. These findings suggest that disruption of *SSN20* is lethal in a haploid.

We also constructed a substitution mutation at the chromosomal *SSN20* locus. The substitution in pCN212 (Fig. 1) was introduced into the genome by using the *Sph*I fragment from pCN212 to transform the diploid strain MCY1257 to histidine independence (22). Integration of this fragment at the *SSN20* locus by homologous recombination resulted in the replacement of the wild-type sequence with the substitution. The presence of this mutant allele, designated *ssn20-Δ1::HIS3*, on one chromosome homolog of a diploid transformant was confirmed by gel-transfer hybridization analysis. This diploid transformant was subjected to tetrad analysis. Two viable spore clones were recovered from each of 19 four-spored asci, and all displayed a His$^-$ phenotype, indicating that the *HIS3* marker was tightly linked to the lethal mutation. Ten tetrads were allowed to germinate at 25°C and nine tetrads at 30°C; for both sets, the extent of budding observed for spores that failed to form clones was similar to that described above. These experiments suggest that disruption of the *SSN20* locus results in a recessive lethal mutation.

Verification that the cloned gene is *SSN20*. To confirm the identity of the cloned gene, we constructed diploids of the genotype *ssn20-14/ssn20*::pCN213 to show that the mutation generated by gene disruption was allelic to an *ssn20* missense mutation. Disruption of the wild-type *SSN20* locus in an *SSN20/ssn20-14* dipploid (MCY1529) was accomplished by transformation with pCN213 as described above. Eight temperature-sensitive transformants were recovered from among the Ura$^+$ transformants, and gel-transfer hybridization analysis verified that each carried a disrupted (*ssn20*::pCN213) locus. Tetrad analysis showed 2:2 segregation of a lethal mutation tightly linked to the *URA3* marker in eight four-spored asci from each of five of these diploids; all viable spore clones were temperature-sensitive for growth, confirming that the wild-type *SSN20* locus was disrupted in these diploids. Tetrad analysis of the remaining three diploids yielded some asci with three and four viable spores, most of which were Ura$^-$; these were not analyzed further. Diploids of the genotype *ssn20-7/ssn20*::pCN213 and *ssn20-22/ssn20*::pCN213 were constructed in parallel experiments, and these diploids were also temperature sensitive for growth. These experiments in conjunction with the complementation and linkage data prove that the cloned gene is *SSN20*.

From these experiments we also recovered six diploids of the genotype *SSN20/ssn20*::pCN213, as shown by gel-transfer hybridization analysis and tetrad analysis. These diploids were not temperature sensitive for growth; the null mutation is therefore recessive for this phenotype.

Genetic mapping. The chromosome carrying the *SSN20* locus was identified by gel-transfer hybridization analysis of chromosome-sized DNAs separated by the method of Schwartz and Cantor (27). Radiolabeled pCC211 DNA hybridized to the doublet of chromosome VII and XV (3). Standard meiotic linkage analysis revealed linkage of *ssn20* to the *ade6* locus on the right arm of chromosome VII. Analysis of the mapping cross MCY939 × MCY847 established a linkage distance of 53 centimorgans (cM) between *ssn20* and *ade6* and 10 cM between *ssn20* and *cly8* (Table 3). Examination of 11 tetrads recombinant for the *ssn20* and *cly8* markers showed that 10 were also recombinant for *ssn20* and *ade6*, indicating that the gene order is *ade6-cly8-ssn20*.

TABLE 3. Linkage data

Gene pair	No. of tetrads[a]			Map distance[b] (cM)
	PD	NPD	T	
ssn20-ade6	7	2	42	53
ssn20-cly8	42	0	11	10
cly8-ade6	18	2	28	42[c]

[a] PD, Parental ditype; NPD, nonparental ditype; T, tetratype. Scoring of *ssn20* and *cly8* was done by complementation for temperature sensitivity.

[b] Genetic map distances in centimorgans were calculated from the tetrad data by the equation of Perkins (19): cM = 100(T + 6 NPD)/2(PD + NPD + T).

[c] This value agrees with our previous value of 43 cM (9) but is greater than the value of 18.2 cM calculated by Mortimer and Schild (16).

***SSN20* is the same gene as *SPT6*.** The map position and temperature-sensitive lethal phenotype of *ssn20* suggested the possibility of allelism with *spt6*, although not all *spt6* mutations conferred temperature sensitivity (34). Mutations in *SPT6* were isolated as extragenic suppressors of δ insertions into the 5′ noncoding region of *HIS4* (34). The restriction maps of the cloned *SSN20* and *SPT6* (10) genes were identical, and the alleles *ssn20-7* and *spt6-140* failed to complement for the temperature-sensitive lethal phenotype.

To determine whether the *spt6-140* allele suppressed the defects in *SUC2* derepression caused by *snf2*, we crossed strain CC7 (*spt6-140*) to MCY935 (*snf2*). Tetrad analysis of the resulting diploid showed 2:2 segregations for temperature sensitivity and growth on glycerol in seven tetrads (*snf2* causes a failure to grow on glycerol that is not suppressed by *ssn20*). In contrast, 4+:0−, 3+:1−, and 2+:2− segregations for ability to utilize raffinose were observed, indicating that *spt6-140* suppressed the invertase derepression defect of *snf2*. All glycerol-nonutilizing, raffinose-fermenting spore clones were also temperature sensitive for growth, and all glycerol-nonutilizing, temperature-sensitive spore clones were raffinose fermenters.

We also tested the ability of the *spt6-167* allele to suppress *snf2*. Strain FW147 (*spt6-167*) was crossed to MCY1318 (*ssn20-1 snf2*), and the resulting diploid was subjected to tetrad analysis. In five tetrads, glycerol utilization segregated 2+:2−, and raffinose fermentation segregated 4+:0−. These data confirm the tight linkage of *ssn20* and *spt6* and indicate that *spt6-167* suppressed the raffinose fermentation defect of *snf2*.

The map position and conditional lethal phenotype of *ssn20* also suggested the possibility of allelism with *cdc62* (13), although the *ssn20* mutations tested did not confer a cell-cycle defect; however, *ssn20-1* and *cdc62-1* complemented for temperature sensitivity of growth.

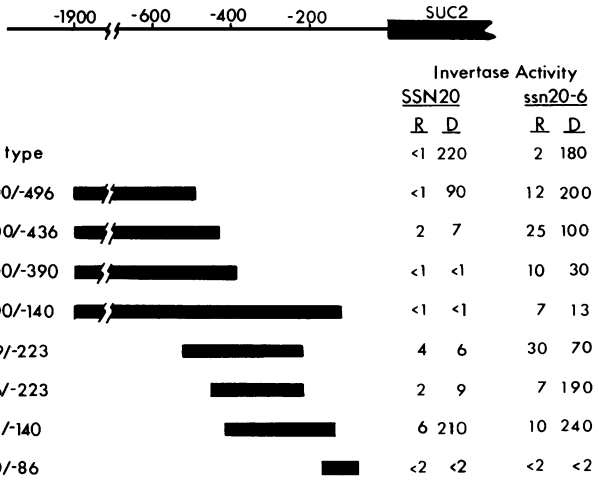
***ssn20* restores expression from *SUC2* upstream regulatory region deletions.** Previously, a series of deletions of the upstream regulatory region was constructed at the *SUC2* locus (23). To examine the effects of an *ssn20* mutation on expression of these deletions, we constructed double mutants carrying *ssn20-6* and each deletion by genetic crossing. Segregants from these crosses were assayed for secreted invertase activity after growth under glucose-repressing and -derepressing conditions (Fig. 3).

The *ssn20-6* mutation did not suppress deletion Δ−180/−86, which removes the TATAAATA sequence at position −133 (relative to the translational start for the

precursor to secreted invertase). In contrast, *ssn20* did, at least partially, restore expression from every other deletion tested. The two most dramatic examples were deletions Δ−1900/−436 and Δ−456/−223. These two deletions in a wild-type (*SSN20*) background reduce the derepression of secreted invertase to less than 5% of the wild-type level; introduction of *ssn20* restored derepression to 50 and 100%, respectively. An *ssn20* mutation also partially restored invertase expression from two deletions that completely prevent invertase derepression in an *SSN20* strain (Δ−1900/−140 and Δ−1900/−390); this expression was not significantly glucose repressible. In fact, *ssn20* caused some constitutive (glucose insensitive) expression of many of the deletions. It is likely that in some cases the deletion mutation contributes to the constitutivity because some of the deletions show constitutive expression in a wild-type (*SSN20*) genetic background.

The *ssn20* allele used in these studies, *ssn20-6*, is a partially dominant suppressor of *snf2*. Another allele, *ssn20-1*, which is a recessive suppressor of *snf2*, was also tested for suppression of the *SUC2* deletions Δ−1900/−436 and Δ−456/−223. Diploids heterozygous at both the *SSN20* and *SUC2* loci were subjected to tetrad analysis. In each cross temperature sensitivity segregated 2+:2−, and ability to utilize raffinose segregated 4+:0−, 3+:1−, and 2+:2−. No raffinose-nonfermenting spore clone was temperature sensitive for growth. These data indicate that *ssn20-1* reverses the raffinose-nonfermenting phenotype of both of these deletions.

***ssn20* suppresses defects in derepression of *SUC2* mRNA.** In the wild type, secreted invertase is encoded by a glucose-repressible 1.9-kb *SUC2* mRNA, and the constitutively synthesized intracellular invertase is encoded by an unregulated 1.8-kb *SUC2* mRNA with a different 5′ end (4). To determine whether *ssn20* restored secreted invertase expression from *SUC2* deletion mutants by restoring expression of the 1.9-kb mRNA, we examined the mRNAs produced from deletion Δ−1900/−390 in both *ssn20* mutant and wild-type



	Invertase Activity			
	SSN20		ssn20-6	
	R	D	R	D
Wild type	<1	220	2	180
Δ-1900/-496	<1	90	12	200
Δ-1900/-436	2	7	25	100
Δ-1900/-390	<1	<1	10	30
Δ-1900/-140	<1	<1	7	13
Δ-529/-223	4	6	30	70
Δ-456/-223	2	9	7	190
Δ-418/-140	6	210	10	240
Δ-180/-86	<2	<2	<2	<2

FIG. 3. Effects of *ssn20* on synthesis of secreted invertase by strains carrying deletions of the *SUC2* upstream regulatory region. The bar indicates the deleted sequence. Nucleotide positions are relative to the translational start site (+1). Invertase activity is expressed as micromoles of glucose released per minute per 100 mg (dry weight) of cells. Values are the average of determinations for at least two segregants from the cross used to construct each double mutant. Standard errors were <10%. R, Glucose repressed; D, derepressed.

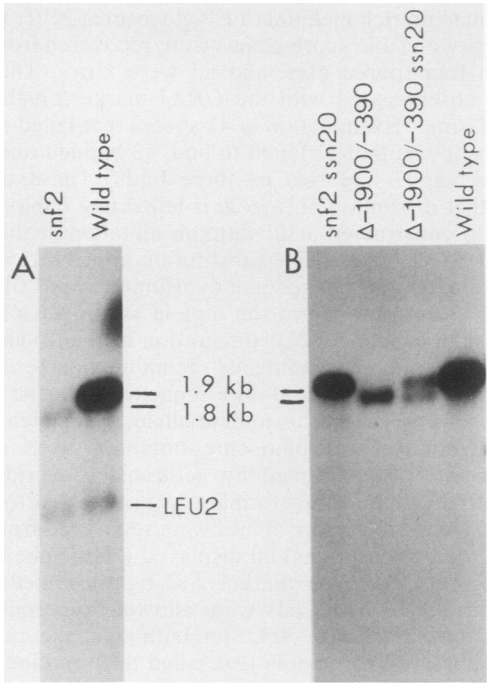


FIG. 4. An *ssn20* mutation restores expression of *SUC2* mRNA in mutants. Poly(A)-containing RNAs prepared from glucose-derepressed cells were subjected to electrophoresis on a 1.5% (A) or a 1.2% (B) agarose gel containing formaldehyde (15) and transferred to nitrocellulose (33). (A) RNAs were prepared from strains MCY1145 (*snf2*) and MCY501 (wild type). The *SUC2* and *LEU2* mRNAs were detected with probes prepared from pRB117 (4) and YEp351 (gift of J. Hill and T. J. Koerner), respectively. This panel is taken from reference 1. (B) RNAs were prepared from strains MCY863 (*snf2 ssn20*), MCY1147 (*suc2*-Δ−1900/−390), MCY1146 (*suc2*-Δ−1900/−390 *ssn20*), and MCY501 (wild type). The *SUC2* mRNAs were detected with a probe prepared from pRB59 (4). Subsequent hybridization of this filter with a probe specific for the *LEU2* RNA confirmed that equal amounts of RNA were loaded in all lanes.

(*SSN20*) genetic backgrounds. Poly(A)-containing RNA was prepared from derepressed cultures and examined by Northern blot hybridization analysis (Fig. 4B). The *SSN20 suc2*-Δ−1900/−390 cells contained the 1.8-kb mRNA but little or no 1.9-kb mRNA, as expected from the failure of this strain to produce secreted invertase. In contrast, the 1.9-kb mRNA was detected in derepressed *ssn20 suc2*-Δ−1900/−390 cells.

Previously, *snf2* and *snf5* mutants were shown to fail to derepress the 1.9-kb *SUC2* mRNA (1) (Fig. 4A). Analysis of RNA from derepressed *snf2 ssn20* cells showed that *ssn20* restored derepression of the 1.9-kb *SUC2* mRNA in a *snf2* mutant (Fig. 4B).

Suppression by multiple copies of *SSN20*. To determine whether multiple copies of *SSN20* would suppress the defects in *SUC2* expression caused by *snf2* mutations or *SUC2* regulatory region deletions Δ−1900/−436 and Δ−456/−223, strains carrying each of these mutations were transformed with the multicopy plasmid pLN206 and the vector YEp24. Transformants were grown under glucose-repressing or derepressing conditions with selection for plasmid maintenance, and secreted invertase was assayed (Table 2). pLN206 partially suppressed the invertase derepression defects caused by *snf2* and both deletions, and the slight increase in derepression of *suc2*−Δ−1900/−436 was sufficient to result in an improved growth phenotype on sucrose.

This suppression, however, was not as efficient as that observed with *ssn20* missense mutations. Plasmid pLN206 had no significant effect on the regulation of invertase expression in the wild type (*SUC2 SNF*) (Table 2).

Effects of reduced *SSN20* gene dosage. Clark-Adams and Winston (10) found that a diploid hemizygous at the *SPT6* (*SSN20*) locus showed an Spt$^-$ phenotype. Their findings prompted us to examine hemizygous strains for suppression of the defect in invertase derepression caused by the *SUC2* upstream region deletion Δ−1900/−436. A diploid homozygous for *suc2*-Δ−1900/−436 and *ura3* was transformed with pCN213, and Ura$^+$ transformants carrying a disruption of the *SSN20* locus on one homolog were identified by gel-transfer hybridization analysis. Tetrad analysis of two such transformants showed the expected 2:2 segregation of a lethal mutation tightly linked to the *URA3* marker on the plasmid. Both of these hemizygous diploids (*SSN20/ssn20*::pCN213) grew noticeably better on sucrose than did the parent diploid (*SSN20/SSN20*) or other transformants from this experiment in which the integration event occurred at the *ura3* locus. Assays of secreted invertase activity showed that the diploid transformants carrying one disrupted *SSN20* locus derepressed invertase to slightly higher levels than did diploids with two wild-type *SSN20* alleles (Table 2). Diploids of the genotype *SUC2/SUC2 SSN20/ssn20*::pCN213 showed normal regulation of invertase expression (data not shown).

DISCUSSION

We cloned the *SSN20* gene and showed that it is an essential gene in *S. cerevisiae*: disruption of the gene proved lethal to a haploid cell. All of 42 *ssn20* missense mutations previously isolated as suppressors of *snf2* and *snf5* caused temperature sensitivity for growth (18).

We showed here that mutations at the *SSN20* locus also partially alleviated the requirement for *SUC2* upstream regulatory sequences that are normally essential for derepression of secreted invertase. We previously identified a region between −650 and −418 that was required for high-level *SUC2* expression (23); in an *ssn20* mutant much of this region was dispensable. An *ssn20* mutation restored high-level secreted invertase expression in mutants with partial deletions of this upstream regulatory region and allowed some expression in mutants carrying complete deletions of this region. Examination of *SUC2* mRNAs showed that synthesis of secreted invertase resulted from expression of the expected 1.9-kb *SUC2* mRNA in *ssn20 suc2*-Δ−1900/−390 strains and also in *ssn20 snf2* mutants. Thus, *ssn20* mutations permitted transcription from the promoter for the 1.9-kb mRNA in the absence of some of the *cis*- and *trans*-acting elements that are normally critical. The TATA box, however, was still required.

Previous studies have identified a 7-base-pair repeated element within the *SUC2* upstream region and shown that tandem copies of a 32-base-pair sequence containing one of these 7-base-pair elements activate expression of the heterologous yeast promoter of a *LEU2-lacZ* fusion (26). The pattern of expression from *SUC2* deletions suggested that these repeated elements were still important for expression in an *ssn20* mutant. One possibility to account for the effects of *ssn20* on expression of SUC2 upstream deletions was that a mutant *ssn20* gene product enhanced the activating function of the remaining repeated elements; however, no effect of *ssn20* on activation of the *LEU2-lacZ* fusion by tandem 32-base-pair sequences could be detected (L. Sarokin and M. Carlson, unpublished data).

Interestingly, both increased and reduced gene dosage of the wild-type *SSN20* gene also caused suppression, although not as effectively as *ssn20* missense mutations. A possible explanation for this finding is that the *SSN20* gene product is part of a complex. The isolation of *ssn20* alleles with both recessive and dominant suppression phenotypes led us previously to suggest that the *SSN20* product functions as a multimer (18).

Evidence that *SSN20* is the same gene as *SPT6* is presented here and in the accompanying paper (10). Mutations in *SPT6* were isolated as suppressors of a δ insertion in the 5′ noncoding region of *HIS4* (34), and *spt6* mutations have been found to alter transcription from this mutant *HIS4* locus (10). There is no obvious relationship between the selection used to obtain *ssn20* and *spt6* mutations except that in both cases changes in transcription were selected. There is no evidence that transcription of δ elements is regulated by glucose repression, although gene activation by Ty elements is affected by the carbon source (32). The *SUC2* locus does not contain any δ element (5). Suppression of *snf2* and *snf5* cannot involve δ insertions at these loci because nonsense alleles are suppressed.

In summary, dominant and recessive *ssn20* missense mutations alleviate requirements for some *cis*- and *trans*-acting elements for expression of the 1.9-kb *SUC2* mRNA. These suppressor phenotypes, the gene dosage effects, the essentiality of the gene, and the identity with *SPT6* suggest that *SSN20* plays a role in general transcriptional processes. Perhaps the *SSN20* gene product interacts with RNA polymerase or with promoter elements such as the TATA box. It is also possible that *SSN20* mediates interactions between factors associated with upstream activating sequences and factors acting at downstream promoter sequences.

ACKNOWLEDGMENTS

We thank C. D. Clark-Adams and F. Winston for communicating unpublished results. We thank C. R. Cantor for providing a chromosome blot and M. S. Swanson for carrying out studies of the relationship between *ssn20* and *spt6*.

This work was supported by Public Health Service grant GM34095 from the National Institutes of Health and an Irma T. Hirschl Research Career Award to M.C.

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