Molecular and Genetic Analysis of the *SNF7* Gene in *Saccharomyces cerevisiae*

Jiangling Tu, Laura G. Vallier and Marian Carlson

Institute of Cancer Research and Department of Genetics and Development, Columbia University, New York, New York 10032

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ABSTRACT

Mutations in the *SNF7* gene of *Saccharomyces cerevisiae* prevent full derepression of the *SUC2* (invertase) gene in response to glucose limitation. We report the molecular cloning of the *SNF7* gene by complementation. Sequence analysis predicts that the gene product is a 27-kDa acidic protein. Disruption of the chromosomal locus causes a fewfold decrease in invertase derepression, a growth defect on raffinose, temperature-sensitive growth on glucose, and a sporulation defect in homozygous diploids. Genetic analysis of the interactions of the *snf7* null mutation with *ssn6* and *spt6/ssn20* suppressor mutations distinguished *SNF7* from the *SNF2*, *SNF5* and *SNF6* genes. The *snf7* mutation also behaved differently from mutations in *SNF1* and *SNF4* in that *snf7 ssn6* double mutants displayed a synthetic phenotype of severe temperature sensitivity for growth. We also mapped *SNF7* to the right arm of chromosome *XII* near the centromere.

IN the yeast *Saccharomyces cerevisiae*, the availability of glucose, or another preferred carbon source, has a profound effect on patterns of gene expression. In response to glucose, the cell coordinately represses expression of genes necessary for utilization of alternate carbon sources and for respiration. This phenomenon is referred to as glucose (or catabolite) repression. When glucose is limiting, repression is relieved.

A number of regulatory genes have been identified that are necessary for glucose repression and derepression (for review, JOHNSTON and CARLSON 1992; TRUMBLY 1992). Genes required for release from glucose repression include *SNF1* (*CAT1*, *CCR1*), *SNF4* (*CAT3*), *SNF2* (*SWI2*), *SNF5* and *SNF6*. These five genes fall into two groups: *SNF1*, *SNF4*; and *SNF2*, *SNF5*, *SNF6*. These groupings were first recognized genetically by suppressor analysis and later confirmed by molecular analysis of function. *SNF1* encodes a protein serine/threonine kinase required for release from glucose repression (CELENZA and CARLSON 1986; SCHULLER and ENTIAN 1987), and *SNF4* encodes a protein that is associated with *SNF1* and acts as a positive effector of the kinase (CELENZA, ENG and CARLSON 1989; SCHULLER and ENTIAN 1988). *SNF2*, *SNF5* and *SNF6* are involved in general transcriptional activation and affect an array of genes that are regulated in response to various signals besides glucose (ABRAMS, NEIGEBORN and CARLSON 1986; HAPPEL, SWANSON and WINSTON 1991; LAURENT, TREITEL and CARLSON 1991; LAURENT and CARLSON 1992; PETERSON and HERSKOWITZ 1992). *SNF2* and *SNF5* also affect expression of protease B activity (MOEHLE and JONES 1990). (*SNF3* encodes a glucose transporter that does not appear to have a regulatory role (MARSHALL-CARLSON *et al.* 1990)).

Five other genes required for derepression of the *SUC2* gene, encoding invertase, were identified in a recent mutant search: *SNF7*, *SNF8*, *SNF9*, *SNF10* and *GAL11* (VALLIER and CARLSON 1991). The three *snf7* alleles recovered in this screen caused a partial reduction in invertase production, a growth defect on raffinose (invertase catalyzes the hydrolysis of sucrose and raffinose) and failure of homozygous diploids to sporulate. These *snf7* alleles did not cause growth defects on galactose or glycerol, but none was known to be a null mutation. To determine the role of *SNF7* in the regulatory response to glucose, we have undertaken further genetic and molecular characterization of the gene.

We report here the cloning, sequence analysis and disruption of *SNF7*. We examined the genetic interaction of a *snf7* null mutation with *spt6* and *ssn6*, which are suppressors showing distinctive patterns of interactions with two previously defined groups of *snf* mutations. Finally, we genetically mapped the *SNF7* locus.

MATERIALS AND METHODS

Yeast strains: The *S. cerevisiae* strains used are listed in Table 1. All strains were constructed in this laboratory and have the S288C genetic background.

General genetic methods and media: Standard methods for mating, sporulation and tetrad analysis were used (ROSE, WINSTON and HIETER 1990). Yeast cells were transformed by the lithium acetate method (ITO *et al.* 1983). Utilization of glucose and raffinose was assessed on solid medium containing YEP (1% yeast extract, 2% bacto-peptone), 1 μg/ml antimycin A (Sigma; prepared as a stock solution of 1

TABLE 1

Strains used in this study

Strain	Genotype
MCY1093	*MATa his4-539 lys2-801 ura3-52 SUC2*
MCY1366	*MATa ssn20-1 ade2-101 lys2-801 ura3-52 SUC2*
MCY1514	*MATa his4-539 ura3-52 gal2 SUC2*
MCY1751	*MATa/MATα ade2-101/+ his4-539/+ lys2-801/+ ura3-52/ura3-52 SUC2/SUC2*
MCY1802	*MATa his4-539 lys2-801 ssn6Δ9 SUC2*
MCY1823	*MATa ade2-101 lys2-801 ssn6Δ9 SUC2*
MCY1958	*MATa his4-539 lys2-801 ura3-52 ssn6Δ9 SUC2*
MCY2220	*MATα snf7-12 ade2-101 ura3-52 SUC2*
MCY2221	*MATa snf7-12 his4-539 lys2-801 ura3-52::pLS11-URA3 SUC2*
MCY2455	*MATα snf7Δ1::URA3 SUC2 ura3-52*
MCY2458	*MATα snf7Δ1::URA3 his4-539 lys2-801 ura3-52 SUC2*
MCY2924	*MATa ade2-101 ura3-52 trp1Δ1 SUC2*
MCY2937	*MATα snf7Δ1::URA3 his4-539 lys2-801 ura3-52 gal2 SUC2*

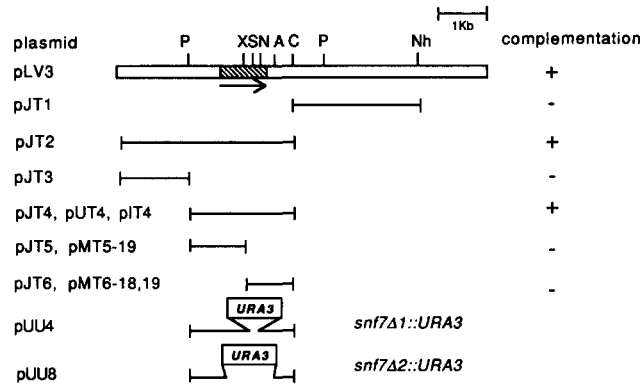

FIGURE 1.—Restriction maps of *SNF7* plasmids. Yeast genomic DNA cloned in plasmid pLV3 is shown by the bar. The *Nhe*I site in the vector YEp24 is on the left. The coding region of *SNF7* is shaded, and the direction of transcription is indicated by the arrow. The ability of plasmids to complement *snf7* for growth on raffinose is shown. Restriction sites: *Acc*I (A), *Cla*I (C), *Nar*I (N), *Nhe*I (Nh), *Pvu*II (P), *Stu*I (S), *Xba*I (X).

mg/ml in 95% ethanol) and 2% glucose (YPDaa) or 2% raffinose (YPRaa). Antimycin A is an inhibitor of respiration. The Raf$^-$ phenotype of *snf7* mutant could also be scored on YPR medium with anaerobic incubation in Gas Paks (BBL). Utilization of galactose was scored on YEP medium containing 2% galactose with anaerobic incubation. Utilization of glycerol was scored on YEP medium containing 3% glycerol (YPGly). Synthetic medium was 0.67g/liter yeast nitrogen base supplemented with histidine, lysine and adenine and containing 2% glucose (SD-Ura) or containing 1 μg/ml antimycin A and 2% glucose (SDaa-Ura) or 2% raffinose (SRaa-Ura). Except where noted otherwise, all scoring was performed by spotting cell suspensions onto the appropriate medium and incubating the plates at 30°. To assess sporulation proficiency, diploid cells were grown in YEP containing 2% glucose (YPD) overnight, collected by centrifugation, resuspended in a small volume, spotted on solid sporulation medium (ROSE, WINSTON and HIETER 1990) and incubated at room temperature for 8 days after which they were examined for the presence of asci.

Cloning of the *SNF7* gene: DNA from a YEp24 library (CARLSON and BOTSTEIN 1982) was used to transform MCY2220 to uracil prototrophy on SD-Ura, and transformants were replica-plated onto SDaa-Ura and SRaa-Ura. Five complementing transformants were recovered that retained the ability to complement the growth defect on raffinose after retesting. Plasmid DNA was recovered from two transformants by a modification of the method of HOFFMAN and WINSTON (1987) and transformed into *Escherichia coli* HB101 strain by the calcium chloride method. Plasmids were prepared from bacteria by a modification of the alkaline lysis method (MANIATIS, FRITSCH and SAMBROOK 1982).

Construction of subclones: First, the 7-kb *Nhe*I fragment from pLV3 (extending from a site in the YEp24 vector sequence into the insert) was subcloned into the *Spe*I site of the centromere-containing vector pRS316 (SIKORSKI and HIETER 1989), yielding pLV8. Plasmids pJT1, pJT2, pJT3, pJT4, pJT5 and pJT6 (Figure 1) are subclones in pRS316. pJT2 was derived from pLV8 by deleting the *Cla*I fragment extending into the polylinker. pJT3 and pJT4 contain 1.4 kb and 2.3 kb *Pvu*II fragments from pJT2, extending to vector sites, subcloned into the *Sma*I site of pRS316. pJT5 and pJT6 contain 1.3-kb *Xba*I-*Hin*dIII (polylinker site) and 1-kb *Xba*I-*Xba*I (polylinker site) fragments from pJT4 cloned into the cognate sites of pRS316; and pMT5-19, pMT6-18 and pMT6-19 contain these fragments cloned into M13 mp18 and M13 mp19 (YANISCH-PERRON, VIEIRA and MESSING 1985). pUT4 and pIT4 contain the *Eco*RI-*Sal*I fragment (polylinker site) from pJT4 cloned into pUC19 (NORRANDER, KEMPE and MESSING 1983) and pRS306 (SIKORSKI and HIETER 1989), respectively.

Disruption of chromosomal *SNF7* locus: To disrupt *SNF7* at the chromosomal locus, we first constructed pUU4 by replacing the *Xba*I-*Stu*I fragment in pUT4 with the 1.1-kb *Xba*I-*Sma*I fragment from pAC100 (E. ABRAMS, unpublished data) carrying the *URA3* gene. The *Xba*I site lies at codon 101 of *SNF7*. We then used the *Pvu*II-*Cla*I fragment purified from pUU4 to disrupt the *SNF7* locus of the wild-type diploid strain MCY1751 using the one-step gene replacement method (ROTHSTEIN 1983) selecting for uracil prototrophs. The presence of the disrupted allele on one homolog of the diploid was confirmed by Southern blot analysis (SOUTHERN 1975) using a probe prepared from pJT4. The resulting allele was designated *snf7Δ1::URA3*.

To generate a second disruption, *snf7Δ2::URA3*, we constructed pUU8. First, we used the polymerase chain reaction with pUT4 as template to amplify a fragment extending from the polylinker *Eco*RI site to a primer that included nucleotides −1 to −20 of *SNF7* and introduced a *Bam*HI site at codon 1. The resulting *Eco*RI-*Bam*HI fragment was cloned with the *Bam*HI-*Cla*I *URA3* gene fragment from a derivative of pIC-19R (MARSH, ERFLE and WYKES 1984) and the *Acc*I-*Sal*I fragment from pUT4 into pUC19 between the *Eco*RI and *Sal*I sites. The *Pvu*II-*Cla*I fragment purified from pUU8 was used to disrupt the *SNF7* locus of MCY1751 as described above.

Invertase assays: Glucose-repressed cultures were grown to mid-log phase in YEP containing 2% glucose at 25° or 30°; derepressed cultures were prepared by shifting to YEP containing 0.05% glucose for 2.5 hr at the same temperature. Invertase activity was assayed as previously described (VALLIER and CARLSON 1991).

Sequence analysis: Restriction fragments were cloned into M13mp18 or M13mp19 (YANISCH-PERRON, VIEIRA and MESSING 1985), yielding pMT5-19, pMT6-18 and pMT6-19. The nucleotide sequence was determined using the Sequenase (United States Biochemical) enzyme, the 17-mer sequence primer and several synthetic primers with a-^{35}S dATP purchased from Amersham Corp. The sequence

across the *Xba*I site was also determined from pJT4 using a synthetic primer. Both strands were sequenced (SANGER, NICKLEN and COULSON 1977) from position −147 to +1069.

Nucleotide sequence accession number: The sequence reported has been submitted to GenBank under accession number L09751.

RESULTS

Cloning of the *SNF7* gene: Plasmids carrying the *SNF7* gene were isolated from a multicopy genomic library by complementation of the *snf7* growth defect on raffinose, as described in MATERIALS AND METHODS. Two plasmids were recovered and found to carry overlapping segments of cloned yeast DNA. The restriction map of the smaller plasmid, pLV3, is shown in Figure 1.

To delimit the complementing gene on the cloned DNA, we constructed subclones of pLV3 in the centromere-containing vector pRS316 (SIKORSKI and HIETER 1989) and tested for complementation of the raffinose nonfermenting (Raf$^-$) phenotype of the *snf7* mutant MCY2220. Subclones pJT2 and pJT4 complemented the Raf$^-$ growth defect, but subclones pJT5 and pJT6 did not (Figure 1). These results suggest that the *SNF7* gene lies within the 2-kb region between the *Pvu*II and *Cla*I sites and spans the *Xba*I site.

We next tested the linkage of the cloned DNA to the chromosomal *SNF7* locus. Subclone pIT4 was digested with *Nar*I, to target integration, and was used to transform wild-type strain MCY1093 to uracil prototrophy. Southern blot analysis confirmed that pIT4 had integrated into its cognate locus. Four Ura$^+$ tranformants were then crossed to strain MCY2220 (*snf7-12 ura3*). Tetrad analysis of the diploids showed 2+:2− segregation for growth on raffinose in a total of 32 tetrads. In all tetrads the *URA3* marker cosegregated with ability to use raffinose. Thus, the cloned gene is tightly linked to the *SNF7* locus.

Nucleotide sequence of *SNF7*: Nucleotide sequence analysis of 1.2 kb within the *Pvu*II-*Cla*I fragment revealed a long open reading frame of 240 codons, which was subsequently shown to correspond to the *SNF7* gene (see below). The nucleotide sequence and the amino acid sequence of the predicted gene product are shown in Figure 2. A 1-kb RNA was detected by Northern analysis (data not shown); this size is consistent with that of the open reading frame. The amino acid sequence of the predicted 27-kDa protein was compared with the sequences in Protein Identification Resource (release V19.0) of the National Biochemical Research Foundation by using the FASTP program (PEARSON and LIPMAN 1988) and also with the sequences in GenBank (release 58.0) translated in all six reading frames by using the GCG program TFASTA (PEARSON and LIPMAN 1988). No significant homology to other proteins was found. The protein is highly charged and overall acidic, containing 21% acidic and 14% basic residues. The charged residues are clustered in three areas: at the N-terminus, residues 1–80 are 24% basic and at the C-terminus, amino acids 122 to 178 and 211 to 240 are 39% and 50% acidic, respectively. SNF7 has a potential nuclear localization signal, KKKK, starting at position 68 (BURGLIN and DE ROBERTIS 1987). No other informative signature sequences were identified.

Disruption of chromosomal *SNF7* locus: To determine the phenotype of a *snf7* null mutation, we disrupted the *SNF7* locus on one chromosome in the wild-type diploid strain MCY1751, as described in MATERIALS AND METHODS. Tetrad analysis of the resulting diploid, heterozygous for the *snf7Δ1::URA3* allele (Figure 1), showed 2+:2− segregations for growth on raffinose with cosegregation of the *URA3* marker. Derepression of invertase was reduced threefold relative to wild-type segregants (Table 2). The mutant segregants seemed slightly impaired for growth on glycerol, but grew well on galactose and glucose at 30°. The mutants were also able to utilize low concentration of glucose (YEP-0.05% glucose) as effectively as wild type. We noted a defect in growth on glucose at 37° that cosegregated with the *snf7Δ1::URA3* mutation. The mutant strains showed only a mild growth defect when cell suspensions were spotted onto rich medium containing glucose (YPD) at 37° but could not form single colonies on YPD at 37° (Figure 3, panels A and B). In addition, mutants homozygous for the disruption did not sporulate, even at room temperature.

Because some *SNF7* coding sequence remained in the *snf7Δ1::URA3* allele, we also constructed a second disruption, *snf7Δ2::URA3* (Figure 1), which deleted the entire gene. This mutation caused an identical phenotype with respect to carbon source utilization, invertase derepression and growth at 37°. In addition, we scored anaerobic growth on sucrose, and mutant segregants grew more slowly than wild type.

To confirm that the *SNF7* locus was disrupted, we tested the *snf7Δ1::URA3* and *snf7-12* mutations for complementation. Heteroallelic diploids displayed a Raf$^-$ phenotype and failed to sporulate, thereby supporting the identity of the cloned gene as *SNF7*.

Genetic interaction of *snf7* with *spt6* suppressor: Five of the previously characterized *SNF* genes fall into two distinct groups on the basis of phenotype and patterns of interaction with extragenic suppressors, including the *spt6/ssn20* mutation. The *SPT6* gene encodes an essential gene product that appears to be involved in transcription (CLARK-ADAMS and WINSTON 1987; NEIGEBORN, CELENZA and CARLSON 1987; SWANSON, CARLSON and WINSTON 1990). The raffinose growth defect of *snf2*, *snf5* or *snf6* mutants is suppressed by *spt6/ssn20* mutations, and double mutants show normal regulation of invertase; in contrast,

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-147                                    AGCCGTTACGAAGCACTGTGCTGGATT
-120  TAATAACGGTGGGTTTTCGCCCATGAATAGTTGCAGACCAGGGAAGTCGAGCTCCTAAGG
 -60  GGTAAGATATTGTATTTCGGCCGGAAGCAGCAGAAACATAACAGTATTGATAAATAAGGC
   1  ATGTGGTCATCACTTTTTGGTTGGACATCAAGTAATGCCAAGAATAAAGAGTCACCAACA
       M  W  S  S  L  F  G  W  T  S  S  N  A  K  N  K  E  S  P  T     20
  61  AAGGCCATAGTGCGGTTGAGGGAGCATATCAACCTTCTATCCAAAAAGCAATCGCATTTA
       K  A  I  V  R  L  R  E  H  I  N  L  L  S  K  K  Q  S  H  L     40
 121  CGTACTCAAATTACAAATCAAGAGAATGAAGCTAGAATCTTTTTGACGAAGGGCAATAAA
       R  T  Q  I  T  N  Q  E  N  E  A  R  I  F  L  T  K  G  N  K     60
 181  GTAATGGCGAAGAATGCACTTAAAAAGAAGAAGACAATCGAACAACTTTTAAGTAAGGTA
       V  M  A  K  N  A  L  K  K  K  K  T  I  E  Q  L  L  S  K  V     80
 241  GAAGGCACAATGGAGTCTATGGAACAGCAGCTATTCTCTATAGAAAGTGCAAACTTAAAT
       E  G  T  M  E  S  M  E  Q  Q  L  F  S  I  E  S  A  N  L  N    100
 301  CTAGAGACAATGAGGGCTATGCAGGAAGGTGCTAAGGCAATGAAAACTATTCACAGTGGC
       L  E  T  M  R  A  M  Q  E  G  A  K  A  M  K  T  I  H  S  G    120
 361  CTTGACATAGATAAAGTGGATGAAACTATGGACGAGATAAGGGAGCAAGTCGAATTAGGA
       L  D  I  D  K  V  D  E  T  M  D  E  I  R  E  Q  V  E  L  G    140
 421  GATGAAATAAGCGACGCTATATCCAGGCCTTTAATTACTGGGGCAAACGAGGTGGATGAA
       D  E  I  S  D  A  I  S  R  P  L  I  T  G  A  N  E  V  D  E    160
 481  GATGAGCTGGACGAGGAATTGGACATGCTGGCTCAAGAAAATGCTAACCAAGAAACGTCC
       D  E  L  D  E  E  L  D  M  L  A  Q  E  N  A  N  Q  E  T  S    180
 541  AAGATCGTTAATAATAATGTTAATGCGGCGCCTATCTCAGAGAACAAAGTCTCACTACCT
       K  I  V  N  N  N  V  N  A  A  P  I  S  E  N  K  V  S  L  P    200
 601  AGTGTTCCAAGTAATAAAATTAAACAAAGTGAGAACTCTGTGAAGGACGGGGAAGAGGAA
       S  V  P  S  N  K  I  K  Q  S  E  N  S  V  K  D  G  E  E  E    220
 661  GAGGATGAAGAAGATGAAGATGAAAAAGCATTAAGAGAACTACAAGCAGAAATGGGGCTT
       E  D  E  E  D  E  D  E  K  A  L  R  E  L  Q  A  E  M  G  L    240
 721  TGAACGTGTTCTATGCGGTTTAGATGAAAGAAAAAAAAAAAGGTGTTCTTACTTTCCATGT
       •
 928  TCTGTATACGCTCTTTTATATACATGAAGTTCATAATGCTAAGTCATAATTAATAGTTTT
 988  TATTTTTTTGTTGTCTTTTTTTTTTGCGTTCATAGGTATAGAAGGGAATGTGCATTCATT
1048  ATGTTATTGCTATATTATTGTACAGAAAAGATGAGAGAAGGAAAAAAAATAAATAATCAA
1108  ATATGTATCTTTTTTTCTTTTACTTTGCTTTTAATTGACTAAAACCCAAATAACGAAAAA
1168  TACAAAGATTATAAAAAAAACCTTTGCGGCTAACCATCTAT
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FIGURE 2.—Nucleotide sequence of the *SNF7* gene and predicted amino acid sequence of the gene product. Amino acids are indicated in standard single-letter code. Dot (•) indicates the termination codon. Nucleotides are numbered on the left and amino acids on the right.

TABLE 2

Invertase activity in mutants

Experiment	Relevant genotype	Invertase activity	
		Repressed	Derepressed
A	Wild type	1	270
	snf7Δ1::URA3	1	90
B	*ssn6Δ9*	680	2060
	snf7Δ1::URA3	<1	54
	ssn6Δ9 snf7Δ1::URA3	240	330

Invertase activity is expressed as μmol glucose released/min/100 mg cells (dry weight). All values are averages of two to six assays. Standard errors were less than 25%. (A) Strains were grown and derepressed at 30°. (B) Strains were grown and derepressed at room temperature.

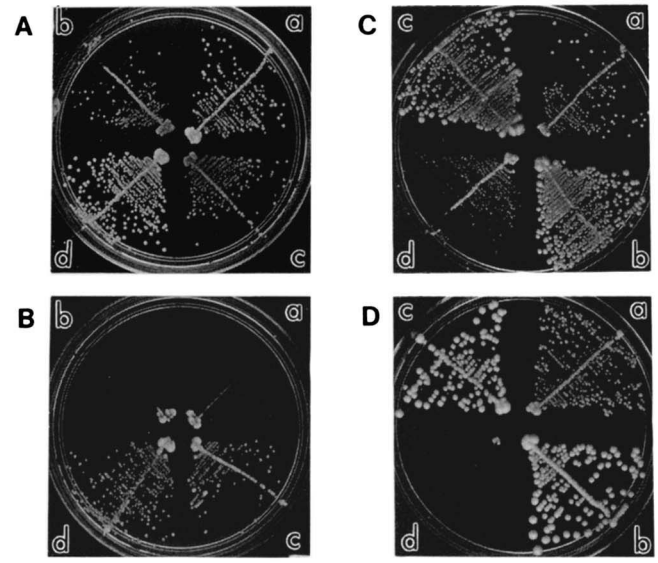

FIGURE 3.—Growth phenotype of the *snf7Δ1::URA3* and *snf7Δ1::URA3 ssn6Δ9* mutants. (A, B) MCY1751 was disrupted at the *SNF7* locus, and the resulting heterozygous dipoid was subjected to tetrad analysis. Two mutant segregants carrying the *snf7Δ1::URA3* allele (a, b) and two wild-type segregants (c, d) from one tetrad were streaked for single colonies on YPD plates. Plates were incubated 42 hr at (A) 30° or (B) 37°. (C, D) The diploid MCY1802 (*ssn6Δ9*) × MCY2455 (*snf7Δ1::URA3*) was subjected to tetrad analysis. Four segregants from a tetratype tetrad were streaked for single colonies on YPD plates. Relevent genotypes: (a) *ssn6Δ9*, (b) wild type, (c) *snf7Δ1::URA3*, (d) *snf7Δ1::URA3 ssn6Δ9*. Plates were incubated 5 days at (C) room temperature or (D) 30°.

defects of *snf1* and *snf4* mutants are not suppressed by *spt6/ssn20* mutations (NEIGEBORN, RUBIN and CARLSON 1986). Subsequent molecular studies supported the classification of these *SNF* genes into two functional groups (JOHNSTON and CARLSON 1992). We therefore tested the interaction of *snf7* with a *ssn20* suppressor mutation in an effort to assign the gene to the *SNF1*, *SNF4* group or the *SNF2*, *SNF5*, *SNF6* group.

We crossed strain MCY2458 (*snf7Δ1::URA3*) to strain MCY1366 (*ssn20-1*). Tetrad analysis of this cross showed 2+:2− segregation for ability to utilize raffinose and cosegregation of the *URA3* marker in 10 tetrads examined, indicating that the Raf⁻ defect of *snf7Δ1::URA3* is not suppressed by the *ssn20-1* mutation. The *ssn20-1* mutation segregated 2+:2−, independently from *snf7Δ1::URA3*, as expected from their different chromosomal map positions (see below) (CLARK-ADAMS and WINSTON 1987; NEIGEBORN, CE-

LENZA and CARLSON 1987); the *ssn20* mutation could be followed because it causes a much more severe temperature sensitive phenotype than *snf7* (NEIGEBORN, RUBIN and CARLSON 1986). Thus, *snf7* resembles *snf1* and *snf4*, rather than *snf2*, *snf5* and *snf6*, in its interaction with *spt6/ssn20*.

Synthetic phenotype of *snf7 ssn6* double mutants: We next examined the interaction of *snf7* with an *ssn6* mutation. The *SSN6* gene encodes a global repressor protein affecting a variety of differently regulated genes (KELEHER *et al.* 1992). Mutations in *SSN6* cause loss of glucose repression of *SUC2* and other genes, inappropriate expression of cell-type specific genes, flocculence, temperature sensitivity for growth at 37°, and other phenotypes (SCHULTZ and CARLSON 1987; TRUMBLY 1988). Mutations in *SSN6* suppress defects of the five previously characterized *snf* mutations, but show distinct interactions with the two different groups (ESTRUCH and CARLSON 1990; NEIGEBORN and CARLSON 1984). The Raf$^-$ phenotype in *snf1* or *snf4* mutants is suppressed by *ssn6*, and double mutants show the high-level constitutive invertase expression and extreme clumpiness characteristic of *ssn6* single mutants. In contrast, double mutants carrying *snf2*, *snf5* or *snf6* in combination with *ssn6* show partially regulated invertase expression and only slight clumpiness; thus, the phenotype of the double mutants is intermediate between those of the single mutant parents.

To test the interaction of *snf7Δ1::URA3* with the *ssn6Δ9* null mutation, we crossed strains MCY2458 and MCY1823. Germination of this cross at 30° gave poor spore viability: the 40 four-spored asci dissected yielded 10 with four spore clones, 21 with three spore clones, and 9 with two spore clones. Microscopic examination of 12 inviable spore clones in 12 tetrads revealed in each case a microcolony containing four to eight cells. The spore clones from 31 tetrads, each yielding three or four viable meiotic products, were analyzed. In none of the tetrads with four spore clones were any *snf7Δ1::URA3 ssn6Δ9* double mutants recovered. In the tetrads containing three spore clones, it could be inferred that the inviable spore clone was the *snf7Δ1::URA3 ssn6Δ9* double mutant. In no case was a *snf7Δ1::URA3 ssn6Δ9* double mutant recovered, thus indicating that the double mutant fails to form viable clones at 30°. Tetrad analysis of crosses from three different *ssn6Δ9* strains (MCY1802, MCY1823, MCY1958) to two different *snf7Δ1::URA3* strains (MCY2458, MCY2455) produced the same patterns of spore clone viability.

Further experiments revealed that germination of spores at room temperature, rather than at 30°, partially rescued the inviability. When the dissected tetrads from two of the crosses above were germinated at room temperature, *snf7Δ1::URA3 ssn6Δ9* double mutants were recovered, although at a low frequency; from 19 dissected tetrads, 6 double mutants formed visible spore clones. We recovered two of these and confirmed their Ura$^+$ and clumpy phenotypes. After growth at 25°, these spore clones were tested for growth on YPD at 30°; growth of the double mutants was much slower than that of either single mutant, as judged either by spotting cell suspensions or by streaking for single colonies, (Figure 3, panels C and D). The double mutants exhibited the high level constitutive invertase production and clumpiness characteristic of *ssn6* single mutants (Table 2). The lower invertase activity in the *snf7 ssn6* strains relative to *ssn6* mutants may simply reflect the poor health of the double mutants.

Thus, *snf7 ssn6* double null mutants are more temperature sensitive for growth than either single mutant parent. These synthetic phenotypes are not found in double mutants carrying *ssn6* in combination with other *snf* mutations. However, the *snf7 ssn6* strains resemble *snf1 ssn6* and *snf4 ssn6* strains in their invertase constitutivity and clumpiness (NEIGEBORN and CARLSON 1984).

TABLE 3

Linkage data

Cross	Gene pair	Number of tetrads PD	NPD	T	Genetic distance (cM)
MCY1514 × MCY2458	*snf7-gal2*	9	1	38	
MCY2924 × MCY2937		5	2	30	
		14	3	68	51
MCY2924 × MCY2937	*snf7-trp1*	7	19	11	15

PD = parental ditype, NPD = nonparental ditype, T = tetratype. Genetic distance in centimorgans (cM) between *snf7* and *gal2* was calculated by the formula of Perkins (1949). The distance of *snf7* from its centromere was calculated as the frequency of second division segregation from the centromere marker *trp1*.

Genetic map position of *SNF7*: We mapped the location of *SNF7* using a collection of mapped yeast DNA fragments available as lambda clone grids (OLSON *et al.* 1986). These grids were probed with the *Xba*I-*Cla*I fragment from pJT4, and the results indicated that *SNF7* is located on the right arm of chromosome *XII* between *gal2* and *ppr1* (L. RILES and M. OLSON, unpublished data). Since *ppr1* is near the centromere (6.5 cM; LILJELUND *et al.* 1984), we carried out tetrad analysis of a mapping cross heterozygous for *snf7* and *trp1*. In 37 tetrads, 11 tetratype tetrads were recovered, placing *snf7* approximately 15 cM from the centromere (Table 3). The physical mapping data are consistent with these genetic data for the position of *snf7*. We attempted to determine the relative order of *snf7* and *asp5* with respect to the centromere but were not successful because of low spore viability in the cross. Genetic linkage data for

gal2 and *snf7* indicated a distance of 51 cM (Table 3). Taken together, the physical and genetic mapping data suggest that the correct order is: *CEN12-ppr1-snf7-gal2*. The *spt8* locus also lies in this region (WINSTON *et al.* 1987), but sequence analysis of the cloned genes indicates that *SPT8* is different from *SNF7* (F. WINSTON, unpublished data).

DISCUSSION

We have cloned the *SNF7* gene, and sequence analysis predicts a 27-kDa acidic protein. Disruption of the gene caused a fewfold decrease in derepression of invertase in response to glucose limitation, defective raffinose utilization, temperature-sensitivity for growth on glucose, and a sporulation defect in homozygous diploids. This null phenotype differs from that of previously characterized *SNF* genes (*SNF1, 2, 4, 5, 6*) in several respects. First, the *snf7* defect in invertase derepression is less severe. Second, *snf7* does not cause pleiotropic defects in utilization of other nonpreferred carbon sources, such as galactose and glycerol. Third, *snf7* is unique in causing a temperature sensitive phenotype: at 37°, the null mutant grows when spotted as a cell suspension but cannot form single colonies on YPD.

Analysis of interactions with extragenic suppressors also distinguishes *SNF7* from both the *SNF1, SNF4* and the *SNF2, SNF5, SNF6* classes of genes. The genetic interactions between *snf7Δ1::URA3* and the *ssn20/spt6* and *ssn6* suppressors are distinct from those of *snf2, snf5* and *snf6*. The genetic behavior of *snf7* more closely resembles that of *snf1* and *snf4*: the *snf7 spt6* double mutants fail to grow on raffinose, and *snf7 ssn6* double mutants display high-level constitutivity for invertase and clumpiness. Nonetheless, the genetic behavior of *snf7* is distinct from that of *snf1* and *snf4* in that the combination of *snf7* with *ssn6* results in a synthetic phenotype of temperature sensitivity for growth. Even at room temperature, the *snf7Δ1::URA3 ssn6Δ9* double mutants are markedly more unhealthy than either single mutant. We note that these phenotypes are specific to this combination of mutations. For example, the *ssn20* mutation, like *ssn6*, causes temperature-sensitive growth, but the *snf7 ssn20* double mutants are neither more unhealthy nor more temperature-sensitive than either parent. Moreover, *snf1* mutants are less healthy than *snf7* mutants, but *snf1 ssn6* double mutants do not exhibit similar synthetic phenotypes.

SNF7, then, appears to have a functional role different from those of the *SNF1, SNF4* and *SNF2, SNF5, SNF6* groups. The partial defect in invertase derepression in *snf7* mutants is reminiscent of the defect seen in *gal11/spt13* null mutants, which derepress invertase to approximately 30% of the wild-type level (VALLIER and CARLSON 1991). However, except for the sporulation defect, these mutants do not share other phenotypes (FASSLER and WINSTON 1989; NISHIZAWA *et al.* 1990; NOGI and FUKASAWA 1980).

The mildness of the invertase defect in *snf7* null mutants was unexpected. Disruption of a gene frequently causes a more severe phenotype, as was the case for the *SNF6* gene (ESTRUCH and CARLSON 1990); moreover, two of the original mutants (*snf7-12* and *snf7-215*) had lower invertase activity than the null mutant. We suggest that these mutants carried a secondary mutation that further compromised invertase production; the variation in invertase activity reported for *snf7-215* mutant segregants derived from a backcross (VALLIER and CARLSON 1991) is consistent with this explanation.

The finding that a *snf7* null mutant has only a partial defect in invertase expression indicates that the SNF7 protein contributes to derepression of *SUC2* but is not absolutely required. One possibility is that SNF7 acts in a pathway that is only partly responsible for derepression of *SUC2*. Alternatively, a functionally redundant protein may partially compensate for the loss of SNF7 and together the two proteins may provide a function essential for derepression. Southern blot analysis of yeast genomic DNA with a probe including the *SNF7* sequence (*Cla*I-*Pvu*II fragment from pJT4) showed a single strongly hybridizing band and additional faint bands, which could correspond to sequence homologues (J. TU, unpublished data). Further studies are needed to distinguish between these two possibilities.

Overall, it seems likely that the major functional role of *SNF7* in the cell is unrelated to invertase expression or glucose repression. The most striking phenotype of the null mutation is the temperature-sensitivity for growth resulting from its combination with an *ssn6* mutation. Further analysis of the effects of *SSN6* on transcriptional repression may yield insight into possible roles for *SNF7* in cellular regulatory processes.

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