MOLECULAR AND CELLULAR BIOLOGY, Nov. 1990, p. 5616–5625
0270-7306/90/115616-10$02.00/0

Vol. 10, No. 11

The SNF5 Protein of *Saccharomyces cerevisiae* Is a Glutamine- and Proline-Rich Transcriptional Activator That Affects Expression of a Broad Spectrum of Genes

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Received 30 May 1990/Accepted 30 July 1990

The *Saccharomyces cerevisiae SNF5* gene affects expression of both glucose- and phosphate-regulated genes and appears to function in transcription. We report the nucleotide sequence, which predicts that *SNF5* encodes a 102,536-dalton protein. The N-terminal third of the protein is extremely rich in glutamine and proline. Mutants carrying a deletion of the coding sequence were viable but grew slowly, indicating that the *SNF5* gene is important but not essential. Evidence that *SNF5* affects expression of the cell type-specific genes *MFα1* and *BAR1* at the RNA level extends the known range of *SNF5* function. *SNF5* is apparently required for expression of a wide variety of differently regulated genes. A bifunctional SNF5–β-galactosidase fusion protein was localized in the nucleus by immunofluorescence. No DNA-binding activity was detected for SNF5. A LexA-SNF5 fusion protein, when bound to a *lexA* operator, functioned as a transcriptional activator.

Many transcription factors in *Saccharomyces cerevisiae* have been identified by a combination of genetic and biochemical approaches. Some affect a narrow subset of genes, and others have a very broad range of action; for example, GAL4 activates transcription of genes controlled by a specific regulatory mechanism, whereas RAP1/GRF1/TUF affects transcription of a broad variety of genes (for a review, see reference 53).

The *SNF5* gene of *S. cerevisiae* was originally identified as a gene required for expression of *SUC2* (encoding invertase) and other glucose-repressible genes (38). The *snf5* mutants showed growth defects on raffinose, galactose, and glycerol, and homozygous diploids failed to sporulate. The defect in *SUC2* expression lies at the RNA level (1). Further studies revealed that *SNF5* is required not only for expression of glucose-repressible genes but also for derepression of acid phosphatase in response to phosphate starvation (1). Mutations in *SNF5* also cause increased expression of protease B in stationary-phase cells (34). This evidence argues against a role for *SNF5* in a specialized signal transduction pathway and suggests rather that *SNF5* has a more general function in transcription.

Genetic evidence also supports the notion that *SNF5* functions in the transcriptional process. Mutations in *SPT6/SSN20*, an essential gene that appears to affect transcription (9, 12, 39, 54, 61), restore regulated invertase expression in *snf5* mutants (40). The *spt6/ssn20* suppressors restore derepression of *SUC2* mRNA in mutants lacking both *cis*- and *trans*-acting elements that are normally required (39). The suppression of *snf5* defects by *spt6* mutations is consistent with an involvement of *SNF5* in transcription.

SNF5 was one of six *SNF* genes identified in a search for mutants (38). Genetic evidence suggests that *SNF5* is a member of a group of three functionally related genes. The *SNF2*, *SNF5*, and *SNF6* genes share similarly pleiotropic mutant phenotypes and show similar patterns of genetic interactions with two extragenic suppressors, *spt6/ssn20* and *ssn6* (14, 38, 40). The properties of this group are distinct from those of the remaining *SNF* genes.

Previously, the *SNF5* gene was cloned (Fig. 1), its 3-kilobase (kb) RNA was identified, and the chromosomal locus was disrupted (1). In this study, we have undertaken a more extensive molecular analysis of the gene and its product in an effort to elucidate its function. We report here the sequence of the gene, which encodes a glutamine- and proline-rich protein, and the localization of a bifunctional SNF5–β-galactosidase fusion protein in the nucleus. We show that in addition to affecting glucose- and phosphate-regulated genes, *SNF5* also affects the expression of two cell type-specific genes. Several assays detected no DNA-binding activity for SNF5. Finally, we show that a LexA-SNF5 fusion protein, when bound to DNA at a *lexA* operator, functions as a transcriptional activator.

MATERIALS AND METHODS

Yeast strains and genetic methods. Strains of *S. cerevisiae* used are listed in Table 1. Standard genetic methods were followed (23, 49).

Sequence analysis. Restriction fragments were cloned into M13mp18 or M13mp19 (41) and sequenced by the method of Sanger et al. (45) using Sequenase (U.S. Biochemical), a 17-mer sequencing primer (Amersham Corp.), and three synthetic 18-mer oligonucleotides (Research Genetics) complementary to nucleotides 635 to 652, 652 to 635, and 876 to 893. The sequence was determined for both strands for the region −825 to +3634.

Plasmids. Cloned *SNF5* DNA was derived from pJW34 (1). pLY14 carries the *Eco*RI-*Bam*HI fragment in the centromere-containing vector pRS316 (50). pLY15 is a derivative of pLY14 with a deletion of the *Acc*I-*Bal*I fragment; the 5′ overhanging end at the *Acc*I site was rendered blunt by treatment with the Klenow fragment of DNA polymerase I and then ligated to the *Bal*I end, thereby adding a CTC (Leu) codon at the site of deletion. pLY17 was constructed by deleting the *Kpn*I fragment from pLY16, a derivative of YIp5 (3).

Plasmids pSH2-1 (20), pLR1Δ1 (59), 1840 (identical to

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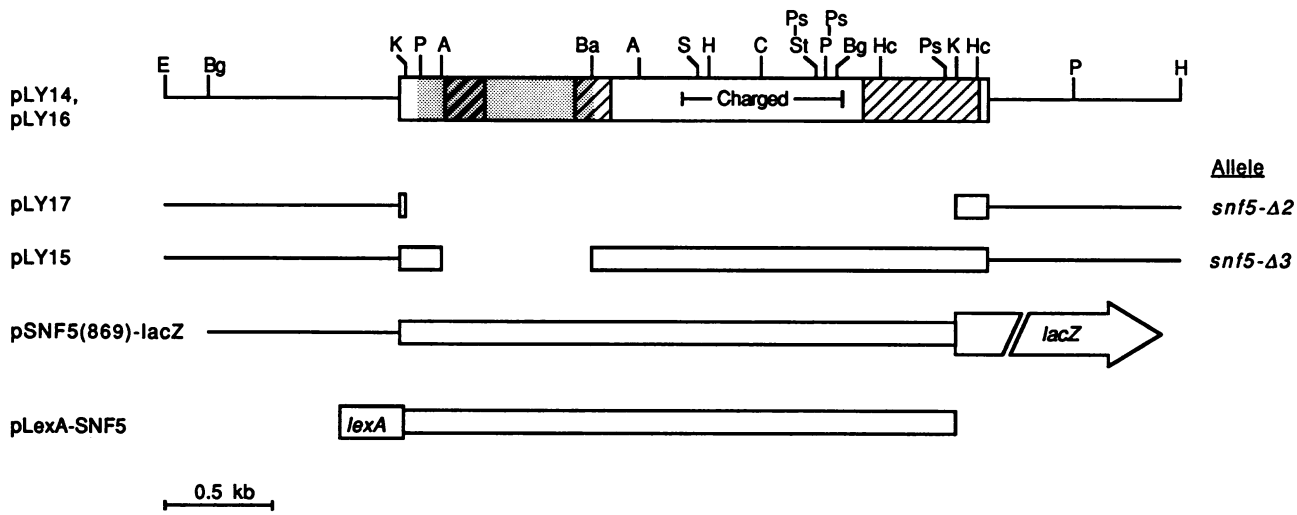

FIG. 1. Restriction maps of *SNF5* gene and plasmids. ——, Yeast DNA; ▭, *SNF5* coding sequence; ▩, glutamine-rich region; ▨, proline-rich regions (heavy lines, 37% proline; light lines, 23 and 18% proline). The region containing 37% charged residues is marked Charged. Boxes representing *lacZ* and *lexA* coding sequences are labeled. Plasmid constructions are described in the text. Vector sequences are not shown. Allele designations are indicated. The first ATG codon of *SNF5* is upstream of the *Kpn*I site, and the 3-kb *SNF5* RNA was detected by using probe prepared from the *Eco*RI-*Pvu*II fragment (data not shown); thus, the 5′ end of the RNA lies slightly to the left of the previous tentative placement (1). Restriction sites: A, *Acc*I; Ba, *Bal*I; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; Hc, *Hin*cII; K, *Kpn*I; P, *Pvu*II; Ps, *Pst*I; S, *Sau*I; St, *Stu*I. Not all sites for *Acc*I, *Hin*cII, and *Stu*I are shown.

1145 in reference 5), and pSH18-18 (S. D. Hanes and R. Brent, personal communication) contain the 2μm replicator and selectable markers (*HIS3* for pSH2-1 and *URA3* for the others) and were kindly provided by S. D. Hanes and R. Brent. pSH2-1 encodes the N-terminal 87 amino acids of LexA plus additional residues encoded by polylinker sequence under the control of the *ADH1* promoter and terminator. To construct pLexA-SNF5, the *Kpn*I fragment encoding residues 9 to 868 of SNF5 was blunted with T4 DNA polymerase and ligated to pSH2-1 that had been digested with *Eco*RI and treated with Klenow fragment. This construction regenerated the *Eco*RI site and yielded an in-frame fusion between codon 87 of *lexA* and codon 9 of *SNF5* separated by 5 codons contributed by polylinker sequence. A translational stop codon is located 11 codons distal to *SNF5* codon 868.

Gel mobility shift assays with in vitro-synthesized SNF5 protein. pBLU2 contains the *SNF5* sequence from the *Kpn*I site at +24 to the *Hin*dIII site 0.7 kb 3′ to the gene cloned into the Bluescript KS vector (Stratagene). pBLU2 (3 μg) was linearized with *Eco*RI and transcribed with T3 RNA polymerase (Stratagene) as described elsewhere (25) in the presence of G5′ppp5′G (Pharmacia). The RNA (2 μg) was translated by using [^{35}S]methionine (1,000 Ci/mmol; Amersham) in a rabbit reticulocyte lysate system (Promega Biotec). The first ATG codon in this RNA is codon 29 of *SNF5*. A protein of the expected size was synthesized. Additional experiments showed that the deleted N-terminal sequence is not essential for function: both pLexA-SNF5 and the *Kpn*I fragment from *SNF5* cloned into the centromere-containing vector pRS316 (50) complemented a *snf5* mutation. DNA binding assays were carried out essentially as described by Hope and Struhl (21), except that poly(dI · dC) was used as carrier. Preparation of crude protein extracts will be described elsewhere (J. H. New, J. Schultz, and M. Carlson, unpublished data).

TABLE 1. List of *S. cerevisiae* strains

Strain[a]	Genotype
MCY829	*MATα his3-Δ200 lys2-801 ura3-52 SUC2*
MCY946	*MATα snf5-5::URA3 his4-539 lys2-801 ade2-101 (ura3?) SUC2*
MCY1093	*MAT***a** *his4-539 lys2-801 ura3-52 SUC2*
MCY1094	*MATα ura3-52 ade2-101 SUC2*
MCY1751	MCY1093 × MCY1094
MCY1939	*MATα snf5-Δ2 ura3-52 SUC2*
MCY2062	*MAT***a** *snf5-Δ2 ura3-52 his4-539 ade2-101 SUC2*
MCY2063	*MATα snf5-Δ2 ura3-52 SUC2*
MCY2064	*MAT***a** *snf5-Δ2 his3-Δ200 lys2-801 ura3-52 ade2-101 SUC2*
RC634[b]	*MAT***a** *sst1-3 ade2 his6 met1 ura1 rme1*
DC17[c]	*MATα his1*

[a] MCY strains have the S288C genetic background.
[b] Obtained from R. Chan.
[c] Obtained from J. Hicks.

Invertase assays. Glucose-repressed and derepressed cells were prepared as described elsewhere (38). Cells carrying plasmids were grown in supplemented synthetic medium to select for plasmid maintenance. Secreted invertase activity was assayed (18) in whole cells.

β-Galactosidase assays. Transformants were grown to mid-log phase in synthetic complete medium (49) containing 2% glucose and lacking uracil and histidine to maintain selection for both expression and target plasmids. β-Galactosidase activity was assayed in cells permeabilized with sodium dodecyl sulfate and chloroform (19). Units of activity normalized for the optical density of the culture at 600 nm were calculated as described by Miller (33): 1,000 × [OD_{420}/(time in minutes)(volume of culture in milliliters) (OD_{600} of culture)], where OD_{420} and OD_{600} are the optical densities at 420 and 600 nm.

Immunofluorescence microscopy. Cells were prepared and stained as described elsewhere (6) except that incubations with antibody were at 4°C overnight. Cells were examined and photographed as described before (6).

Nucleotide sequence accession number. The GenBank accession number for the sequence described here is M36482.

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-825                AGCCCTTGAAGTTCTCCAGGGAACAATCTCTTCATTTTCGATTAGTGAATTACTACCACGAGAAATCTGATTTTGTTGTAGCTGCTGTTGCCTTTGTTGTGGGAG
-720  TTGCACAGCCTGCTGTAAATTATGGGCTGTAGAACTTATGAACTTCGATAAATCCGATCTTTCAGGAACAGCTTCTTTAATTTTATTAACATAGAGCATATCACTCACATTTGGTTCACC
-600  CGCTTCGACGCAACAGTTAAAAATCAAACTGATTCTATCATAATTATTAACAATTTTCTTGATACTTAATTTATCCTTGTCAAAATATTCTAACAGGATACGATCTATAGTCTCTAAAAA
-480  GGGTAAAACAATCAAGCGGGGCCTTTTGACTTCGAAGGTGGAGGGCTAAGCACCAATAATTGAGCTTATTTATAACTGAGAATACTTATAGACCTCTAAATCTCTTCCAACCATTGAATT
-360  GGTCTAAATAATCATCACTACTGCTATCTTCGAGCAATTGAGGACATGTGGACTGAACGCGGGTCCACAGGTGCTTGAAGGAGGGAGCTGTTGCACCTAAAAGATACTGGAAAATAAGTT
-240  TGTTCTTTGTATCAGTGATATAGAATGACAAATACATCTATTTTGGTTGGGTTGGTAAGGTTTACAGCCTCTGTTGTTGCCCAAGTCCTGTTATCGCCAACTTTAAATAAATCTCTTCTT
-120  GTTCTTTGACCAAAAATTTCATTTTTCGTCGCATTTAAAAGAAACTGAAATTTCAAACATAAACACCAAAACAAAGCATCATCAAGGGAACATATAGTAAAGAACTACACAAAAGCAACA
                                  KpnI
   1  ATG AAT AAT CAG CCG CAG GGT ACC AAC AGC GTT CCA AAT AGT ATT GGA AAT ATA TTT AGC AAC ATT GGA ACT CCA TCT TTT AAC ATG GCG
      Met Asn Asn Gln Pro Gln Gly Thr Asn Ser Val Pro Asn Ser Ile Gly Asn Ile Phe Ser Asn Ile Gly Thr Pro Ser Phe Asn Met Ala   30
                                                                                                                  AccI
  91  CAA ATT CCG CAA CAG CTG TAT CAG AGC CTC ACA CCA CAA CAA TTG CAG ATG ATT CAG CAA CGA CAC CAA CAG TTA CTG AGG AGT CGT CTA
      Gln Ile Pro Gln Gln Leu Tyr Gln Ser Leu Thr Pro Gln Gln Leu Gln Met Ile Gln Gln Arg His Gln Gln Leu Leu Arg Ser Arg Leu   60

 181  CAA CAA CAA CAA CAA CAA CAA CAA CAA ACT TCA CCG CCA CCG CAA ACG CAT CAA TCT CCA CCC CCT CCT CCG CAA CAA TCT CAA CCC ATT
      Gln Gln Gln Gln Gln Gln Gln Gln Gln Thr Ser Pro Pro Pro Gln Thr His Gln Ser Pro Pro Pro Pro Pro Gln Gln Ser Gln Pro Ile   90

 271  GCT AAT CAA TCA GCG ACT TCT ACC CCT CCT CCT CCT CCA GCA CCA CAC AAC TTA CAT CCC CAA ATT GGT CAA GTG CCC TTA GCT CCA GCG
      Ala Asn Gln Ser Ala Thr Ser Thr Pro Pro Pro Pro Pro Ala Pro His Asn Leu His Pro Gln Ile Gly Gln Val Pro Leu Ala Pro Ala  120

 361  CCT ATT AAT TTG CCT CCA CAA ATT GCT CAG TTA CCT TTG GCT ACA CAG CAA CAA GTT TTG AAC AAG TTG AGG CAG CAG GCC ATA GCA AAA
      Pro Ile Asn Leu Pro Pro Gln Ile Ala Gln Leu Pro Leu Ala Thr Gln Gln Gln Val Leu Asn Lys Leu Arg Gln Gln Ala Ile Ala Lys  150

 451  AAT AAT CCA CAG GTT GTG AAT GCA ATT ACT GTT GCA CAA CAA CAA GTG CAA CGC CAA ATT GAG CAG CAA AAG GGA CAG CAA ACG GCA CAA
      Asn Asn Pro Gln Val Val Asn Ala Ile Thr Val Ala Gln Gln Gln Val Gln Arg Gln Ile Glu Gln Gln Lys Gly Gln Gln Thr Ala Gln  180

 541  ACT CAG CTA GAA CAG CAG AGG CAA TTG CTG GTT CAG CAG CAA CAG CAG CAG CAA CTT AGA AAC CAA ATA CAG CGA CAA CAG CAA CAA CAG
      Thr Gln Leu Glu Gln Gln Arg Gln Leu Leu Val Gln Gln Gln Gln Gln Gln Gln Leu Arg Asn Gln Ile Gln Arg Gln Gln Gln Gln Gln  210

 631  TTT AGG CAT CAT GTG CAA ATA CAA CAG CAG CAA CAA AAG CAA CAA CAA CAG CAG CAG CAG CAT CAG CAA CAA CAA CAA CAA CAA CAG CAA
      Phe Arg His His Val Gln Ile Gln Gln Gln Gln Gln Lys Gln Gln Gln Gln Gln Gln Gln His Gln Gln Gln Gln Gln Gln Gln Gln Gln  240

 721  CAG CAG CAA CAG CAA CAG CAA CAG CAG CAG CAA CAA CAA CAG CAA CAA CAA CAA CAA CAG CAG CAG CAG CAG CAG CAG CAG CAA GGA CAA
      Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln Gln  270
                                                                                                          BalI
 811  ATA CCG CAA TCT CAG CAA GTT CCT CAA GTT AGA TCC ATG AGT GGA CAA CCT CCC ACC AAT GTT CAG CCA ACT ATT GGC CAA CTT CCT CAA
      Ile Pro Gln Ser Gln Gln Val Pro Gln Val Arg Ser Met Ser Gly Gln Pro Pro Thr Asn Val Gln Pro Thr Ile Gly Gln Leu Pro Gln  300

 901  CTT CCA AAA TTA AAC TTA CCC AAG TAC CAA ACT ATT CAA TAC GAT CCA CCA GAA ACC AAG CTA CCA TAT CCA ACC TAT TGG TCA GAC AAA
      Leu Pro Lys Leu Asn Leu Pro Lys Tyr Gln Thr Ile Gln Tyr Asp Pro Pro Glu Thr Lys Leu Pro Tyr Pro Thr Tyr Trp Ser Asp Lys  330

 991  AAA GCA GAT ACG GAT ACT TTG TTG TAC GAA CAA ATT ATC CAG CGT GAT AAA ATT AAC AAA TAT TCG CTA ATA AGA GAA ACC AAT GGT TAC
      Lys Ala Asp Thr Asp Thr Leu Leu Tyr Glu Gln Ile Ile Gln Arg Asp Lys Ile Asn Lys Tyr Ser Leu Ile Arg Glu Thr Asn Gly Tyr  360

1081  GAT CCG TTT AGC ATT TAT GGA TTT AGT AAT AAA GAG TAT ATT AGT AGA CTG TGG CAT ACA CTG AAG TAT TAT CAA GAT TTG AAG AAC ACT
      Asp Pro Phe Ser Ile Tyr Gly Phe Ser Asn Lys Glu Tyr Ile Ser Arg Leu Trp His Thr Leu Lys Tyr Tyr Gln Asp Leu Lys Asn Thr  390

1171  AGA ATG AAA TCT ATC ACA AGC ACT TCT CAG AAG ATT CCT TCG GCA AGT ATT TGG GGA AAT GGT TAC TCA GGG TAT GGT AAT GGG ATT ACG
      Arg Met Lys Ser Ile Thr Ser Thr Ser Gln Lys Ile Pro Ser Ala Ser Ile Trp Gly Asn Gly Tyr Ser Gly Tyr Gly Asn Gly Ile Thr  420

1261  AAT ACA ACT ACC AGA GTT ATT CCA CAA GTA GAA GTT GGA AAT AGG AAG CAT TAC CTA GAG GAT AAA TTA AAA GTC TAT AAA CAG GCC ATG
      Asn Thr Thr Thr Arg Val Ile Pro Gln Val Glu Val Gly Asn Arg Lys His Tyr Leu Glu Asp Lys Leu Lys Val Tyr Lys Gln Ala Met  450

1351  AAT GAG ACA TCG GAA CAG TTA GTT CCC ATA AGA TTG GAG TTC GAT CAA GAT CGT GAC AGA TTC TTC CTC AGG GAC ACT TTG TTA TGG AAC
      Asn Glu Thr Ser Glu Gln Leu Val Pro Ile Arg Leu Glu Phe Asp Gln Asp Arg Asp Arg Phe Phe Leu Arg Asp Thr Leu Leu Trp Asn  480
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FIG. 2. Nucleotide sequence of *SNF5* gene and deduced amino acid sequence of gene product. Nucleotides are numbered on the left, and amino acids are numbered on the right. The first ATG codon of the open reading frame was assigned the +1 position. Asterisks mark the termination codon. *Kpn*I, *Acc*I, and *Bal*I sites relevant to the construction of plasmids are marked.

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1441  AAA AAT GAC AAG CTT ATT AAA ATT GAA GAC TTT GTG GAC GAC ATG TTG CGA GAT TAC CGA TTT GAG GAC GCT ACG AGA GAG CAA CAC ATT
      Lys Asn Asp Lys Leu Ile Lys Ile Glu Asp Phe Val Asp Asp Met Leu Arg Asp Tyr Arg Phe Glu Asp Ala Thr Arg Glu Gln His Ile  510

1531  GAT ACT ATT TGT CAA TCT ATA CAA GAG CAG ATT CAG GAG TTT CAA GGA AAT CCA TAT ATA GAG TTG AAT CAG GAC CGT CTA GGC GGT GAT
      Asp Thr Ile Cys Gln Ser Ile Gln Glu Gln Ile Gln Glu Phe Gln Gly Asn Pro Tyr Ile Glu Leu Asn Gln Asp Arg Leu Gly Gly Asp  540

1621  GAC TTG AGA ATT AGA ATC AAG CTG GAT ATT GTC GTG GGA CAA AAC CAG TTA ATC GAT CAA TTT GAG TGG GAC ATC TCT AAT AGT GAT AAC
      Asp Leu Arg Ile Arg Ile Lys Leu Asp Ile Val Val Gly Gln Asn Gln Leu Ile Asp Gln Phe Glu Trp Asp Ile Ser Asn Ser Asp Asn  570

1711  TGT CCA GAA GAG TTT GCA GAG TCC ATG TGT CAA GAA TTA GAA CTA CCA GGT GAG TTT GTG ACT GCC ATT GCT CAC TCC ATA AGA GAG CAA
      Cys Pro Glu Glu Phe Ala Glu Ser Met Cys Gln Glu Leu Glu Leu Pro Gly Glu Phe Val Thr Ala Ile Ala His Ser Ile Arg Glu Gln  600

1801  GTT CAT ATG TAT CAT AAA TCA CTG GCA CTG TTA GGT TAC AAT TTT GAT GGA TCA GCG ATA GAA GAT GAT GAC ATT AGA AGC AGA ATG CTC
      Val His Met Tyr His Lys Ser Leu Ala Leu Leu Gly Tyr Asn Phe Asp Gly Ser Ala Ile Glu Asp Asp Asp Ile Arg Ser Arg Met Leu  630

1891  CCA ACG ATT ACT CTT GAT GAT GTT TAT AGG CCT GCA GCG GAA AGC AAA ATT TTT ACT CCA AAC CTA TTA CAG ATT TCA GCT GCA GAG TTA
      Pro Thr Ile Thr Leu Asp Asp Val Tyr Arg Pro Ala Ala Glu Ser Lys Ile Phe Thr Pro Asn Leu Leu Gln Ile Ser Ala Ala Glu Leu  660

1981  GAG AGA TTG GAT AAA GAT AAG GAC AGA GAC ACA AGA AGG AAA AGA AGA CAA GGT AGA TCT AAT AGA CGT GGT ATG CTC GCA TTG TCC GGC
      Glu Arg Leu Asp Lys Asp Lys Asp Arg Asp Thr Arg Arg Lys Arg Arg Gln Gly Arg Ser Asn Arg Arg Gly Met Leu Ala Leu Ser Gly  690

2071  ACA TCT GCA AGT AAT ACA TCT ATG AAC GGC GTT CAC AAC ACA GTA GCA GCA GGA AAT GCT TCA TCG TTG CCA CCA GGA GAG ATT TTA CTG
      Thr Ser Ala Ser Asn Thr Ser Met Asn Gly Val His Asn Thr Val Ala Ala Gly Asn Ala Ser Ser Leu Pro Pro Gly Glu Ile Leu Leu  720

2161  CCA GAT ATT GCA GAT ATT CCA AGA ACT TTC AGG ACT CCA GTA CCT AGC ACT TTA ATG CCT GGT GGT GTT GAC GTA GGC CCT TCT GTG GAA
      Pro Asp Ile Ala Asp Ile Pro Arg Thr Phe Arg Thr Pro Val Pro Ser Thr Leu Met Pro Gly Gly Val Asp Val Gly Pro Ser Val Glu  750

2251  TCG TAC GAA TTG AGA AAC ACA ACC ACT TAT AAA AGC AGG CCA GAT AGA CCT AAG CCA GTT TCA CCT CCT TGT TAT ATT ATT GAC CAT ATT
      Ser Tyr Glu Leu Arg Asn Thr Thr Thr Tyr Lys Ser Arg Pro Asp Arg Pro Lys Pro Val Ser Pro Pro Cys Tyr Ile Ile Asp His Ile  780

2341  CCG GGT CAT TCG CTA CTA CTT TCT ATT AAA CTG CCT GGG AAA GTT AAT ACA AAA GAA GAG TTC GCA GCA GCG CCC AAT GAC ACA AGT AGT
      Pro Gly His Ser Leu Leu Leu Ser Ile Lys Leu Pro Gly Lys Val Asn Thr Lys Glu Glu Phe Ala Ala Ala Pro Asn Asp Thr Ser Ser  810

2431  GGC ACC AAT GCA ATG CTT CCG AGT CCA GAA TCG CTG AAA ACT AAG CTG AAT AGT AAC ATT CGC GCT GGT GTG ACG ATA CCT TCA ATC CCA
      Gly Thr Asn Ala Met Leu Pro Ser Pro Glu Ser Leu Lys Thr Lys Leu Asn Ser Asn Ile Arg Ala Gly Val Thr Ile Pro Ser Ile Pro  840
                                                                                                                  KpnI
2521  AAC CCG ATT GCC AAT CAC ACT GTT ACT AAT TCA CCC AAT CCC ACA CTG CAG CCA GTA ATC CCA GGT GGG GCA GCT AGT AAA TCG GTA CCT
      Asn Pro Ile Ala Asn His Thr Val Thr Asn Ser Pro Asn Pro Thr Leu Gln Pro Val Ile Pro Gly Gly Ala Ala Ser Lys Ser Val Pro  870

2611  ACA CCT AGT CTT CCT ATA GCA CCT CCA GTA GCA CCA CAT GAT AGC GAA GCG ACA TTG TTG ACT AAT AGC AAT AAT GGT AGC AGT AAC AAT
      Thr Pro Ser Leu Pro Ile Ala Pro Pro Val Ala Pro His Asp Ser Glu Ala Thr Leu Leu Thr Asn Ser Asn Asn Gly Ser Ser Asn Asn  900

2701  AAC ACA CAG AAT ACA TAG AAAATATATACACATATATAAAATATACCGGAGATGTAAATAACCGTGGAAGAATTTGTATTATCATCGTGTTATACGATGCGGAGTCCCTTTGCG
      Asn Thr Gln Asn Thr ***

2816  CCTACTTTTTTTAGAAGAATAGTACATAACCTTTATAAAAATCTTCCAATTTAGATACATAAAAAGAGCATGAGAATAACAAGAACACGTAGTCTAGGAAACTAAGCGCTATGCCAGAGC
2936  AAGAACTACTTATAGGGCAAGAAATGAATACACTTCATGCAGGTTCATCTACTGATGGCATAAATGTCGGAAACGCAGGACGAACTAGAGACACACAAACTGGCGTAGGAAGGGGAAACG
3056  GAAATAGGGTCTGACGAGGAAGATAGCATAGAGGACGAGGGAAGCAGCAGTGGGAGGAAATAGTACGACAGAAAGACTAGTACCACACCAGCTGAGGGAACAAGCAGCCAGACATATAGG
3176  AAAAATAGGAAGACATTTTAATATTCTTGATAGACTTTTTAAGAAACGTACACAACAGTCTTCGGATATACAACAAGGTGCCATGTTTGATGGTGTCTTTAGCAATTTAAGCGCAAAACC
3296  AGATACCACAGAGACTGAAGGTAATAACGAACAAGACATACCACCTACATACGACGAAGCTGTGCTGATATGGCCCCCTCGTATTATGGAATGGATTTGAACAATTCAGATATCTACTAC
3416  GACGAAATATGCATTGAGGGGCTTCCTGTAGGAAATATATCAAATTTATTATGGAATATCATCGTAAGCACGAGTTTTCAGTTCATTGGATTTTTAATAACCTACATTTTACACACATCT
3536  CACGCAGCAAAGCAAGGTTCAAGGTTTGGATTAGGGTTAACGTTCATTGGATATGGTTATTCAATGATTCCCAATGATGTTACTTCAAAAGTCAAAAAT
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RESULTS AND DISCUSSION

Sequence of the *SNF5* gene. The nucleotide sequence of the *SNF5* gene was determined (Fig. 2), and a single open reading frame containing 905 codons was identified. The amino acid sequence of the predicted 102,536-dalton protein is shown in Fig. 2. The size of the coding region was confirmed by showing that a protein of the appropriate size was produced by in vitro translation of RNA transcribed in vitro from a bacteriophage T3 promoter (25) (data not shown). The predicted amino acid sequence was compared with the sequences in GenBank (Release 58.0) translated in all six reading frames by using the program TFASTA (42). No significant homology to other proteins (except those containing glutamine-rich regions) was found in this search.

The most striking feature of the predicted SNF5 protein is the highly glutamine-rich N terminus. Of the 270 amino acids between positions 31 and 300, 46% are glutamine. This region includes two long, homogeneous stretches of glutamine residues (positions 61 to 69 and 218 to 268). The protein also has three regions with high proline content: residues 72 to 132, 272 to 324, and 714 to 882 (36, 23, and 18% proline, respectively). The first two stretches of prolines are interspersed with glutamines. Thus, the region comprising residues 31 to 324 can be viewed as a glutamine- and proline-rich region.

Glutamine-rich regions are present in transcription factors from various organisms and have been shown to mediate transcriptional activation (11). Proline-rich sequences have also been reported in transcription factors, and a region containing 25% proline in the human CTF-1 CCAAT box binding factor has been identified as a transcriptional activation domain (32). Regions with high content of both proline and glutamine have been found in the enhancer-binding factor AP-2 (60) and the lymphoid-specific transcription factor OTF-2 or Oct-2 (10, 35, 48).

Most of the charged residues lie in the C-terminal two-thirds of the predicted SNF5 protein. The segment from amino acid 435 to 683 is highly charged (37%) and includes an acidic stretch (net charge, −17 over 99 amino acids from 490 to 588). Acidic regions have been shown to function in activation of transcription (22, 28). SNF5 also contains a striking cluster of charged residues at positions 659 to 683; of the 25 residues, 12 are basic and 6 are acidic. Brendel and Karlin (4) have observed that charge clusters are common among nuclear transcription factors. Another noteworthy feature is the predominantly basic region comprising amino acids 755 to 798 (net charge, +8 over 44 residues).

Deletion of most of the *SNF5* coding sequence is not lethal. Previously, the chromosomal *SNF5* locus was disrupted with an insertion of the *URA3* gene at the *Hin*dIII site, generating the *snf5-5::URA3* allele (1). The nucleotide sequence analysis allowed us to construct a deletion removing most of the *SNF5* coding sequence. Plasmid pLY17 (Fig. 1), carrying a deletion of codons 9 to 869, was integrated into the genome of diploid strain MCY1751 at the *SNF5* locus. Excision of the plasmid from Ura$^+$ transformants was selected (2), and a diploid heterozygous for the deletion, designated *snf5-Δ2*, was identified by blot hybridization (51). Tetrad analysis of the diploid showed 2:2 segregations for anaerobic growth on raffinose and galactose and for aerobic growth on glycerol. Small spore clone size cosegregated with these phenotypes. The slow-growth phenotype is consistent with a general transcriptional defect. This analysis shows that *SNF5* is not an essential gene.

Part of the N-terminal glutamine- and proline-rich region is dispensable. The N-terminal glutamine- and proline-rich region of SNF5 is extremely large. To determine whether the entire region is required for SNF5 function, we deleted codons 60 to 296 (*snf5-Δ3*; Fig. 1), thereby removing 110 glutamine and 28 proline residues. The 60 codons surrounding the deletion still include 15 glutamines and 9 prolines. To test this deleted allele for function, the centromere-containing plasmid pLY15 (Fig. 1) was used to transform MCY1939 (*snf5-Δ2*). pLY15 restored anaerobic growth on raffinose and galactose (in supplemented synthetic medium lacking uracil) and aerobic growth on glycerol (in rich medium) in the eight Ura$^+$ transformants tested. Control transformants carrying pLY14 grew only slightly better. pLY15 also restored sporulation in homozygous mutant diploids. Strains carrying pLY15 derepressed invertase to about 80% of the level seen for strains carrying pLY14 (Table 2). Thus, the large glutamine- and proline-rich region can be greatly reduced in size without impairing SNF5 function with respect to the phenotypes assayed.

TABLE 2. Invertase activity in *snf5* mutants

Relevant genotype	Plasmid	Invertase activity[a]	
		Repressed	Derepressed
SNF5		2	545
snf5-Δ2		2	24
snf5-Δ2	pRS316	2	31
snf5-Δ2	pLY14	2	497
snf5-Δ2	pLY15	2	387
snf5-18	pSNF5(869)-lacZ	1	757

[a] Micromoles of glucose released per minute per 100 mg (dry weight) of cells. Values for pLY14 and pLY15 are the averages of determinations for three transformants of MCY1939; standard errors were <5%.

SNF5* affects cell type-specific gene expression.** The *SNF5* gene is required for expression of genes controlled by both glucose and phosphate availability. We have observed that *snf5* mutants form homozygous diploids less readily than wild type, suggesting that *SNF5* may also affect cell type-specific gene expression. To examine this possibility, we first tested *MATα snf5* mutant strains for production of the mating pheromone α-factor by using the halo assay (24). A suspension of cells was spotted on a lawn of *MATa** *sst1* cells, which are supersensitive to α-factor (7). Cells that produce α-factor cause a zone of growth inhibition in the surrounding lawn. The *snf5* mutants elicited a much smaller halo than the wild-type control (Fig. 3A). *MAT***a** *snf5* mutants did not elicit halos (data not shown).

We also tested *snf5* mutants for expression of barrier activity, the **a**-specific product of the *BAR1* gene that inactivates α-factor (7, 52). Strains to be tested were patched adjacent to an α-factor-producing strain on a lawn of *MAT***a** *sst1* cells (Fig. 3B). *MAT***a** cells, both *snf5* mutant and wild type, protected the cells in the lawn from growth inhibition by α-factor. However, the *MATα snf5* cells also provided protection, indicating that they aberrantly produced barrier activity.

To examine the molecular basis for the altered regulation of α-factor and barrier in *snf5* mutants, we determined the levels of the RNAs encoding these proteins by Northern (RNA) blot hybridization (Fig. 4). In *MATα snf5* mutants, the RNA transcribed from *MFα1*, the major α-factor structural gene (26), was greatly reduced in abundance relative to wild-type levels. *BAR1* RNA was not detectable, although barrier activity was clearly detected in the more sensitive bioassay. The diminished *MFα1* RNA levels and the inap-

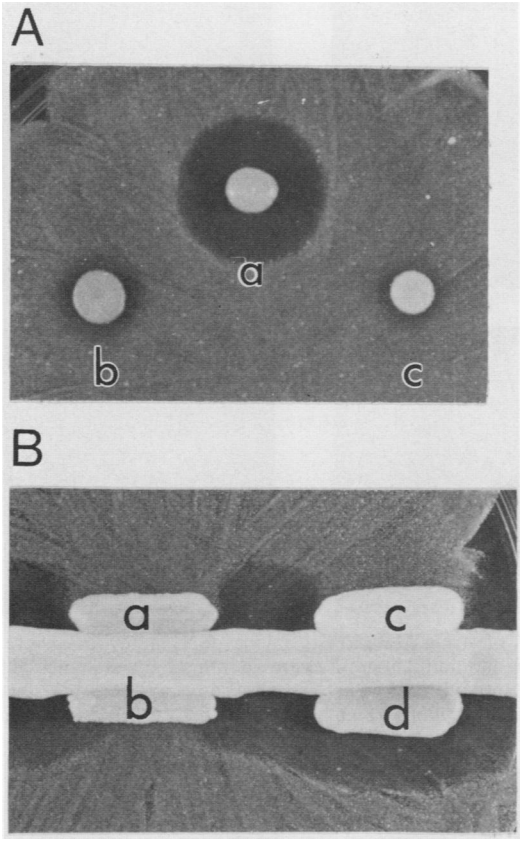

FIG. 3. Bioassays for α-factor and barrier activity. (A) Production of α-factor by a strain was assayed by the ability to elicit a halo, corresponding to an area of inhibited growth, on a lawn of strain RC634 (*MAT***a** *sst1*) (24). *MAT*α strains MCY1094 (*SNF5*) (a), MCY946 (*snf5-5::URA3*) (b), and MCY1939 (*snf5-Δ2*) (c) were grown to mid-log phase in YEP–2% glucose (49). Cells were collected by centrifugation and suspended in a small volume. Equal amounts of cells were spotted on a lawn of 5×10^5 cells of strain RC634 on YEP–2% glucose medium. The plate was incubated at 30°C for 2 days. (B) Production of the *BAR1* gene product (barrier) by a strain was assayed by ability to permit growth of a lawn of RC634 cells in the presence of α-factor. The α-factor-producing strain, DC17, was streaked on a freshly spread lawn of strain RC634 on a YEP–2% glucose plate, and the strains to be assayed were patched next to the DC17 cells. Strains and relevant genotypes: (a) MCY2062 (*MAT***a** *snf5-Δ2*), (b) MCY2063 (*MAT*α *snf5-Δ2*), (c) MCY1093 (*MAT***a** *SNF5*), (d) MCY1094 (*MAT*α *SNF5*). The plate was incubated at 30°C for 2 days.

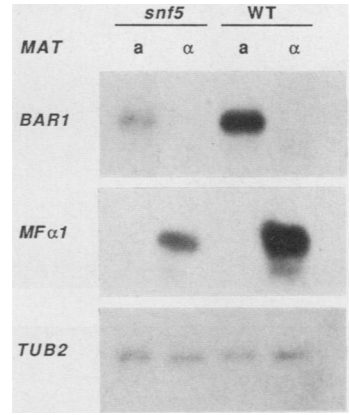

FIG. 4. Northern analysis of cell type-specific RNAs in *snf5* mutants. Poly(A)-containing RNA was prepared (47) from wild-type (WT) and *snf5* mutant strains of both *MAT***a** and *MAT*α cell type. RNAs (5 μg) were separated by electrophoresis in a 1.5% agarose gel containing formaldehyde (30) and transferred to nitrocellulose (55). The filter was successively hybridized to ^{32}P-labeled probes prepared by nick translation (44) of the following fragments: a 1-kb fragment from the *BAR1* coding region (29; gift of S. Kurtz), a 0.8-kb fragment containing most of the *MFα1* coding region (26; gift of D. Julius), and a 1.6-kb *Eco*RI fragment from *TUB2* (37). The *MFα1* probe also hybridized weakly to the slightly smaller *MFα2* RNA. Hybridization to *TUB2* RNA provided a control for the amount of RNA in each lane. Strains and relevant genotypes were as follows: MCY2062 (*MAT***a** *snf5-Δ2*), MCY2063 (*MAT*α *snf5-Δ2*), MCY1093 (*MAT***a** *SNF5*), and MCY1094 (*MAT*α *SNF5*).

propriate expression of barrier activity probably together contributed to the impaired halo in the α-factor halo assay.

In *MAT***a** *snf5* strains, the level of *BAR1* RNA was significantly decreased relative to that of the *MAT***a** wild-type strain, although the strain produced sufficient barrier activity to give a normal phenotype in the bioassay.

Thus, *SNF5* is required for appropriate expression of at least two cell type-specific genes at the RNA level. This evidence extends the known range of *SNF5* function. The finding that *SNF5* is required for expression of a wide variety of differently regulated genes argues that *SNF5* affects general transcriptional processes rather than a specific signal transduction system. In addition, other laboratories have recently shown that *SNF5* is required for transcription of Ty elements (A. M. Happel, M. S. Swanson, and F. Winston, personal communication) and that *snf5* is allelic to *tye4* (M. Ciriacy, personal communication), which reduces Ty-mediated constitutive expression of *ADH2* (8). Further pleiotropic defects undoubtedly remain to be identified.

Nuclear localization of a bifunctional SNF5–β-galactosidase fusion protein. If *SNF5* affects transcription by a direct mechanism, the protein might be expected to reside in the nucleus. To facilitate its localization, we tagged the SNF5 protein with β-galactosidase. A *SNF5-lacZ* gene fusion between codon 869 of *SNF5* and codon 8 of the *Escherichia coli lacZ* gene was constructed in the multicopy vector YEp357R (36), yielding plasmid pSNF5(869)-lacZ (Fig. 1). Proteins expressed from the gene fusion were examined by immunoblot analysis (56), and the major protein detected with anti-β-galactosidase antibody was the full-length fusion protein (data not shown). This plasmid complements the defects in invertase derepression (Table 2) and raffinose utilization of a *snf5* mutant and therefore encodes a bifunctional protein. A fusion protein that provides SNF5 function must be present at the normal site of action of the native SNF5 protein.

Wild-type cells expressing SNF5–β-galactosidase were examined by immunofluorescence microscopy, and the fusion protein was clearly localized in the nucleus (Fig. 5). The staining pattern was the same in both glucose-repressed and derepressed cells and in *snf5* mutant cells carrying the gene fusion (not shown). This finding is consistent with the idea that SNF5 has a fairly direct role in the transcriptional process.

Nuclear localization is independent of *SNF2* and *SNF6*. Previous evidence suggests that *SNF5* is functionally related to two other genes, *SNF2* and *SNF6* (14, 38, 40). *SNF2* and *SNF6* both encode nuclear proteins, as judged by the nuclear localization of bifunctional SNF2– and SNF6–β-galactosidase fusion proteins (B. C. Laurent, unpublished results; 14). It seemed possible that the SNF5 protein is localized in the nucleus by virtue of association with the SNF2 or SNF6

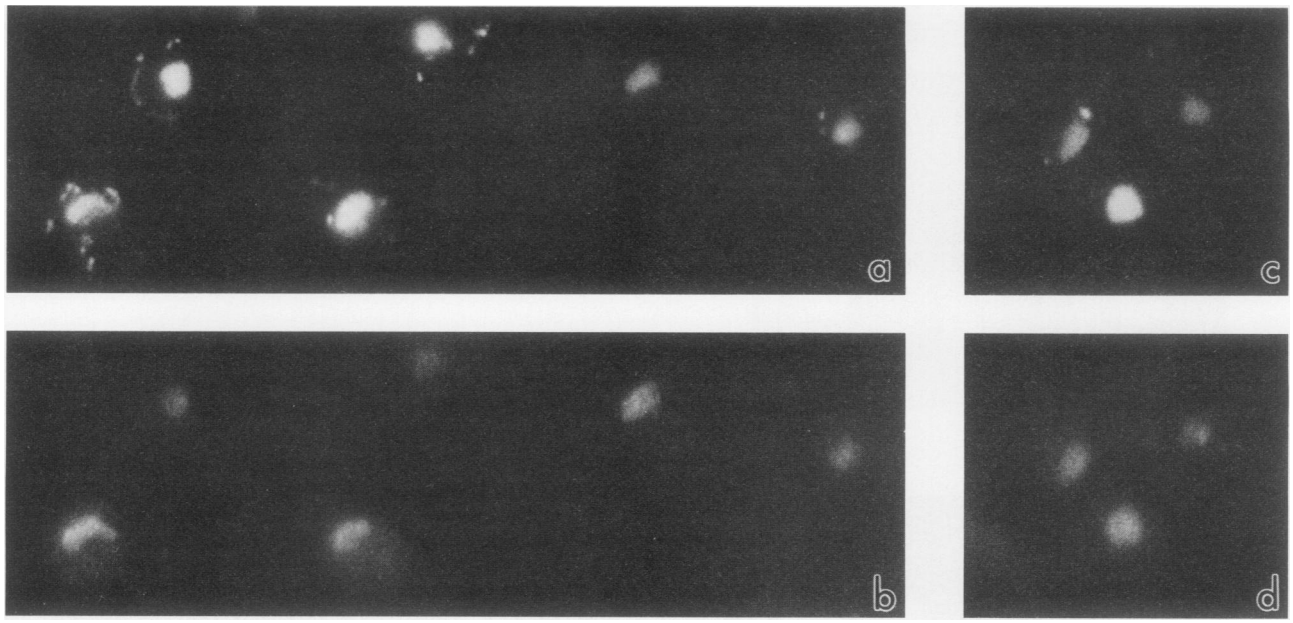

FIG. 5. Nuclear localization of SNF5–β-galactosidase fusion protein. Cells of strain MCY1093 carrying pSNF5(869)-lacZ were grown under glucose-repressing (c, d) and derepressing (a, b) conditions. Nuclei and mitochondria were identified by staining cells with 4′,6-diamidino-2-phenylindole (a, c). Cells were stained with mouse monoclonal antibody to β-galactosidase (Promega Biotec) and fluorescein isothiocyanate-conjugated $F(ab')_2$ fragment of sheep antibody to mouse immunoglobulin G (Sigma Chemical Co.) (b, d). No staining was detected in cells lacking the gene fusion.

protein. To test this hypothesis, *snf2* and *snf6* null mutants (*snf2-Δ1::HIS3*, *snf6-Δ2*) were transformed with pSNF5 (869)-lacZ and examined by immunofluorescence microscopy. In each case, the fusion protein was detected in the nucleus, and the staining pattern was indistinguishable from that observed in a wild-type strain (data not shown). These results suggest that the SNF5 protein is directed to the nucleus by a mechanism that is independent of the SNF2 and SNF6 proteins.

Assays of DNA-binding activity. A simple model casts SNF5 as a DNA-binding protein that functions as a transcriptional activator. However, using several methods, we obtained no evidence for DNA-binding activity. Gel mobility shift assays (16, 17) revealed no differences in the formation of DNA-protein complexes with *SUC2* 5′ sequences in extracts prepared from wild type and a *snf5* null mutant (data not shown; New et al., unpublished data). The *SUC2* DNA fragments tested contained nucleotides −542 to −381, −390 to −166, and −166 to −26; a TATA box is located at −133 (46).

We also synthesized SNF5 protein in vitro (see Materials and Methods) and tested the ^{35}S-labeled protein for binding to *SUC2* fragments containing the sequences −650 to −26, −542 to −381, and −166 to −26 by the method of Hope and Struhl (21). Incubation with excess, unlabeled DNA did not shift the mobility of the protein, even when crude protein extract from wild-type cells was added (data not shown). Finally, addition of in vitro-synthesized SNF5 protein to protein extracts did not alter the pattern observed in gel mobility shift assays with labeled DNA fragments.

Although this evidence did not exclude the possibility that SNF5 binds DNA, it provided no support for such a model. Nonetheless, the idea that SNF5 functions as a transcriptional activator was still tenable. The SNF5 protein could be tethered to DNA by association with a DNA-binding activity.

LexA-SNF5 fusion protein activates transcription when bound to DNA. To test the SNF5 protein for function as a transcriptional activator, we applied the method of Brent and Ptashne (5). First, we fused SNF5 to a known DNA-binding domain, that of the *E. coli* LexA protein. Then we determined whether the LexA-SNF5 fusion protein, when bound to DNA containing a *lexA* operator, could activate transcription of a nearby promoter. Previous studies have shown that fusions between LexA and transcriptional activators such as GAL4, GCN4, and bicoid and likely activators such as the cellular and viral *fos* products activate transcription in this assay (5, 20, 22, 27). In contrast, no activation was effected by native LexA or by fusions to the *MATα2* product or bacteriophage 434 repressor (5, 27).

The first step was to construct a gene fusion between the N-terminal 87 codons of *lexA* and codons 9 to 868 of *SNF5*. The fusion, on plasmid pLexA-SNF5 (Fig. 1), is under the control of the *ADH1* promoter. The LexA-SNF5 fusion protein expressed from this plasmid provided SNF5 function in *S. cerevisiae*, as assayed by complementation. The control for these experiments was provided by pSH2-1, which expresses the first 87 residues of LexA in the $LexA_{(1-87)}$ protein (20) and is the parent plasmid from which LexA-SNF5 was derived.

We then sought to determine whether the LexA-SNF5 fusion protein, when bound to DNA containing a *lexA* operator, could activate transcription of a promoter. A set of "target" plasmids was obtained from Hanes and Brent (Fig. 6). Plasmid 1840 contains a *lexA* operator located 5′ to the promoter of a *GAL1-lacZ* fusion from which UAS_G was removed (20). 1840 was derived from pLR1Δ1, which has no *lexA* operator (59). Plasmid pSH18-18 contains six *colE1* overlapping *lexA* operators (13) and is otherwise identical to 1840 (Hanes and Brent, personal communication).

Both wild-type (*SNF5*) and *snf5* mutant strains were doubly transformed with each combination of expression

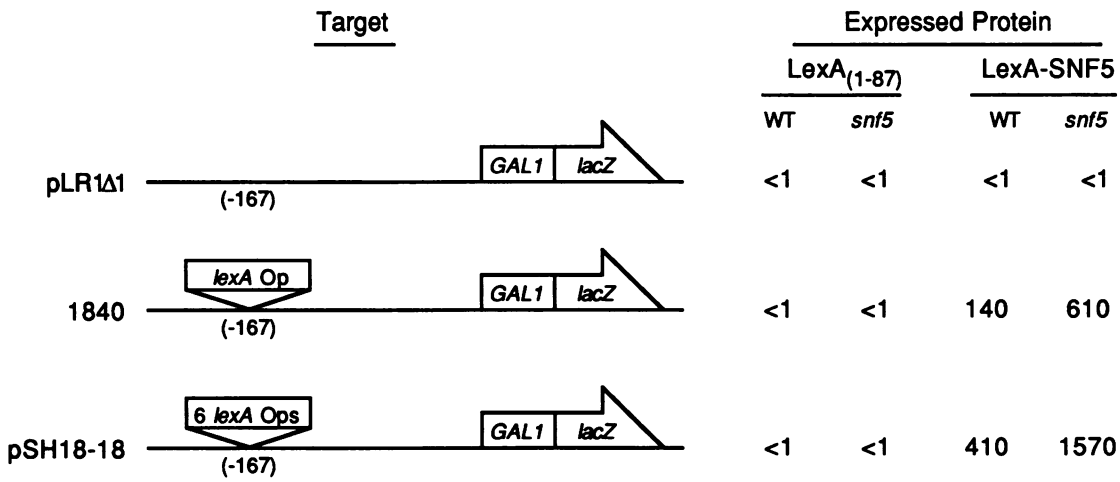

FIG. 6. *GAL1-lacZ* gene activation by DNA-bound LexA-SNF5 fusion protein. Strains MCY829 (wild type [WT]) and MCY2064 (*snf5*) were doubly transformed with both expression and target plasmids. The $LexA_{(1-87)}$ protein was expressed from pSH2-1 (20), and the LexA-SNF5 fusion protein was expressed from pLexA-SNF5 (Fig. 1). Target plasmids are represented schematically. Plasmid 1840 is an autonomously replicating plasmid with a single copy of the 22-base-pair *lexA* operator (*lexA* Op) inserted at the *Xho*I site 167 base pairs 5′ to the transcriptional start site for the *GAL1-lacZ* gene fusion; UAS_G has been removed (5). pLR1Δ1 is identical to 1840 except that it has no *lexA* operator (59). pSH18-18 is identical to 1840 except that it contains six *colE1* overlapping *lexA* operators (13) inserted at the *Xho*I site (Hanes and Brent, personal communication). Transformants carrying each plasmid combination were grown with selection for both plasmids and assayed for β-galactosidase activity. Numbers denote units of β-galactosidase activity normalized to cell density. Values are averages of assays of four transformants, except for the four control experiments (values <1) in the *snf5* mutant host. Standard errors were <10%.

plasmid and target plasmid. *GAL1-lacZ* gene activation was assessed by assaying expression of β-galactosidase (Fig. 6). The LexA-SNF5 fusion protein served as a highly effective activator, stimulating expression from plasmid 1840 more than 100-fold in the wild-type strain and 600-fold in the *snf5* mutant. The LexA-SNF5 protein stimulated expression from pSH18-18 nearly 1,600-fold in the *snf5* mutant. Its function was dependent on the SNF5 sequence in the fusion protein, as no stimulation by the $LexA_{(1-87)}$ protein was detected. Activation was also dependent on the presence of a *lexA* operator in the target plasmid, as judged by the lack of expression from the target with no operator (pLR1Δ1). This finding is consistent with the idea that the fusion protein is bound to DNA via recognition of the *lexA* operator by the LexA DNA-binding domain. The SNF5 moiety may contribute to dimerization of the LexA-SNF5 protein, as it has been argued that the ability of a fusion protein to recognize the operator efficiently suggests that the sequence fused to LexA contributes to dimerization (20, 27).

Thus, these experiments indicate that the SNF5 protein is capable of functioning as a transcriptional activator when it is brought close to a promoter. This evidence, together with the genetic data, the sequence, and the nuclear localization, strongly suggests that SNF5 functions as a transcriptional activator of a broad spectrum of genes.

It is possible that other, closely associated proteins are required for the activation function. The negative effect of the wild-type chromosomal *SNF5* gene on the ability of the fusion to activate transcription, even though the gene fusion is on a multicopy plasmid, suggests a competition between the wild-type and fusion proteins for some limiting factor. The SNF2 and SNF6 proteins are candidates for associated proteins. Because the SNF5 protein contains motifs characteristics of transcriptional activators (glutamine- and proline-rich regions and an acidic region), it seems likely that SNF5 provides an activating domain. The SNF2, SNF5, and SNF6 proteins could form a heteromeric complex similar to the HAP2-HAP3-HAP4 DNA-binding complex, in which HAP4 is thought to provide the transcriptional activation domain (15).

Our data suggest that the SNF5 protein does not have DNA-binding activity. Although this possibility cannot be excluded, we instead favor the idea that SNF5 is brought into close proximity to a promoter by association with another protein(s) that binds DNA directly. A precedent is provided by the herpes simplex virus protein VP16, a potent transcriptional activator that does not bind DNA directly but rather forms a complex with cellular DNA-binding proteins (31, 43, 57, 58). As yet there is no evidence that SNF2 and SNF6 are responsible for DNA binding, and mutations in *SNF2* and *SNF6* do not affect DNA-protein complexes formed with the *SUC2* sequences −542 to −381 or −166 to −26 (New et al., unpublished data; J. H. New, B. C. Laurent, and F. Estruch, unpublished results). Therefore, we suggest that SNF5, or a SNF2-SNF5-SNF6 complex, interacts with an unidentified DNA-binding protein(s) that recognizes the promoters subject to activation.

ACKNOWLEDGMENTS

We gratefully acknowledge generous gifts of plasmids and communication of unpublished results from Steve Hanes and Roger Brent. We thank Linda Marshall-Carlson for assistance with immunofluorescence microscopy, Shannon Fitzgerald for constructing pBLU2, Janet Schultz for suggesting assays of α-factor, and Paco Estruch for helpful discussions. We also thank Steve Kurtz and David Julius for gifts of plasmids. We thank Anne Happel, Michele Swanson, Fred Winston, and Michael Ciriacy for permission to cite unpublished results.

This work was supported by Public Health Service grant GM34095 from the National Institutes of Health and by an American Cancer Society Faculty Research Award to M.C. B.C.L. was the recipient of National Research Service Award postdoctoral fellowship F32 GM11933.

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