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Genetics

Functional interdependence of the yeast SNF2, SNF5, and SNF6 proteins in transcriptional activation

(transcriptional activators/*Saccharomyces cerevisiae*/LexA fusion proteins)

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ABSTRACT The *SNF2*, *SNF5*, and *SNF6* genes of *Saccharomyces cerevisiae* are required for expression of a variety of differently regulated genes. Previous evidence implicated the SNF5 protein in transcriptional activation, and a DNA-bound LexA-SNF5 fusion protein was shown to activate expression of a nearby promoter. Here, we examine the functional relationship of the SNF2, SNF5, and SNF6 proteins. Activation by DNA-bound LexA-SNF5 fusion protein was greatly reduced in *snf2* and *snf6* mutants, indicating that activation by LexA-SNF5 requires SNF2 and SNF6 function. An *spt6* mutation, which suppresses transcriptional defects caused by *snf2*, restored activation by LexA-SNF5 in a *snf2* mutant. The *SNF2* gene was sequenced and encodes a 194-kDa protein that is targeted to the nucleus. DNA-bound LexA-SNF2 fusion protein also activated transcription, dependent on SNF5 and SNF6. These findings suggest that SNF2, SNF5, and SNF6 function interdependently in transcriptional activation, possibly forming a heteromeric complex.

The *SNF2*, *SNF5*, and *SNF6* genes of *Saccharomyces cerevisiae* are required for transcription of many genes subject to different regulatory systems: glucose-repressible genes, acid phosphatase, cell type-specific genes, and *Ty* elements (refs. 1–4; A. Happel, M. Swanson, and F. Winston, personal communication). *SNF2* and *SNF5* also affect expression of protease B activity (5). All three genes are required for healthy growth and for sporulation of diploids, although none is essential for viability (1–4). Their similar mutant phenotypes suggest that *SNF2*, *SNF5*, and *SNF6* have related functions. Further genetic evidence for this view is that mutations in these genes show similar interactions with the suppressor mutations *ssn6* and *spt6* (1, 3, 6).

Previous evidence suggests that *SNF5* encodes a transcriptional activator (4). The predicted SNF5 protein has extremely glutamine- and proline-rich regions and an acidic region, which are characteristic of activators (7–10), and the *SNF5* product is located in the nucleus (4). Using the method of Brent and Ptashne (11), we showed that a LexA-SNF5 fusion protein, when bound to a *lexA* operator via the LexA DNA-binding domain, activates expression of a nearby promoter (4). Fusions of LexA to various authentic transcriptional activators function in this assay, whereas fusions to the *MAT*α*2* product or bacteriophage 434 repressor do not (10–13).

What is the functional relationship among the SNF2, SNF5, and SNF6 proteins? One model is that they function sequentially in a cascade of events. Another is that the three proteins are physically associated and function together as a unit. A third possibility is that two of the proteins function together, and the third protein either affects or is affected by the other two. Previous studies showed that none of these *SNF* genes affects expression of the others, nor does increased dosage of one compensate for a mutation in another (2, 3).

We examine here the functional relationship of the SNF2, SNF5, and SNF6 proteins in transcriptional activation. We tested activation by DNA-bound LexA-SNF5 fusion protein in *snf2* and *snf6* mutants, thereby showing that LexA-SNF5 function requires the SNF2 and SNF6 proteins. We report here the sequence of the *SNF2* gene† and the nuclear localization of its product. A LexA-SNF2 fusion protein was constructed and found to activate gene expression, dependent on SNF5 and SNF6. Finally, we examined the effects of an *spt6* mutation, a suppressor of *snf2*, *snf5*, and *snf6*, on transcriptional activation.

MATERIALS AND METHODS

Strains and Genetic Methods. All strains of *S. cerevisiae* were derivatives of S288C. Genetic methods were as described (4). A deletion was constructed at the chromosomal *SNF2* locus of a diploid by transformation with the *Nru* I–*Xho* I fragment from pLY21 (Fig. 1). A transformant heterozygous for the mutation, *snf2*-Δ2::*URA3*, was identified by blot hybridization analysis.

β-Galactosidase Assays. β-Galactosidase activity was assayed in permeabilized cells (14) and is expressed as described by Miller (15).

Plasmids. pLY21 was constructed by replacing the *Bst*EII–*Xba* I fragment of *SNF2* (2) with *URA3*. pSNF2(1554)-lacZ (Fig. 1) contains *SNF2* sequences cloned in YEp358 (16). To create pSNF2(1423–1554)-lacZ, the *Eag* I–*Xba* I fragment containing codons 1423–1554 was provided with a translational initiation codon from the pUC19 polylinker and placed under control of the *GAL10* promoter in YEp52 (17); the *GAL10-SNF2* sequence was then fused to *lacZ* in YEp358 (16). pLexA-SNF2 carries the *Hinc*II–*Xho* I fragment encoding residues 14–1696 of *SNF2* cloned into pSH2-1 (12) between the *Bam*HI and *Sal* I sites. Codons 1–87 of *lexA* and seven codons derived from the pSH2-1 polylinker are fused in frame to codon 14 of *SNF2*. A translational stop codon lies 14 residues 3′ to codon 1696 of *SNF2*.

DNA Sequencing. The sequence was determined for both strands of the cloned (2) *SNF2* gene by the method of Sanger *et al.* (18) using Sequenase (United States Biochemical) and the universal primer (Amersham) and 11 synthetic 18-mer oligonucleotides (Research Genetics, Huntsville, AL).

RESULTS

Activation by LexA-SNF5 Requires SNF2 and SNF6. Previously, we expressed a LexA-SNF5 fusion protein containing the LexA DNA-binding domain (residues 1–87) from the

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M55906).

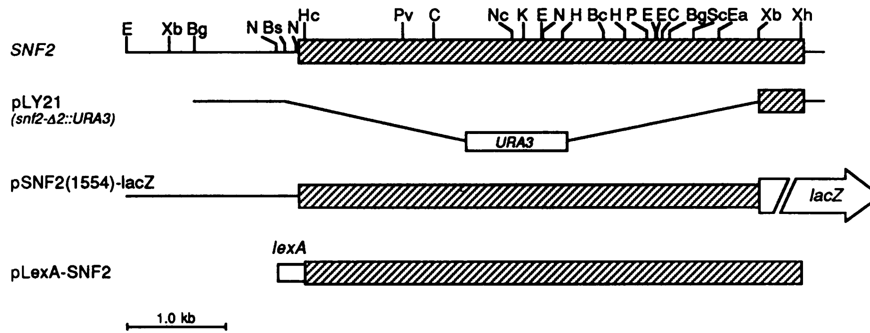

FIG. 1. Maps of the *SNF2* gene and plasmids. ▨, *SNF2* coding region. The direction of transcription is left to right. Plasmids are described in the text. Restriction sites: Bc, *Bcl* I; Bg, *Bgl* II; Bs, *Bst*EII; C, *Cla* I; E, *Eco*RI; Ea, *Eag* I; H, *Hin*dIII; Hc, *Hin*cII; K, *Kpn* I; N, *Nru* I; Nc, *Nco* I; P, *Pst* I; Pv, *Pvu* II; Sc, *Sac* I; Xb, *Xba* I; Xh, *Xho* I.

ADH1 promoter in plasmid pLexA-SNF5. The fusion protein activated expression of *GAL1-lacZ* target genes that contained single or multiple *lexA* operators 5′ to the *GAL1* promoter (Fig. 2). No activation was detected from a *GAL1-lacZ* target gene that lacked any *lexA* operator. The LexA DNA-binding domain alone did not activate expression from the target plasmids (values <1; ref. 4). These results suggest that the SNF5 protein, when bound to DNA, functions as a transcriptional activator (4).

To test whether activation by LexA-SNF5 requires SNF2 or SNF6 function, we cotransformed *snf2* or *snf6* mutants with pLexA-SNF5 and each of three target plasmids. Gene activation was measured by assaying β-galactosidase activity (Fig. 2). Mutations in *SNF2* and *SNF6* dramatically reduced activation by the LexA-SNF5 fusion protein (10- to 50-fold).

To rule out the possibility that the *snf* mutations merely prevented expression of the fusion protein by blocking transcription from the *ADH1* promoter, we examined *ADH1* RNAs by RNA blot hybridization (21). Levels were the same in the three *snf* mutants as in wild type (data not shown). Moreover, the same amount of LexA-SNF5 protein was present in *snf2* and *snf6* mutants as in wild type, as detected by immunoblot analysis with anti-LexA antibody (data not shown). In addition, a LexA-GAL4 fusion protein expressed from the *ADH1* promoter (12) activated a *GAL1-lacZ* target gene with multiple *lexA* operators (pSH18-18 in Fig. 2) in the *snf* mutants; β-galactosidase activities in the mutants were reduced at most by a factor of 2 relative to the wild type (data not shown). Thus, the SNF2 and SNF6 proteins appear to be required for transcriptional activation by DNA-bound LexA-SNF5 protein.

An *spt6* Mutation Suppresses the Requirement for SNF2. To obtain further genetic evidence that this assay monitors biologically relevant activities of the SNF proteins, we examined the effects of an *spt6* mutation on activation. *SPT6* is an essential gene, encoding a nuclear protein that appears to play a role in transcription (22–24). Mutations in *SPT6* were first isolated as suppressors of a *delta* insertion mutation 5′ to the *HIS4* gene (25), and additional alleles (called *ssn20*) were isolated as suppressors that restore high-level, regulated *SUC2* (invertase) gene expression in *snf2*, *snf5*, and *snf6* mutants (6). The mutations also suppressed deletions of *SUC2* sequences 5′ to the TATA box (22).

Because *spt6* mutations suppress the requirement for *SNF2* and *SNF6* function for expression of the *SUC2* promoter, we tested whether *spt6* would similarly suppress the dependence of LexA-SNF5 on SNF2 and SNF6 in the gene activation assay. An *spt6* mutation restored activation by LexA-SNF5 to wild-type levels in a *snf2* mutant (Fig. 2). The *spt6* mutation did not significantly affect activation in a wild-type (*SNF*) background. This parallel genetic behavior suggests that SNF2, SNF5, and the mutant *spt6* product function in this activation system in a manner that parallels their function at the *SUC2* promoter.

In contrast, the *spt6* allele failed to restore activation by LexA-SNF5 in a *snf6* mutant (Fig. 2). This lack of suppression could reflect differences in the function of these gene products in the two systems; alternatively, the ability of *spt6* to suppress *snf6* for activation of the *SUC2* promoter could

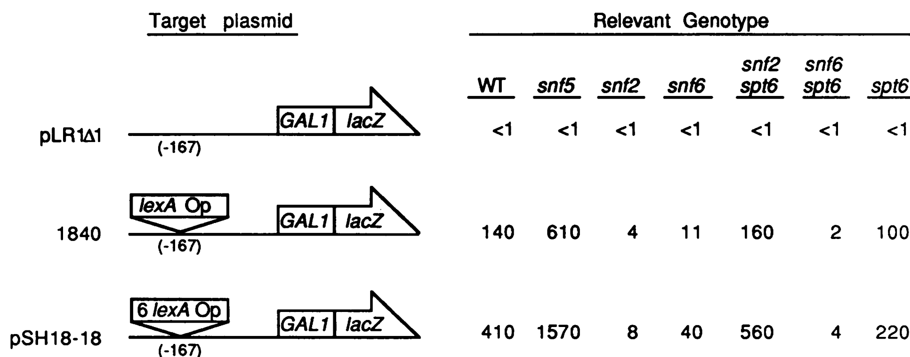

FIG. 2. Activation by the LexA-SNF5 fusion protein. The relevant genotype of the host strain is indicated. Alleles used were *snf2-141* (ochre), *snf5-Δ2*, *snf6-Δ2*, and *ssn20-1* (an *spt6* allele). All strains carried *his3-Δ200* and *ura3-52* mutations. The *snf6 spt6* strain was tested for suppression of the sucrose-nonfermenting phenotype. Transformants were grown in synthetic complete medium lacking histidine and uracil to maintain selection for the multicopy plasmid pLexA-SNF5 and the target plasmids, as described (4). pLexA-SNF5 is a derivative of pSH2-1 (12). The target plasmids 1840 and pSH18-18 (refs. 11 and 12; S. Hanes and R. Brent, personal communication) are derived from pLR1Δ1 (19) and contain the *lexA* operator and six overlapping *lexA* operators (20), respectively, inserted at position −167 relative to the *GAL1* transcriptional start site. The 331 bp of *GAL* sequence 5′ to this site, including the upstream activation sequence (UAS_G), are deleted (19). Values are the average of assays of four transformants. Standard errors were <25%. Values for wild-type (WT) and *snf5* mutant strains are taken from ref. 4. The LexA DNA-binding domain expressed from pSH2-1 did not activate expression from the target plasmids in any strain (values < 1). Op, operator(s).

simply reflect the involvement of other factors at the chromosomal *SUC2* locus.

Analysis of the Role of SNF2. Various roles can be envisioned for SNF2 and SNF6 in activation by the LexA-SNF5 protein. However, they do not simply mediate the association of SNF5 with DNA, as this is carried out by the LexA DNA-binding domain, and neither SNF2 nor SNF6 is required for nuclear localization of SNF5 (4). One possible model is that SNF2 or SNF6 participates in a heteromeric complex with SNF5 that is essential for the activation function.

To facilitate functional analysis of the SNF2 protein, we determined the nucleotide sequence of the gene (Fig. 3). An open reading frame of 1703 codons could encode a protein of 194,036 daltons. Three noteworthy features of the SNF2 protein are a glutamine-rich region (42% glutamine; residues 45–68), a stretch of glutamine and alanine (residues 207–239), and nine arginine-glycine repeats (residues 1505–1522). The protein contains 30% charged residues and includes several short basic and acidic regions. The acidic regions include residues 730–743 (net charge, −8), 1269–1293 (−10), 1407–1437 (−9), and 1654–1700 (−10). A search of the sequences in the GenBank data base (release 58.0) translated with the program TFASTA (26) revealed that the translated noncoding strand of the 3′ untranslated region of the *S. cerevisiae LYS2* gene (27) is 37% identical to 125 amino acids (1132–1263) of SNF2.

LexA-SNF2 Fusion Protein Bound to a *lexA* Operator Activates Transcription. If the SNF2 protein participates in a heteromeric complex with SNF5, a LexA–SNF2 fusion protein might also effect activation. To test this idea, we constructed a derivative of pSH2-1 (12) containing the DNA-binding domain of *lexA* fused to codons 14–1696 of *SNF2*. The resulting plasmid, pLexA-SNF2 (Fig. 1), complemented the growth defects of a *snf2* mutant, indicating that the fusion protein provides SNF2 function.

Both wild-type and *snf2* mutant strains were cotransformed with pLexA-SNF2 and each of the three target plasmids described above. The fusion protein strongly stimulated expression of *GAL1-lacZ* target genes that carried upstream *lexA* operators: expression from pSH18-18 was stimulated 900-fold in a *snf2* mutant (Table 1). Greater activation was observed in a *snf2* mutant than in wild type, suggesting that the native SNF2 protein competes with the LexA-SNF2 fusion protein for some limiting factor required for transcriptional activation or forms mixed oligomers incapable of binding the *lexA* operator.

Function of LexA-SNF2 Requires SNF5 and SNF6. To test whether activation by LexA-SNF2 requires *SNF5* and *SNF6* function, appropriate mutants were cotransformed with expression and target plasmids. Activation from the target plasmids was reduced substantially, by factors of 40–100, in *snf5* and *snf6* mutants (Table 1). The amount of LexA-SNF2 protein detected by immunoblot analysis was similar in the *snf5* and *snf6* mutants and wild type (data not shown).

Because *spt6* suppresses the requirement for *SNF5* for transcription of *SUC2*, the effect of *spt6* in this assay was examined. The *spt6* allele that was tested restored activation by LexA-SNF2 in a *snf5* mutant to 25% of the wild-type level (Table 1). Thus, *spt6* suppresses the dependence of LexA-SNF2 on SNF5, as well as the dependence of LexA-SNF5 on SNF2, for transcriptional activation. This *spt6* mutation did not suppress the requirement for *SNF6*.

Nuclear Localization of a Bifunctional SNF2-β-Galactosidase Fusion Protein. If the SNF2 protein functions in transcriptional activation, one would predict a nuclear location. To test this hypothesis, the protein was tagged with β-galactosidase for localization by immunofluorescence microscopy. We fused codon 1554 of *SNF2* to the *Escherichia coli lacZ* gene (Fig. 1). The gene fusion, *SNF2(1554)-lacZ*, encodes a bifunctional protein that complements *snf2* for invertase expression and has the expected size, as detected by immunoblotting. Immunofluorescent staining of cells carrying the gene fusion revealed that the fusion protein was located in the nucleus (Fig. 4). Previous studies showed that β-galactosidase is not localized in the nucleus (28).

Additional evidence suggested that the C terminus of SNF2 contains a nuclear targeting signal: a similar fusion protein bearing the N-terminal 1333 residues of SNF2 was not localized in the nucleus (data not shown). We therefore constructed pSNF2(1423-1554)-lacZ, containing *SNF2* codons 1423–1554 fused to *lacZ* under the control of the *GAL10* promoter. Wild-type cells carrying this plasmid were induced with galactose, and the fusion protein was localized in the nucleus (Fig. 4). A sequence that may serve as a nuclear targeting signal (29) is Gly-Arg-Pro-Arg-Gly-Arg-Pro-Lys-Lys-Val-Lys-Leu-Glu at position 1446.

Deletion of *SNF2* Is Not Lethal. Previously, the *SNF2* gene was disrupted in the C-terminal half of the sequence (2). To verify the null phenotype, a diploid heterozygous for a deletion of nearly the entire *SNF2* coding sequence (*snf2-Δ2::URA3*; Fig. 1) was constructed and subjected to tetrad analysis. Four viable spores were obtained from each tetrad. The two Ura^+ spore clones grew slowly on glucose and showed growth defects on raffinose, galactose, and glycerol. Thus, *SNF2* is not an essential gene, but it is important for normal growth.

DISCUSSION

We show here that DNA-bound LexA-SNF2 and LexA-SNF5 fusion proteins activate expression from a nearby promoter. In each case, activation is dependent on the other two SNF proteins: LexA-SNF2 requires SNF5 and SNF6, and LexA-SNF5 requires SNF2 and SNF6. These results suggest that the SNF2, SNF5, and SNF6 proteins function interdependently in transcriptional activation. These findings are in accord with previous genetic evidence suggesting that the three genes have related functions (1, 3, 6). The ability of both LexA-SNF2 and LexA-SNF5 proteins to activate transcription suggests that the SNF2 and SNF5 proteins form a heteromeric complex, perhaps similar to the HAP2–HAP3–HAP4 complex (30). Thus, fusion of either SNF protein to a LexA DNA-binding domain may suffice to recruit both SNF2

Table 1. Activation by the LexA-SNF2 fusion protein

Target plasmid	*lexA* operator	Relevant genotype						
		wt	*snf2*	*snf5*	*snf6*	*snf5 spt6*	*snf6 spt6*	*spt6*
pLR1Δ1	None	<1	<1	<1	<1	<1	<1	<1
1840	1	120	360	2	1	30	3	30
pSH18-18	6	350	910	8	5	90	6	90

This experiment was carried out as described for Fig. 2. Standard errors were <20%. In control experiments, no stimulation by the LexA DNA-binding domain was detected in strains carrying pSH2-1 and each target plasmid (values < 1). wt, Wild type.

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-895                                                                   TATCAGTAAGTCTGGGCTCCTTTTTATTTTAAAGAAGAATCTATTGACTCTGAAC
-840   TATTTTCAATCAAAAATAAATTCACAAGTCCAACACCTCCAACTCTATGGTATTGCGTTCTCTTTTTGCCCTTTTTTCCTTTTTTTCTTTTTTTCACTACTCTTTCCTTGATTTCCATTT
-720   TTTTTCATTTTTCACTCTTCTATCACCTCTTCATACCCATACCCCCTGTTGGCCGCGGCTGCCCTAAATACACACATATATATATATAAAACAAACAAGGCATTTTTCAAATTAATAAAA
-600   CTAATAAAAAAAGCTATAGAGCCTTTTCTTAGAACAACGTGTTGAAATATTCGCTTTTTTCTTTTTATTGAAACTCCTTCACTTCCAAAAAGACACGATTCTTAATGGCTATTCTGAGTG
-480   AACATAAGGACACTAAGAACTAACAAGTAATTGAAAAAAAAAGAAGAAGACATGATGAAGAAGAATTAGTGAGCAAAATACAGTTGAAGTTCCTAACGCTTATTTCCTTGCAGATTAAT
-360   TCCAATACATTCGCCACTATTGGAATTTTGCAGGTAGCCAATTTTCGCGTATATATACTAGGTTTAGGAAAAATAGCCGCCGGTAAAAATAGTGATAGGCTGTTACCCGAATACCGTTGT
-240   TCGCGATATGTTAAAACCGCGTTTGAAAGACAAATATCAACAAATTGATTGACGAAACGGGGCCATCCTGAGGCGGTAGGACAATAAGTTAAAAGAAAGGTTACCTCTAGTCTCAATAAA
-120   GAAGATATTTGTGACGTACGTGGACCTTTTGTATATAAATCATCGGGAAGGTCAGCTATTCTGTTGTTTCTCTAATCGCGACTTTCTGCTATTTTCACGACTTTCGATTAATTATCTGCC
        M  N  I  P  Q  R  Q  F  S  N  E  E  V  N  R  C  Y  L  R  W  Q  H  L  R  N  E  H  G  M  N  A  P  S  V  P  E  F  I  Y  L    40
1      ATGAACATACCACAGCGTCAATTTAGCAACGAAGAGGTCAACCGCTGCTATTTAAGATGGCAGCATTTGAGAAATGAACACGGAATGAACGCTCCGAGTGTGCCGGAATTTATCTATTTG
        T  K  V  L  Q  F  A  A  K  Q  R  Q  E  L  Q  M  Q  R  Q  Q  Q  G  I  S  G  S  Q  Q  N  I  V  P  N  S  S  D  Q  A  E  L    80
121    ACTAAAGTGTTGCAATTTGCTGCCAAGCAGCGACAAGAATTACAAATGCAACGCCAACAGCAAGGCATTTCAGGTTCTCAGCAAAATATTGTTCCAAATTCTTCAGATCAAGCAGAACTG
        P  N  N  A  S  S  H  I  S  A  S  A  S  P  H  L  A  P  N  M  Q  L  N  G  N  E  T  F  S  T  S  A  H  Q  S  P  I  M  Q  T   120
241    CCGAATAATGCTTCCTCGCACATTTCTGCTTCTGCTTCCCCGCATTTAGCTCCGAATATGCAATTGAATGGAAATGAGACCTTTAGCACTTCTGCTCATCAATCACCAATCATGCAAACA
        Q  M  P  L  N  S  N  G  G  N  N  M  L  P  Q  R  Q  S  S  V  G  S  L  N  A  T  N  F  S  P  T  P  A  N  N  G  E  N  A  A   160
361    CAGATGCCATTAAATAGCAATGGTGGTAATAACATGCTACCACAACGGCAATCTTCGGTTGGTTCATTAAATGCAACTAATTTTTCACCAACACCAGCAAACAATGGAGAAAATGCTGCG
        E  K  P  D  N  S  N  H  N  N  L  N  L  N  N  S  E  L  Q  P  Q  N  R  S  L  Q  E  H  N  I  Q  D  S  N  V  M  P  G  S  Q   200
481    GAGAAACCAGATAACTCAAACCATAATAACTTAAACCTTAATAATTCCGAGCTTCAACCGCAAAATCGATCATTACAAGAACACAACATACAGGATTCAAATGTGATGCCAGGTTCGCAG
        I  N  S  P  M  P  Q  Q  A  Q  M  Q  Q  A  Q  F  Q  A  Q  Q  A  Q  Q  A  Q  Q  A  Q  Q  A  Q  Q  A  Q  A  R  L  Q  Q  G   240
601    ATAAATTCTCCTATGCCCCAGCAGGCGCAGATGCAGCAGGCACAATTCCAAGCACAACAGGCGCAACAAGCGCAACAGGCACAACAGGCACAACAAGCACAAGCGAGACTGCAACAGGGG
        R  R  L  P  M  T  M  F  T  A  E  Q  S  E  L  L  K  A  Q  I  T  S  L  K  C  L  V  N  R  K  P  I  P  F  E  F  Q  A  V  I   280
721    AGAAGATTACCCATGACAATGTTTACTGCCGAGCAATCCGAACTGTTAAAGGCCCAAATCACATCTCTAAAATGTCTAGTAAATAGAAAGCCTATTCCGTTTGAATTTCAGGCTGTTATC
        Q  K  S  I  N  H  P  P  D  F  K  R  M  L  L  S  L  S  E  F  A  R  R  R  Q  P  T  D  Q  N  N  Q  S  N  L  N  G  G  N  N   320
841    CAAAAATCTATTAACCATCCTCCAGATTTCAAAAGAATGTTACTGTCTTTGAGTGAGTTTGCGAGAAGAAGACAACCCACAGATCAAAATAACCAATCAAATCTCAATGGCGGCAACAAT
        T  Q  Q  P  G  T  N  S  H  Y  N  N  T  N  T  D  N  V  S  G  L  T  R  N  A  P  L  D  S  K  D  E  N  F  A  S  V  S  P  A   360
961    ACGCAGCAACCCGGCACCAATTCCCACTACAATAACACTAACACTGACAACGTTTCTGGGCTTACCAGAAATGCACCTTTAGATAGCAAAGACGAAAATTTTGCATCTGTTTCACCAGCT
        G  P  S  S  V  H  N  A  K  N  G  T  L  D  K  N  S  Q  T  V  S  G  T  P  I  T  Q  T  E  S  K  K  E  E  N  E  T  I  S  N   400
1081   GGTCCATCTAGTGTACATAATGCGAAGAATGGCACCCTCGATAAAAATTCGCAAACTGTAAGTGGCACTCCAATCACGCAAACAGAATCGAAAAAAGAGGAAAACGAAACCATTTCAAAC
        V  A  K  T  A  P  N  S  N  K  T  H  T  E  Q  N  N  P  P  K  P  Q  K  P  V  P  L  N  V  L  Q  D  Q  Y  K  E  G  I  K  V   440
1201   GTAGCTAAAACAGCTCCTAATAGTAATAAGACCCACACCGAGCAAAATAACCCGCCAAAACCGCAGAAACCCGTTCCTTTAAACGTCCTTCAAGATCAATATAAGGAAGGAATAAAAGTG
        V  D  I  D  D  P  D  M  M  V  D  S  F  T  M  P  N  I  S  H  S  N  I  D  Y  Q  T  L  L  A  N  S  D  H  A  K  F  T  I  E   480
1321   GTAGATATAGATGATCCTGATATGATGGTTGATTCTTTTACGATGCCCAATATCTCACATAGTAATATCGATTATCAGACTTTATTGGCCAATTCGGATCATGCAAAGTTTACTATTGAA
        P  G  V  L  P  V  G  I  D  T  H  T  A  T  D  I  Y  Q  T  L  I  A  L  N  L  D  T  T  V  N  D  C  L  D  K  L  L  N  D  E   520
1441   CCTGGCGTTCTACCGGTTGGTATTGACACACATACCGCTACTGACATTTACCAAACTTTAATTGCATTGAACCTAGATACTACGGTAAATGACTGTTTGGATAAGCTACTAAATGACGAA
        C  T  E  S  T  R  E  N  A  L  Y  D  Y  Y  A  L  Q  L  L  P  L  Q  K  A  V  R  G  H  V  L  Q  F  E  W  H  Q  N  S  L  L   560
1561   TGTACGGAATCAACCAGGGAAAATGCTCTTTACGATTACTACGCCTTACAATTATTACCGTTACAAAAAGCCGTAAGAGGTCATGTATTACAATTTGAATGGCATCAAAATTCTTTATTA
        T  N  T  H  P  N  F  L  S  K  I  R  N  I  N  V  Q  D  A  L  L  T  N  Q  L  Y  K  N  H  E  L  L  K  L  E  R  K  K  T  E   600
1681   ACCAATACACATCCAAATTTTCTTTCGAAAATAAGAAACATCAACGTTCAAGATGCATTGCTAACAAACCAACTTTATAAGAATCATGAATTACTTAAGTTAGAAAGGAAGAAGACTGAG
        A  V  A  R  L  K  S  M  N  K  S  A  I  N  Q  Y  N  R  R  Q  D  K  K  N  K  R  L  K  F  G  H  R  L  I  A  T  H  T  N  L   640
1801   GCAGTGGCCAGATTAAAGTCGATGAATAAATCCGCCATCAATCAATATAATAGAAGACAAGATAAAAAGAATAAAAGGTTGAAGTTTGGCCATAGACTCATTGCTACGCATACTAATTTA
        E  R  D  E  Q  K  R  A  E  K  K  A  K  E  R  L  Q  A  L  K  A  N  D  E  E  A  Y  I  K  L  L  D  Q  T  K  D  T  R  I  T   680
1921   GAAAGGGATGAGCAAAAGAGAGCTGAAAAAAAGGCAAAAGAACGTTTACAGGCTTTAAAGGCGAACGATGAGGAAGCCTATATAAAATTATTAGACCAAACCAAAGATACAAGAATTACA
        H  L  L  R  Q  T  N  A  F  L  D  S  L  T  R  A  V  K  D  Q  Q  K  Y  T  K  E  M  I  D  S  H  I  K  E  A  S  E  E  V  D   720
2041   CATCTTCTAAGGCAAACAAACGCATTTTTAGATTCTTTAACAAGAGCCGTGAAAGATCAACAAAAGTACACTAAAGAAATGATTGATTCCCACATAAAGGAAGCATCGGAGGAAGTGGAT
        D  L  S  M  V  P  K  M  K  D  E  E  Y  D  D  D  D  D  N  S  N  V  D  Y  Y  N  V  A  H  R  I  K  E  D  I  K  K  Q  P  S   760
2161   GATTTGTCCATGGTCCCCAAGATGAAGGATGAAGAATATGATGACGACGACGACAATTCTAACGTCGATTATTACAATGTCGCACATAGAATTAAAGAAGATATCAAGAAGCAGCCTTCA
        I  L  V  G  G  T  L  K  D  Y  Q  I  K  G  L  Q  W  M  V  S  L  F  N  N  H  L  N  G  I  L  A  D  E  M  G  L  G  K  T  I   800
2281   ATATTGGTTGGTGGTACCTTAAAGGACTATCAAATCAAGGGTTTACAGTGGATGGTTTCGCTTTTCAATAATCATTTAAATGGTATTTTAGCAGACGAAATGGGTCTTGGTAAGACTATC
        Q  T  I  S  L  L  T  Y  L  Y  E  M  K  N  I  R  G  P  Y  L  V  I  V  P  L  S  T  L  S  N  W  S  S  E  F  A  K  W  A  P   840
2401   CAGACGATATCCTTACTTACATATTTATATGAAATGAAGAATATTCGTGGGCCATATTTAGTCATTGTTCCTTTATCTACACTGTCAAATTGGAGCAGTGAATTCGCGAAATGGGCTCCT
        T  L  R  T  I  S  F  K  G  S  P  N  E  R  K  A  K  Q  A  K  I  R  A  G  E  F  D  V  V  L  T  T  F  E  Y  I  I  K  E  R   880
2521   ACCTTAAGAACTATATCATTTAAAGGTTCGCCAAATGAGAGGAAAGCCAAACAGGCTAAAATAAGGGCAGGTGAATTTGATGTAGTTCTGACTACCTTTGAGTATATTATTAAAGAAAGA
        A  L  L  S  K  V  K  W  V  H  M  I  I  D  E  G  H  R  M  K  N  A  Q  S  K  L  S  L  T  L  N  T  H  Y  H  A  D  Y  R  L   920
2641   GCACTTTTATCCAAGGTCAAGTGGGTCCATATGATTATTGATGAAGGCCATAGAATGAAAAATGCGCAATCCAAGCTTTCTTTAACCTTGAACACACATTACCATGCAGATTATAGATTA
        I  L  T  G  T  P  L  Q  N  N  L  P  E  L  W  A  L  L  N  F  V  L  P  K  I  F  N  S  V  K  S  F  D  E  W  F  N  T  P  F   960
2761   ATTTTGACAGGTACACCACTTCAAAACAACTTGCCAGAATTATGGGCCTTATTGAATTTTGTGTTACCCAAGATTTTCAATTCCGTGAAATCTTTTGATGAATGGTTCAATACACCCTTT
        A  N  T  G  G  Q  D  K  I  E  L  S  E  E  E  T  L  L  V  I  R  R  L  H  K  V  L  R  P  F  L  L  R  R  L  K  K  D  V  E  1000
2881   GCCAACACCGGTGGGCAAGATAAAATTGAGTTGAGTGAAGAAGAGACGCTTTTGGTTATCAGGAGATTGCATAAAGTCTTGAGACCCTTTTTATTGCGTCGTTTGAAAAAGGATGTAGAA
        K  E  L  P  D  K  V  E  K  V  V  K  C  K  M  S  A  L  Q  Q  I  M  Y  Q  Q  M  L  K  Y  R  R  L  F  I  G  D  Q  N  N  K  1040
3001   AAAGAATTACCGGATAAAGTTGAGAAGGTAGTGAAGTGCAAAATGAGCGCTTTACAACAAATTATGTACCAACAAATGTTGAAATATCGTCGTCTTTTTATCGGTGATCAAAATAATAAG
        K  M  V  G  L  R  G  F  N  N  Q  I  M  Q  L  K  K  I  C  N  H  P  F  V  F  E  E  V  E  D  Q  I  N  P  T  R  E  T  N  D  1080
3121   AAAATGGTAGGTTTAAGAGGTTTCAATAATCAAATCATGCAACTAAAGAAAATCTGTAATCATCCTTTTGTATTTGAAGAAGTTGAGGACCAAATCAATCCAACAAGAGAAACGAATGAT
        D  I  W  R  V  A  G  K  F  E  L  L  D  R  I  L  P  K  L  K  A  T  G  H  R  V  L  I  F  F  Q  M  T  Q  I  M  D  I  M  E  1120
3241   GATATTTGGCGTGTTGCTGGTAAATTTGAACTATTAGATAGAATTTTGCCTAAGCTTAAGGCAACTGGGCATAGAGTATTAATTTTTTTTCAAATGACCCAAATTATGGATATTATGGAA
        D  F  L  R  Y  I  N  I  K  Y  L  R  L  D  G  H  T  K  S  D  E  R  S  E  L  L  R  L  F  N  A  P  D  S  E  Y  L  C  F  I  1160
3361   GATTTTTTAAGGTACATTAATATCAAGTACCTGAGGTTAGATGGTCATACAAAATCTGATGAACGTAGTGAGTTGTTACGTCTATTTAACGCCCCTGATTCTGAATACTTGTGCTTTATC
        L  S  T  R  A  G  G  L  G  L  N  L  Q  T  A  D  T  V  I  I  F  D  T  D  W  N  P  H  Q  D  L  Q  A  Q  D  R  A  H  R  I  1200
3481   TTATCAACTAGAGCGGGTGGTTTGGGTTTAAATTTACAAACTGCAGACACTGTCATTATTTTTGATACTGACTGGAATCCTCATCAAGATTTGCAAGCTCAGGATAGAGCGCATAGAATA
        G  Q  K  N  E  V  R  I  L  R  L  I  T  T  N  S  V  E  E  V  I  L  E  R  A  Y  K  K  L  D  I  D  G  K  V  I  Q  A  G  K  1240
3601   GGTCAGAAGAATGAAGTTAGAATTCTAAGATTGATTACTACGAATTCAGTTGAAGAAGTTATCCTAGAGAGAGCCTACAAGAAGCTAGATATCGATGGTAAAGTCATTCAAGCAGGTAAA
        F  D  N  K  S  T  S  E  E  Q  E  A  L  L  R  S  L  L  D  A  E  E  E  R  R  K  K  R  E  S  G  V  E  E  E  E  E  L  K  D  1280
3721   TTCGATAACAAATCTACATCGGAGGAACAAGAAGCGTTGCTTAGATCTTTGCTGGATGCAGAAGAGGAACGTAGAAAGAAAAGAGAGTCTGGTGTTGAAGAAGAAGAGGAGTTAAAAGAC
        S  E  I  N  E  I  L  A  R  N  D  E  E  M  A  V  L  T  R  M  D  E  D  R  S  K  K  E  E  E  L  G  V  K  S  R  L  L  E  K  1320
3841   TCAGAAATCAACGAAATTCTTGCTCGAAATGACGAAGAAATGGCTGTTCTTACAAGGATGGATGAAGATAGATCAAAGAAGGAGGAAGAATTGGGAGTAAAGAGCCGTCTCTTAGAAAAG
        S  E  L  P  D  I  Y  S  R  D  I  G  A  E  L  K  R  E  E  S  E  S  A  A  V  Y  N  G  R  G  A  R  E  R  K  T  A  T  Y  N  1360
3961   AGTGAATTACCTGATATTTACAGTAGAGATATTGGTGCCGAGCTCAAACGTGAAGAATCAGAATCTGCTGCTGTTTACAATGGAAGGGGTGCAAGAGAACGTAAGACGGCAACTTACAAC
        D  N  M  S  E  E  Q  W  L  R  Q  F  E  V  S  D  D  E  K  N  D  K  Q  A  R  K  Q  R  T  K  K  E  D  K  S  E  A  I  D  G  1400
4081   GATAATATGTCTGAAGAACAGTGGTTAAGACAATTTGAAGTTAGCGATGATGAAAAAAATGACAAGCAGGCAAGAAAACAACGAACAAAGAAGGAAGATAAGAGCGAGGCGATTGATGGG
        N  G  E  I  K  G  E  N  I  D  A  D  N  D  G  P  R  I  N  N  I  S  A  E  D  R  A  D  T  D  L  A  M  N  D  D  D  F  L  S  1440
4201   AATGGAGAAATTAAAGGCGAAAACATTGACGCTGATAATGACGGTCCTAGAATTAATAATATATCGGCCGAAGATCGGGCAGATACGGATTTGGCAATGAATGACGATGATTTCTTGTCT
        K  K  R  K  A  G  R  P  R  G  R  P  K  K  V  K  L  E  G  S  E  N  S  E  P  P  A  L  E  S  S  P  V  T  G  D  N  S  P  S  1480
4321   AAAAAGAGAAAGGCAGGAAGGCCCCGTGGAAGACCTAAGAAAGTGAAACTTGAAGGATCTGAAAATTCTGAACCACCAGCGCTTGAATCAAGTCCAGTTACTGGCGATAACTCTCCCAGT
        E  D  F  M  D  I  P  K  P  R  T  A  G  K  T  S  V  K  S  A  R  T  S  T  R  G  R  G  R  G  R  G  R  G  R  G  R  G  R  G  1520
4441   GAGGATTTTATGGACATTCCAAAACCACGTACAGCGGGAAAAACATCTGTTAAATCAGCAAGAACTTCAACAAGGGGCCGTGGTCGCGGTCGTGGTCGTGGTCGAGGGCGAGGACGTGGA
        R  G  R  P  P  K  A  R  N  G  L  D  Y  V  R  T  P  A  A  A  T  S  P  I  D  I  R  E  K  V  A  K  Q  A  L  D  L  Y  H  F  1560
4561   CGAGGAAGGCCGCCAAAAGCAAGAAATGGGCTTGATTATGTCCGCACGCCCGCTGCGGCAACGAGTCCTATTGATATTAGAGAGAAAGTTGCTAAACAAGCTCTAGACTTATACCACTTT
        A  L  N  Y  E  N  E  A  G  R  K  L  S  D  I  F  L  S  K  P  S  K  A  L  Y  P  D  Y  Y  M  I  I  K  Y  P  V  A  F  D  N  1600
4681   GCTCTCAATTATGAAAATGAAGCTGGAAGGAAACTTTCTGACATTTTCCTCTCCAAACCTTCCAAAGCACTATATCCCGACTACTATATGATTATTAAGTACCCTGTCGCCTTTGATAAT
        I  N  T  H  I  E  T  L  A  Y  N  S  L  K  E  T  L  Q  D  F  H  L  I  F  S  N  A  R  I  Y  N  T  E  G  S  V  V  Y  E  D  1640
4801   ATTAACACGCATATTGAAACGCTAGCTTACAACTCCCTTAAAGAGACCCTTCAAGACTTTCATCTGATATTTTCCAATGCTAGGATATACAATACTGAGGGTTCAGTAGTATACGAAGAC
        S  L  E  L  E  K  V  V  T  K  K  Y  C  E  I  M  G  D  N  S  Q  L  D  F  T  E  F  D  E  Q  Y  G  T  R  P  L  V  L  P  P  1680
4921   TCTTTAGAACTGGAAAAAGTGGTTACGAAGAAATACTGTGAGATAATGGGCGATAACTCACAACTCGACTTTACTGAATTTGATGAACAGTATGGCACCAGACCCCTCGTCTTGCCTCCT
        V  V  T  S  S  V  A  E  S  F  T  D  E  A  D  S  S  M  T  E  A  S  V ***                                                   1703
5041   GTTGTTACTTCAAGCGTGGCTGAATCTTTCACAGATGAAGCGGACTCGAGCATGACAGAAGCGAGTGTATAGTACCTTCCTAAAGCAATATAAGTACTTATTCGTTTATACGTAGACAAA
5161   CATTAATCCCTCTTTTTTTTATTGTACCCATTAACCGAGTTGGGATGTTTAATATCTCTAGTATAACGTGCCGTATATTTCCTATATATATATATATATATATTTATATATATATTAGATTT
5281   TAAGTTAATTGTTCAACTAATTGATCCTT
```

FIG. 3. Nucleotide sequence of *SNF2* and predicted amino acid sequence. Nucleotides are numbered on the left, and amino acid residues are numbered on the right. The amino acid sequence is shown in standard single-letter code. The product could be encoded by the 5.7-kb SNF2 RNA (2), and the fusions to *lacZ* at codons 1333 and 1554 confirm the reading frame.

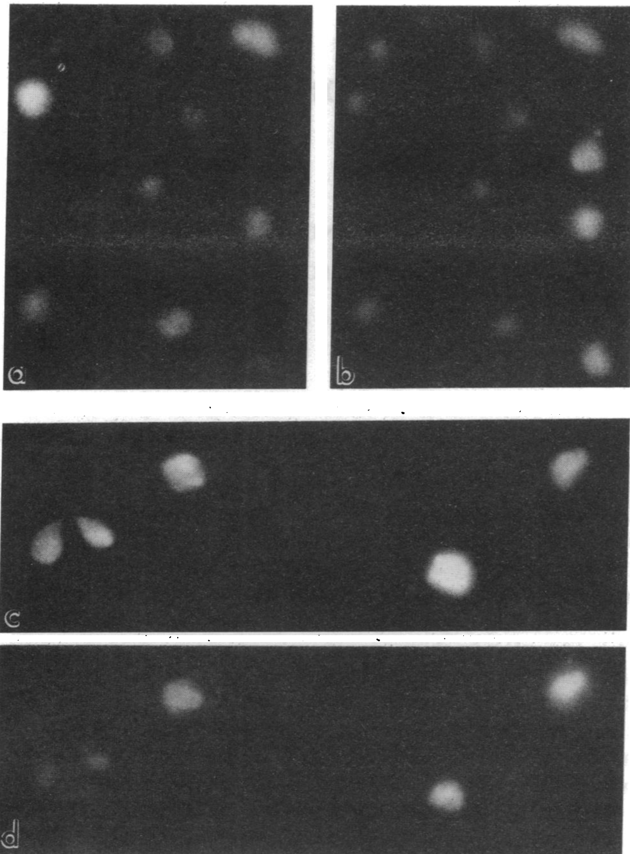

FIG. 4. Nuclear localization of SNF2-β-galactosidase fusion proteins. Glucose-grown cells carrying pSNF2(1554)-lacZ (*a* and *b*) and galactose-grown cells carrying pSNF2(1423-1554)-lacZ (*c* and *d*) were examined by immunofluorescence microscopy as described (4). Cells were stained with mouse monoclonal anti-β-galactosidase antibody (Promega Biotec) and fluorescein isothiocyanate-conjugated $F(ab')_2$ fragment of sheep antibody to mouse immunoglobulin G (Sigma) (*a* and *c*). Nuclei were identified by staining with 4',6-diamidino-2-phenylindole (*b* and *d*). Similar staining patterns were observed for glucose-repressed and -derepressed cells of both *snf2* mutant and wild-type strains carrying pSNF2(1554)-lacZ. Cells appeared intact by phase-contrast microscopy.

and SNF5 to the *lexA* operator. One or both proteins may provide an activation domain.

Although SNF2 and SNF5 behave similarly in the transcriptional activation assay, genetic evidence suggests that they do not perform redundant functions. Increased dosage of one does not substitute for loss of the other (2). In addition, strains with null mutations in both *SNF2* and *SNF5* (*snf2-Δ2::URA3 snf5-Δ2*) are viable and show the same slow growth as the single mutants (B.C.L., unpublished data).

The role of SNF6 in activation by SNF2 and SNF5 is less clear. Previously, *SNF6* was shown to encode a nuclear protein (3). The finding that an *spt6* mutation suppresses the defect in LexA-SNF activation caused by *snf2* and *snf5*, but not *snf6*, suggests that the function of the SNF6 protein is distinct from those of SNF2 and SNF5. It nonetheless remains possible that SNF6 forms a complex with SNF2 and SNF5. Alternatively, SNF6 could affect SNF2/SNF5 function by some other mechanism; for example, the SNF6 protein may modify SNF2 or SNF5 (or another protein in the complex). Some mechanisms have been excluded: SNF6 is not required either for DNA binding in this assay or for nuclear localization of SNF2- or SNF5-β-galactosidase proteins (ref. 4; B.C.L., unpublished data).

The identity of the protein(s) that normally mediate the association of SNF2 and SNF5 with DNA remains unknown. Conceivably, one or both of these SNF proteins could be involved in DNA binding as well as transcriptional activation, but there is as yet no evidence for any DNA binding activity (ref. 4; B.C.L., unpublished data). Another possibility is that these SNF proteins interact with sequence-specific DNA-binding factors that recognize the various genes that are affected by SNF2, SNF5, and SNF6. Precedents for such a mechanism are provided by the herpesvirus VP16 and adenovirus E1a proteins, both potent transcriptional activators that interact with cellular DNA-binding proteins (31–33). It is also possible that the SNF proteins function coordinately with various gene-specific activators to stimulate transcription. Such a mechanism would be consistent with the role of SNF2, SNF5, and SNF6 in transcription of a broad spectrum of differently regulated genes.

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