MOLECULAR AND CELLULAR BIOLOGY, Aug. 1995, p. 4240–4248 Vol. 15, No. 8
0270-7306/95/$04.00+0
Copyright 1995, American Society for Microbiology

SNF11, a New Component of the Yeast SNF-SWI Complex That Interacts with a Conserved Region of SNF2

ISABELLE TREICH,[1] BRADLEY R. CAIRNS,[2] TERESA DE LOS SANTOS,[3] ERIC BREWSTER,[3] AND MARIAN CARLSON[1,3,4]*

Institute of Cancer Research[1] and Departments of Microbiology[3] and Genetics and Development,[4] College of Physicians and Surgeons, Columbia University, New York, New York 10032, and Department of Cell Biology, Stanford University School of Medicine, Stanford, California 94305[2]

Received 16 March 1995/Returned for modification 25 April 1995/Accepted 9 May 1995

The yeast SNF-SWI complex is required for transcriptional activation of diverse genes and has been shown to alter chromatin structure. The complex has at least 10 components, including SNF2/SWI2, SNF5, SNF6, SWI1/ADR6, and SWI3, and has been widely conserved in eukaryotes. Here we report the characterization of a new component. We identified proteins that interact in the two-hybrid system with the N-terminal region of SNF2, preceding the ATPase domain. In addition to SWI3, we recovered a new 19-kDa protein, designated SNF11. Like other SNF/SWI proteins, SNF11 functions as a transcriptional activator in genetic assays. SNF11 interacts with SNF2 in vitro and copurifies with the SNF-SWI complex from yeast cells. Using a specific antibody, we showed that SNF11 coimmunoprecipitates with members of the SNF-SWI complex and that SNF11 is tightly and stoichiometrically associated with the complex. Furthermore, SNF11 was detected in purified SNF-SWI complex by staining with Coomassie blue dye; its presence previously went unrecognized because it does not stain with silver. SNF11 interacts with a 40-residue sequence of SNF2 that is highly conserved, suggesting that SNF11 homologs exist in other organisms.

Transcriptional control involves a complex interplay between gene-specific activators, the general transcription apparatus, and chromatin. The SNF-SWI complex has an important role in this process in the yeast *Saccharomyces cerevisiae*. The SNF-SWI complex is required for transcriptional activation of an array of differently regulated genes (for a review, see reference 4) and has been shown to alter chromatin structure to facilitate binding of transcription factors (7, 15). Components of this complex include SNF2/SWI2, SNF5, SNF6, SWI1/ADR6, SWI3, and at least five additional polypeptides (3, 7, 35). This complex has been widely conserved in eukaryotes. Proteins homologous to several components have been identified in *Drosophila melanogaster* and mammals, and a similar complex has been purified from humans (20).

The yeast *SNF* and *SWI* genes were originally identified by mutations that affect *SUC2* expression (*snf*) and mating-type switching (*swi*) (30, 41). Subsequent genetic studies implicated these genes in transcription of diversely regulated promoters (1, 10, 13, 23, 32, 36). A role in transcriptional activation was suggested by evidence that each of the SNF proteins, when artificially bound to DNA as a LexA fusion, activates transcription of a target gene (23, 24). Moreover, SNF and SWI proteins enhance transcriptional activation by various gene-specific activators expressed in *S. cerevisiae*, including GAL4, LexA-GAL4, LexA-Bicoid, *Drosophila* fushi tarazu, and rat glucocorticoid receptor (21, 36, 46).

Genetic evidence first suggested that SNF/SWI proteins affect transcriptional activation by relieving repressive effects of chromatin (for a review, see reference 45). Transcriptional defects of *snf/swi* mutants are suppressed by mutations in histone genes and genes encoding other chromatin-related proteins (15, 19, 31, 37, 42). In addition, in *snf2* and *snf5* mutants, the chromatin structure at the *SUC2* promoter is altered at sites near the TATA box (15). Recent biochemical studies have shown that the yeast SNF-SWI complex interacts with nucleosomal DNA and stimulates binding of GAL4 derivatives in an ATP-dependent manner (7). Similarly, the human SNF-SWI (hSNF-SWI) complex disrupts nucleosome structure in an ATP-dependent reaction to facilitate binding of GAL4-related activators and TATA-binding protein to sites within a nucleosome core (17, 20). These in vitro studies indicate that the SNF-SWI complex alters chromatin structure to facilitate binding of transcription factors.

To further our understanding of SNF-SWI complex function in vivo, we have taken a genetic approach. We reasoned that the identification of proteins that interact with the SNF-SWI complex in vivo would provide insight into the physiological roles of the complex. Such interacting proteins might include transcriptional activators, chromatin components, or perhaps general transcription factors. To identify proteins that interact with the complex, we used the two-hybrid system (11). In this system, interaction between a protein fused to a DNA-binding domain and a second protein fused to an activation domain is detected by the consequent activation of a target promoter with appropriate binding sites. The SNF2 protein was chosen to construct the initial DNA-binding partner, or bait, because it has been extensively characterized and has functional homologs in *D. melanogaster* and mammals.

SNF2 is a 194-kDa protein that contains a region with motifs similar to those of nucleic acid-stimulated ATPases and helicases (8, 25) (see Fig. 3). Mutations in these motifs impair SNF2 function, and bacterially expressed SNF2 protein has DNA-stimulated ATPase activity in vitro (22); moreover, a DNA-dependent ATPase activity copurifies with the SNF-SWI complex (3, 7). The ATPase-related domain of SNF2 is widely conserved among eukaryotes, and homologous proteins have been identified in yeasts, *D. melanogaster*, mice, and humans (for a review, see reference 4). Several of these proteins are

* Corresponding author. Mailing address: HSC922, 701 W. 168th St., New York, NY 10032. Phone: (212) 305-6314. Fax: (212) 305-1741.

TABLE 1. *S. cerevisiae* strains used

Strain[a]	Genotype
MCY829	*MAT*α *his3*Δ*200 lys2-801 ura3-52 SUC2*
MCY2372	*MAT***a** *his3*Δ*200 leu2-3,112 ura3-52*
MCY3507	*MAT*α *his3*Δ*200 pep4*::*URA3*
MCY1993	*MAT*α *snf2*Δ*2*::*URA3 ura3-52 ade2-101*
MCY3159	*MAT*α *snf11*Δ*2*::*HIS3 his3*Δ*200 leu2-3,112 lys2-801 ura3-52*
BY86[b]	*MAT***a** *HMR***a** *HML***a** *HO-lacZ46 ura3 his3 leu2 trp1 can1 ade2 ade6*? *met*
CTY10-5d[c]	*MAT***a** *ade2-101 his3*Δ*200 leu2-3,112 trp1-901 gal4 gal80 URA3*::*lexAop-lacZ*

[a] All MCY strains have the S288C genetic background.
[b] Gift of L. Breeden.
[c] Constructed by R. Sternglanz.

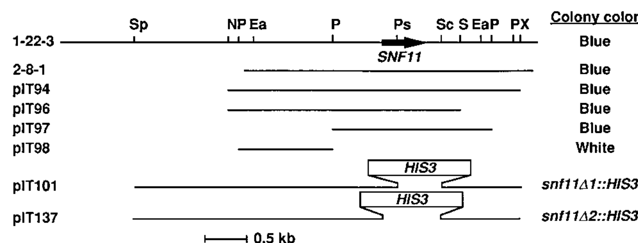

FIG. 1. Restriction map of *SNF11* clones. Only the yeast DNA segment of each plasmid is shown. Arrow shows the *SNF11* coding region. Plasmids 1-22-3 and 2-8-1 were recovered from the two-hybrid screen. Colony color in the two-hybrid system is indicated for each plasmid in combination with pLexA$_{87}$-SNF2$_{14-767}$; blue color results from β-galactosidase expression. Restriction sites: Ea, *Eag*I; N, *Nco*I; Ps, *Pst*I; P, *Pvu*II; Sc, *Sca*I; S, *Spe*I; Sp, *Sph*I; X, *Xba*I.

candidates for functional homologs of SNF2. The brahma (brm) protein of *D. melanogaster* and the human hbrm and BRG1 proteins are involved in transcriptional activation (18, 29, 43), and hybrid SNF2 proteins containing the ATPase domain of brm or BRG1 are functional in yeast cells (9, 18). Furthermore, an hSNF-SWI complex with a DNA-dependent ATPase activity was purified by using an antibody to BRG1 (20). These homologs also share with SNF2 a C-terminal bromodomain and short regions of similarity in the N terminus (43). The N-terminal half of SNF2, preceding the ATPase domain, is required for SNF2 function but does not activate transcription when artificially bound to DNA (22).

We used the two-hybrid system to identify proteins that interact with this N-terminal region of SNF2. We recovered one known member of the SNF-SWI complex, SWI3, and also a new 19-kDa protein, designated SNF11. We showed that SNF11 interacts with a small region of SNF2 that is conserved in the homologs brm, hbrm, and BRG1. Biochemical studies confirmed that SNF11 interacts with SNF2 in vitro. Finally, we showed that SNF11 is a tightly associated component of the SNF-SWI complex.

MATERIALS AND METHODS

Strains and genetic methods. Yeast strains are listed in Table 1. Synthetic complete (SC) medium (38) contained 2% glucose. Standard genetic methods were used. *Escherichia coli* strains were XL1-Blue (Stratagene) and BA1 (*thr leuB6 thi thyA trpC117 hisB* Str^r).

Two-hybrid screen and analysis of clones. *S. cerevisiae* CTY10-5d carrying an integrated *lexAop-GAL1-lacZ* reporter was sequentially transformed with pLexA$_{87}$-SNF2$_{14-767}$ (see below for definitions of the encoded proteins) and three pGAD libraries carrying the GAL4 activation domain (GAD; amino acids 768 to 881) fused to yeast genomic DNA fragments (6). β-Galactosidase activity in transformants was assayed by staining with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) on nitrocellulose filters. Out of 213,000 transformants, 207 turned blue within 24 h. After colony purification, 115 turned blue again. DNA was isolated and used to transform strain BA1 to select for the library plasmid carrying *LEU2*. Eighty-two plasmids were recovered and retested. Thirty-three plasmids caused blue color again in the presence of the LexA$_{87}$-SNF2$_{14-767}$ fusion but not in the presence of LexA$_{87}$ or alone. However, two of these plasmids gave a positive signal in combination with the unrelated LexA$_{87}$-lamin (2) and LexA$_{87}$-ADH1 (gift of R. Sternglanz) fusion proteins. β-Galactosidase activity observed with the 31 clones was not due to suppression of the amber mutation in pLexA$_{87}$-SNF2$_{14-767}$ because all were still positive with pLexA$_{87}$-SNF2$_{14-767\Delta}$ (see below). DNA blots of the 31 positive clones were probed with a 3.5-kb *Bgl*II-*Hpa*I *SWI3* fragment from BD7 (36) and with the 4.3-kb *Xba*I-*Sph*I fragment from 1-22-3 (Fig. 1). No hybridization was detected with probes specific to the *SWI1*, *SNF2*, *SNF5*, *SNF6*, *SPT4*, *SPT5*, *SPT6*, or histone genes. To check that SWI3 was the interacting protein from the GAD-SWI3 clone (3-28-2), the GAD coding sequence was interrupted by filling in the unique *Mlu*I site. Partial sequence analysis of the clones was performed on double-stranded DNA with an oligonucleotide which hybridized to GAD.

Sequence analysis of *SNF11*. Plasmid pIT91, a subclone of the 4.3-kb *Sph*I-*Xba*I fragment from 1-22-3 in M13mp19, was subjected to nested deletion from the *Xba*I site, using a kit from Pharmacia. One entire strand was sequenced by using several deletions and the dideoxy-chain termination method (39). The sequence between *Pvu*II and *Spe*I was confirmed on the other strand by using oligonucleotides. The amino acid sequence of SNF11 was compared with the sequences in GenBank (release 85), using the program TFASTA (34), and a search for motifs was done with the MOTIF program (University of Wisconsin Genetics Computer Group).

Plasmids. pLexA$_{87}$-SNF2$_{14-767}$ (previously called pLexA-SNF2$_{14-767}$ [22]) encodes the N-terminal 87 residues of LexA fused to residue 14 of SNF2; *SNF2* coding sequence is interrupted by an amber mutation at codon 768. pLexA$_{87}$-SNF2$_{14-767\Delta}$ contains the *Eco*RI fragment of pLexA$_{87}$-SNF2$_{14-767}$ cloned into pSH2-1 (12) and therefore lacks SNF2 sequence distal to codon 834. pLexA-SNF2$_{14-767\Delta}$ contains the same *Eco*RI fragment cloned into pEG202 (gift of E. Golemis and R. Brent). All other LexA fusion plasmids are also derivatives of pEG202 and express, from the *ADH1* promoter, LexA (202 residues) fused to the indicated sequences. pLexA-SNF2$_{1-250}$, $_{-1-289}$, $_{-1-616}$, $_{-239-767}$, $_{-277-767}$, $_{-577-767}$, and $_{-239-289}$ contain *Bam*HI-*Sal*I PCR fragments generated by amplification of pLN138-4 (1), using primers OL33 and OL49, OL33 and OL50, OL33 and OL51, OL53 and OL52, OL54 and OL52, OL55 and OL52, and OL53 and OL50 (Table 2), respectively. All plasmids expressed proteins of the expected sizes. To generate YEp24-SNF2ΔD1 and YEp24-SNF2Δ5, the *Kpn*I fragment of pLN138-4 was subcloned into M13mp18, subjected to site-directed mutagenesis with OL61 and OL62, respectively, using the Bio-Rad Muta-Gene kit, and reintroduced into pLN138-4. pLexA-SNF2$_{239-289\Delta5}$ contains a *Bam*HI-*Sal*I PCR fragment amplified from YEp24-SNF2Δ5 with OL53 and OL50. pLexA-hbrm$_{165-215}$ contains a *Bam*HI-*Sal*I PCR fragment generated with OL64 and OL65 on pCG-hbrm (29) cloned in pEG202. pIT94, pIT96, pIT97, and pIT98 are subclones in the *Sma*I site of pRS425 (14) (Fig. 1). pLexA-SNF11 contains the *Bam*HI-*Sal*I fragment generated by PCR amplification of 1-22-3 primed by OL43 and OL47 and encodes the entire SNF11 sequence. pGAD-SNF11 contains the same fragment in pACTII (27). To construct pGST-SNF11, the PCR fragment was cut by *Bam*HI-*Hin*dII and cloned into pGEX-3X (40). To construct pLexA-SWI3, we first cloned the *Bam*HI-*Hin*dIII PCR fragment generated by amplification of BD7 (36) by using OL45 and OL46 together with the 1.7-kb *Hin*dIII-*Hpa*I fragment of BD7 into pBluescript KS-phagemid (Stratagene). A *Bam*HI-*Xho*I fragment was then cloned into pEG202. The resulting plasmid complemented *swi3* for growth.

Disruption of the *SNF11* gene. pIT101 was made by replacing the *Pst*I-*Sca*I fragment from pIT91 with a *Pst*I-*Hin*dII *HIS3* fragment, thereby deleting the sequence C terminal to residue 52 (Fig. 1). To construct pIT137, we created a new *Pst*I site 74 bp 5′ to the initiator ATG by site-directed mutagenesis on pIT101 single-stranded DNA with OL56; the *Pst*I fragment was then deleted. pIT137 and pIT101 were cleaved by *Eag*I and used to transform the diploid MCY829 × MCY2372. Southern blot analysis confirmed the disruptions.

Construction of the *SNF2ΔD1* allele. The 5.6-kb *Afl*III-*Xho*I *SNF2* fragment was released from YEP24-SNF2ΔD1 and used to cotransform MCY1993 (*snf2Δ2*::*URA3*) with an *ADE2* plasmid. Ade$^+$ transformants were plated on 5-fluoro-orotic acid, and Ura$^-$ strains were recovered. The presence of the *SNF2ΔD1* allele was confirmed by Southern blotting.

β-Galactosidase assays. β-Galactosidase activity was assayed either on plates or in liquid as described previously (26). For quantitative assays, transformants were grown to mid-log phase in SC-His-Leu medium to select for plasmids. β-Galactosidase activity is expressed in Miller units (28).

Purification of GST fusion proteins and binding assay. Overnight cultures of *E. coli* XL1-Blue carrying pGEX derivatives were diluted 1:50 in 100 ml of LB medium and grown to an optical density at 600 nm of 0.5. Isopropylthiogalactopyranoside (IPTG) was added to 1 mM, and cultures were incubated for an additional 2 h. Cells were pelleted, resuspended in 4 ml of MTPBS (150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4 [pH 7.3], 1 mM phenylmethylsulfonyl fluoride, 1 μg each of aprotinin, leupeptin, and pepstatin per ml) and lysed by sonication. Fusion proteins were purified on glutathione-agarose beads as described (16). Cultures of MCY3507 carrying LexA fusion plasmids were grown to

TABLE 2. Oligonucleotides used[a]

Name	Sequence
OL33	5′-CCCCGGATCCTTATGAACATACCACAGCG-3′
OL43	5′-CCCCGGATCCTTATGAGCAGTGAAATTGCC-3′
OL45	5′-GGGGGGATCCTTATGGAGAATACACTGGG-3′
OL46	5′-AAGTACTCTCCGAATCAC-3′
OL47	5′-CCCCGTCGACCTACCATATCTAGAACAC-3′
OL49	5′-CCCCGTCGACCTAGGCAGTAAACATTGTCATGG-3′
OL50	5′-CCCCGTCGACCTAATCTGGAGGATGGTTAATAG-3′
OL51	5′-CCCCGTCGACCTAATATTGATTGATGGCGG-3′
OL52	5′-CCCCGTCGACCTATAAGGTACCACCAACC-3′
OL53	5′-CCCCGGATCCAGGGGAGAAGATTACCC-3′
OL54	5′-CCCCGGATCCAGGCTGTTATCCAAAAATC-3′
OL55	5′-CCCCGGATCCAAGATGCATTGCTAAC-3′
OL56	5′-CCCGATCGTGCTGCAGTCTGAG-3′
OL61	5′-CCCATGACAATGTTTACTGGCGAGCCAGATTTCAAAAGAATGTTACTG-3′
OL62	5′-GCCGAGCAATCCGAACTGACATCTCTAAAATGTCTAG-3′
OL64	5′-CCCCGGATCCAGCCCAACAGAGGTCCC-3′
OL65	5′-CCCCGTCGACCTACAAGCCAGGCAACGTCC-3′

[a] Oligonucleotides were synthesized with an Applied Biosystems 394 synthesizer. Restriction sites are underlined.

mid-log phase in SC-His medium. Cells were collected, and crude lysates were prepared as described previously (5) except that the lysis buffer was 20 mM Tris-phosphate (pH 6.7)–5 mM EDTA–200 mM NaCl–2 mM phenylmethylsulfonyl fluoride. Protein extract (1 mg; measured by the Bio-Rad assay) diluted in 500 μl of MTPBS was added to the glutathione-agarose beads with bound GST-SNF11 or GST-SNF4 and rocked for 1 h at 4°C. Beads were washed four times in MTPBS with 1% Triton X-100 and once in MTPBS. Beads were resuspended in 40 μl of MTPBS without NaCl and 80 μl of 2× sample buffer. Samples were boiled for 5 min, and 100 μl of the supernatant was electrophoresed in a sodium dodecyl sulfate (SDS)–7.5% polyacrylamide gel.

Antibody to SNF11. GST-SNF11 protein was prepared from 1 liter of culture essentially as described above. After purification on glutathione-agarose beads, the fusion protein was eluted in 1 ml of 50 mM Tris-HCl (pH 8)–10 mM reduced glutathione. One half was purified on 10% acrylamide gel, and a slice containing GST-SNF11 (400 μg) was used to raise an antibody in rabbits at the Pocono Rabbit Farm & Laboratory.

Purification of the SNF-SWI complex. To purify the SNF-SWI complex, whole cell extracts and the first three chromatographic steps (Bio-Rex 70, DEAE-Sephacel, and hydroxylapatite) were performed as described previously (3). Peak fractions from hydroxylapatite were further resolved on DEAE-Sephacel. Adsorbed proteins were eluted with a linear gradient of 200 to 1,000 mM potassium acetate in buffer A (20 mM Tris-acetate [pH 7.6], 20% glycerol, 1 mM dithiothreitol, 1 mM EDTA, 2 μM chymostatin, 2 μM pepstatin A, 0.6 μM leupeptin, 2 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 0.01% Nonidet P-40 [NP-40]). The peak of the SNF-SWI complex eluted at 480 mM potassium acetate on DEAE-Sephacel. Peak fractions were pooled, dialyzed against buffer B (identical to buffer A except that 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.3] replaces Tris-acetate) containing 100 mM potassium acetate, applied to a TSK-heparin column, and eluted in buffer B with a linear gradient of 200 to 800 mM potassium acetate. The peak of the SNF-SWI complex eluted at 490 mM potassium acetate and was approximately 20% pure, as determined by electrophoretic separation and staining with Coomassie blue dye.

The SNF-SWI complex was also isolated in large scale from extracts of commercial Fleischmann's (Oakland, Calif.) yeast. The first three chromatographic steps were as described above, followed by further fractionation on Mono Q and an immunoaffinity column composed of anti-SNF6 antibodies conjugated to protein A-Sepharose. The SNF-SWI complex was eluted from this column with 5 M urea.

Immunoprecipitation and immunodepletion experiments. The SNF11 antiserum was coupled to protein A-Sepharose essentially as described previously for the SNF6 antiserum (3). Immune complexes were prepared with 20 μl (5 μg) of the peak TSK-heparin fraction. The sample was diluted with 30 μl of buffer A containing 100 mM potassium acetate (100), 25 μl of 50% protein A-Sepharose in buffer A (100) was added, and the sample was rotated at 4°C for 1 h. Samples were sedimented by centrifugation, and the supernatant was charged with 25 μl of 50% protein A-Sepharose in buffer A (100) coupled to either anti-SNF6 or anti-SNF11. Samples were rotated 3 h at 4°C and sedimented by centrifugation. Pellets were washed twice with 1 ml of buffer A containing 200 mM potassium acetate (200) at 4°C and recovered by centrifugation, and all supernatant was removed. Pellets were then subjected to two elutions with 25 μl of buffer A containing 600 mM potassium acetate and 0.2% NP-40, washed twice with 1 ml of this elution buffer, recovered by centrifugation, and subjected to two elutions with 25 μl of 5 M urea.

Immunoblot analysis. Immunoblots were incubated with an anti-LexA antiserum (gift of R. Brent) diluted 1:10,000 or with an anti-SNF11, anti-SNF6 (3), or anti-SWI3 (36) serum diluted 1:500. LexA antibodies were detected with an enhanced chemiluminescence system (Amersham), and other primary antibodies were detected with alkaline phosphatase-conjugated antibody (Bio-Rad).

Nucleotide sequence accession number. The *SNF11* sequence was entered in GenBank by the chromosome IV sequencing project under accession number Z46796.

RESULTS

Identification of proteins that interact with SNF2 in the two-hybrid system. To identify proteins that interact with the N terminus of SNF2, we used the two-hybrid system (11). The DNA-binding partner contained SNF2 residues 14 to 767 fused to the LexA DNA-binding domain. A plasmid library of fusions between GAD and yeast genomic DNA was screened for proteins that interact with $LexA_{87}$–$SNF2_{14-767}$, thereby activating expression of a *lexAop-GAL1-lacZ* reporter. From 213,000 transformants, we recovered 31 plasmids that caused blue color in combination with $LexA_{87}$-$SNF2_{14-767}$ but not with control LexA fusions (see Materials and Methods). Using probes specific for various candidates, including *SNF/SWI*, *SPT*, and histone genes, we identified one clone that hybridized to *SWI3*. Sequence analysis indicated that GAD was fused to residue 245 of SWI3. In combination with $LexA_{87}$-$SNF2_{14-767}$, GAD-$SWI3_{245-825}$ increased β-galactosidase expression 10-fold (Table 3). Sequence analysis of the remaining clones indicated

TABLE 3. Interaction of $SNF2_{14-767}$ with SNF11 and $SWI3_{245-825}$ in the two-hybrid system[a]

DNA-binding protein	Activation protein	β-Galactosidase activity (U)
$LexA_{87}$-$SNF2_{14-767}$	GAD	0.4
$LexA_{87}$	SNF11	0.4
$LexA_{87}$-$SNF2_{14-767}$	SNF11	8
$LexA_{87}$	GAD-SNF11	0.4
$LexA_{87}$-$SNF2_{14-767}$	GAD-SNF11	250
$LexA_{87}$	GAD-$SWI3_{245-825}$	0.4
$LexA_{87}$-$SNF2_{14-767}$	GAD-$SWI3_{245-825}$	4

[a] Proteins were expressed from pLexA$_{87}$-SNF2$_{14-767\Delta}$, pSH2-1 (LexA$_{87}$) (12), pGAD3 (6), 3-28-2, 1-22-3, and pGAD-SNF11. Transformants of CTY10-5d were grown to mid-log phase in SC-His-Leu to select for the plasmids. Values are averages for four independent transformants. Values of <1 corresponded to white colony color. Standard errors were <14%.

```
-608                                          cagctgccgtggccaactgaagtaatctttagccgtttatcgccgcgc
-560 aaaacggcaattgcacttgagaccccaacggcgctgtgcagcagggtattctactacgtgttagcttgccttgcatttcc
-480 ccatgcgtctcgaataggaattattcaagatggattattggcatttacgagtaaccaaggataaccccgctgtgcggtga
-400 aaccaccctcttttcacgtttcttcaaggccagtgcaaacgcgaataaacatatctacgctatatatagatatgacgttt
-320 ctcaagccaacagaagtagataaagcagccaggagggtagagagtgttcaaattatagcaagccttcttctacctgtttt
-240 ttttgatgattgttttgccgggtaacaatcgactttcgggcaaatttttttcctttttctcctaacagtatatacgg
-160 agtggagaacagacttcccataaaagcatattacgtggggtcgtagtaagattgccgtttatgataccctctattcaggg
 -80 ctcagagcgcatcacgatcgggagtgtaaattcaatgtgcatataagcaaaacacacagatttcctttttcccagaaaa
   1 ATG AGC AGT GAA ATT GCC TAC TCG AAT ACG AAC ACC AAC ACT GAA AAC GAG AAC CGC AAT
   1 M   S   S   E   I   A   Y   S   N   T   N   T   N   T   E   N   E   N   R   N

  61 ACT GGC GCT GGC GTA GAT GTA AAT ACA AAT GCA AAT GCA AAT GCA AAT GCA ACT GCA AAT
  21 T   G   A   G   V   D   V   N   T   N   A   N   A   N   A   N   A   T   A   N

 121 GCA ACT GCA AAT GCA ACT GCA AAT GCA ACT GCA GAG CTG AAC CTC CCC ACG GTC GAT GAG
  41 A   T   A   N   A   T   A   N   A   T   A   E   L   N   L   P   T   V   D   E

 181 CAA AGA CAG TAT AAG GTA CAA CTG CTA TTG CAT ATC AAC AGC ATA TTA CTT GCT AGA GTT
  61 Q   R   Q   Y   K   V   Q   L   L   L   H   I   N   S   I   L   L   A   R   V

 241 ATT CAG ATG AAT AAT AGT TTA CAA AAC AAT CTA CAG AAC AAT ATA AAT AAT AGC AAT AAC
  81 I   Q   M   N   N   S   L   Q   N   N   L   Q   N   N   I   N   N   S   N   N

 301 AAT AAC ATC ATC AGG ATA CAG CAA CTT ATA TCT CAG TTC CTT AAA AGG GTT CAT GCC AAT
 101 N   N   I   I   R   I   Q   Q   L   I   S   Q   F   L   K   R   V   H   A   N

 361 CTT CAA TGC ATA TCT CAG ATA AAC CAA GGA GTG CCC TCA GCG AAA CCA CTG ATC CTC ACG
 121 L   Q   C   I   S   Q   I   N   Q   G   V   P   S   A   K   P   L   I   L   T

 421 CCT CCT CAG CTA GCC AAC CAG CAG CAA CCT CCA CAG GAT ATT CTT TCT AAA CTC TAT CTT
 141 P   P   Q   L   A   N   Q   Q   Q   P   P   Q   D   I   L   S   K   L   Y   L

 481 CTC TTG GCA AGA GTG TTC GAG ATA TGG TAG aataactttgtttctcttccctttttatttgtttttt
 161 L   L   A   R   V   F   E   I   W   *
 551 tttttacttttgcaattttaattttgcattcagctacaggtgtgattgcatacccgcagagcaagtacaggaagcataa
 631 gaaaaatagaatacgaaaagcagaaaaagaaaggcaaataacaatcactcaagtactacaactgcatgaatttatcgtat
 711 gtaatgtacccaacgtaaataataatattagtattttaaaatctatgacatcagatgtagttatcttactgccagcccta
 791 ttaatactactttcttattatcttcttcgagagccaccttcgtcatcgtcgcagtaagcattgtcatcttcatcatgcac
 871 gtcggtaacggaagcctaatctcaatatctactagt
```

FIG. 2. Nucleotide sequence of *SNF11* gene and deduced amino acid sequence. The six NA(T/N)A motifs and the five-residue direct repeat are underlined and boxed, respectively. *Pst*I and *Sca*I restriction sites used to create the *snf11Δ1*::*HIS3* allele are at positions 149 and 682, respectively. A putative TATA box is underlined.

that none contained an in-frame fusion to GAD. However, a clone encoding a protein that both interacts with SNF2 and contains a transcriptional activation domain would give a positive signal in this assay, so we proceeded with further characterization.

We determined the restriction maps of two clones causing strong blue color (Fig. 1). These two plasmids overlapped and cross-hybridized to 10 additional clones. The interacting gene was localized to a 1.5-kb region (between the *Pvu*II and *Spe*I sites; Fig. 1), which was present in all 12 plasmids. We mapped the gene on the right arm of chromosome IV, between *REG1* and *RAD55*, by probing lambda clone grid filters (gift of L. Riles; data not shown). Sequence analysis identified a single open reading frame of 169 codons encoding a 18,655-Da protein (Fig. 2), which is not homologous to any other sequence in the GenBank database (release 85). The noteworthy features are the high content of Asn plus Gln (28%), the motif NA(T/N)A repeated six times, and a five-amino acid direct repeat.

The gene seemed likely to encode a transcriptional activator because it was recovered unfused to GAD in our two-hybrid screen. To test this idea, LexA was fused to the coding sequence. The LexA fusion protein activated transcription of *lexAop-GAL1-lacZ* target genes as effectively as LexA-SNF2 and LexA-SNF5; moreover, in *snf2*, *snf5*, and *snf6* mutant strains, activation of a target gene with one *lexA* operator was reduced 45-, 9-, and 6-fold, respectively (data not shown).

This gene was designated *SNF11* because subsequent studies revealed that it encodes a new component of the SNF-SWI complex.

Specific interaction of SNF2 and SNF11 in the two-hybrid system. In the two-hybrid system, $LexA_{87}$-$SNF2_{14-767}$ and SNF11, expressed from its own promoter, stimulated β-galactosidase expression 20-fold (Table 3). To confirm that this signal reflects interaction between the two proteins, we expressed a GAD-SNF11 fusion. Together, $LexA_{87}$-$SNF2_{14-767}$ and GAD-SNF11 activated β-galactosidase expression over 500-fold (Table 3). GAD-SNF11 also interacted with the entire SNF2 sequence. In combination with LexA-SNF2K798R, which has a mutation in the ATP-binding site that impairs function, GAD-SNF11 increased activation 55-fold; with wild-type LexA-SNF2, a 4-fold increase could be detected (Table 4). In control experiments, GAD-SNF11 did not stimulate activation by LexA-GAL4 or LexA-Bicoid (Table 4), and $LexA_{87}$-$SNF2_{14-767}$ did not activate target gene expression

TABLE 4. Interactions of GAD-SNF11 in the two-hybrid system[a]

DNA-binding protein	β-Galactosidase activities for activation protein:		Fold increase in activation
	GAD	GAD-SNF11	
$LexA_{87}$	<0.1	<0.1	
LexA-SNF2	38	164	4.3
LexA-SNF2K798R	2.7	148	55
LexA-SWI3	4.6	20	4.3
LexA-SNF5	43	86	2
LexA-SNF6	505	670	1.3
LexA-Bicoid	108	84	0.8
LexA-GAL4	1,090	920	0.8

[a] Proteins are fused to $LexA_{87}$, except for LexA-SWI3. Proteins were expressed from pSH2-1, pLexA-SNF2 (24), pLexA-SNF2K798R (22), pLexA-SWI3, pLexA-SNF5 (23), pLexA-SNF6 (21), pSH11-1 (LexA-Bicoid) (12), pSH17-4 (LexA-GAL4) (11a), pACTII (GAD) (27), and pGAD-SNF11. Transformants of CTY10-5d were grown to mid-log phase in SC-His-Leu medium. Values are average β-galactosidase units for four transformants. Standard errors were <7%.

A

1 Q D1 D2 Helicase-related RG Br 1703

B

LexA fusion	GAD β-Gal	GAD-SNF11 β-Gal	GAD-SNF11 Fold	GAD-SWI3 β-Gal	GAD-SWI3 Fold
SNF2$_{14\text{-}767\Delta}$	14	290	20X	53	4X
SNF2$_{1\text{-}250}$	11	27	2X	18	2X
SNF2$_{1\text{-}289}$	82	870	11X	95	1X
SNF2$_{1\text{-}616}$	29	440	15X	110	4X
SNF2$_{239\text{-}767}$	7	180	26X	39	6X
SNF2$_{277\text{-}767}$	0.4	0.4	1X	6	15X
SNF2$_{577\text{-}767}$	0.1	0.2	2X	0.1	1X
SNF2$_{239\text{-}289}$	0.3	460	1520X	0.5	2X
SNF2$_{239\text{-}289\Delta5}$	0.2	0.2	1X	ND	

C

LexA fusion	GAD β-Gal	GAD-SNF11 β-Gal	GAD-SNF11 Fold	GAD-SWI3 β-Gal	GAD-SWI3 Fold
hbrm$_{165\text{-}215}$	3.7	690	190X	ND	

FIG. 3. (A) Schematic representation of SNF2. Q, Gln-Gln-Ala repeat; D1 and D2, two domains conserved in SNF2 and brm; Helicase-related, region with 7 motifs that are conserved in nucleic acid-dependent ATPases and helicases; RG, Arg-Gly repeat; Br, bromodomain (24, 43). (B) Interaction of N-terminal subdomains of SNF2 with SNF11 and SWI3 in the two-hybrid system. LexA fusions to the indicated SNF2 residues are described in the text. GAD, GAD-SNF11, and GAD-SWI3$_{245\text{-}825}$ hybrids were expressed from pACTII, pGAD-SNF11, and 3-28-2. Values for β-galactosidase (β-Gal) activity are averages for at least four transformants of CTY10-5d. Standard errors were <15%. Fold indicates the fold increase in activation when each LexA-SNF2 protein was expressed in combination with GAD-SNF11 or GAD-SWI3 compared with GAD. Values for LexA-SNF2 hybrids alone were within twofold of those obtained in combination with GAD. ND, not determined. (C) Interaction of hbrm domain 1 with SNF11. The experiment was performed as described for panel B. All LexA fusion were expressed at comparable levels, as judged by Western blotting, except that LexA-SNF2$_{1\text{-}289}$, -SNF2$_{239\text{-}289}$, and -hbrm$_{165\text{-}215}$ were much less abundant when coexpressed with GAD-SNF11 than when coexpressed with GAD.

when GAL4 was overexpressed (data not shown). Thus, by the criteria of the two-hybrid system, SNF11 interacts specifically with the SNF2 protein.

SNF11 interacts with a small domain conserved in a subset of SNF2 homologs. The N-terminal part of SNF2 contains three noteworthy sequences: a 29-residue Gln-Gln-Ala repeat and two regions, designated domains 1 and 2 (43), that are conserved between SNF2 and the *Drosophila* brm protein (Fig. 3A). To assess the roles of these regions in interaction with SNF11, we constructed LexA fusions containing partial SNF2 sequences. Although some of these fusion proteins activated transcription, all proteins containing domain 1 activated at least 10-fold better in combination with GAD-SNF11 (Fig. 3B). Moreover, a fusion protein containing only domain 1 (LexA-SNF2$_{239\text{-}289}$) showed very strong interaction with GAD-SNF11; β-galactosidase expression was stimulated 1,500-fold. These results indicate that SNF11 interacts with domain 1 of SNF2.

We found that domain 1 is conserved in two human SNF2 homologs, BRG1 and hbrm, that have also been implicated in transcriptional activation (18, 29). Comparison with the human sequences identified a block of five highly conserved amino acids (Fig. 4). Deletion of these five residues from LexA-SNF2$_{239\text{-}289\Delta5}$ abolished interaction with GAD-SNF11 (Fig. 3B). Moreover, a LexA fusion containing the hbrm domain 1 (26% identical to SNF2) showed strong interaction with GAD-SNF11 (Fig. 3C). Thus, the yeast SNF11 protein interacts specifically with a small domain that is conserved in *Drosophila* and human SNF2 homologs. This interaction could be direct or through an intermediary protein.

We noted that coexpression of LexA-SNF2$_{1\text{-}289}$, LexA-SNF2$_{239\text{-}289}$, or LexA-hbrm$_{165\text{-}215}$ together with GAD-SNF11 (but not GAD) impaired growth of yeast cells. Moreover, these LexA fusions were much less abundant when coexpressed with GAD-SNF11 than when coexpressed with GAD, presumably because expression is selected against during growth of the cultures. In contrast, the deleted LexA-SNF2$_{239\text{-}289\Delta5}$ had no deleterious effect. These phenotypes support the conclusion that SNF11 and domain 1 interact in vivo.

Relationship of SNF11 and SWI3. To determine whether SNF11 interacts with SNF2 protein present in the SNF-SWI complex, we sought evidence for interaction of SNF11 with other proteins of the complex. SWI3 was a likely candidate because GAD-SWI3$_{245\text{-}825}$ also interacted with the N terminus of SNF2 in our two-hybrid screen. A complete LexA-SWI3 fusion, which was a weak transcriptional activator, interacted with GAD-SNF11 in the two-hybrid system (Table 4). We also tested for interaction with LexA-SNF5 and LexA-SNF6, but the assay was not conclusive (Table 4).

To localize the region of SNF2 that interacts with SWI3, we tested the series of LexA-SNF2 partial fusions (Fig. 3). GAD-SWI3$_{245\text{-}825}$ stimulated activation by LexA-SNF2$_{277\text{-}767}$ 15-fold and also interacted with other fusions containing residues 277 to 616. Because GAD-SWI3$_{245\text{-}825}$ did not strongly stimulate activation, the region could not be mapped precisely; however, domain 1 was clearly excluded. Thus, SWI3 and SNF11 interact with distinct regions in the N terminus of SNF2.

SNF11 interacts with SNF2 in vitro. To obtain biochemical evidence for interaction of SNF11 and SNF2, we tested a bacterially produced GST-SNF11 fusion protein for binding to LexA$_{87}$-SNF2$_{14\text{-}767}$ in vitro. GST-SNF11 was bound to glutathione-agarose beads and incubated with protein extract from yeast cells expressing LexA$_{87}$-SNF2$_{14\text{-}767}$. After washing, the proteins bound to the beads were analyzed by Western blotting using an anti-LexA antibody. LexA$_{87}$-SNF2$_{14\text{-}767}$ was detected bound to GST-SNF11 (Fig. 5, lane 4) but not to the unrelated protein GST-SNF4 (lane 7). In control experiments, a LexA$_{87}$ fusion to the Bicoid protein (12) did not bind GST-SNF11 or GST-SNF4. Thus, GST-SNF11 binds specifically to LexA$_{87}$-

```
SNF2  239 QGRRLPMTMFTAEQSELLKAQITSLKCLVNRKPIPFEFQAVIQKSINHPPD 289
brm   165 MRATSKHQHLNGNQVNLLRTQITAYRLLARNKPISMQMQQALQAAQQQPPP 215
BRG1  162 GQQNRGPTPFNQNQLHQLRAQIMAYKMLARGQPLPDHLQMAVQGKRPMPGM 212
hbrm  165 SQPNRGPSPFSPVQLHQLRAQILAYKMLARGQPLPETLQLAVQGKRTLPGL 215
                       *  ** **    * **  ***   * ***      *
```

ΔD1

Δ5

FIG. 4. Conservation of domain 1 in *Drosophila* and human homologs of SNF2. Numbers indicate residue positions. Residues identical in at least three of the four sequences are shown in reverse contrast. Residues conserved in the four proteins are marked by asterisks (conservative substitutions are K-R, A-V-I-L). Domain 1 as originally defined by Tamkun et al. (43) includes residues 253 to 277, but the conserved sequence extends farther. Deletions ΔD1 and Δ5 are marked. References: SNF2 (24), brm (43), BRG1 (18), and hbrm (29).

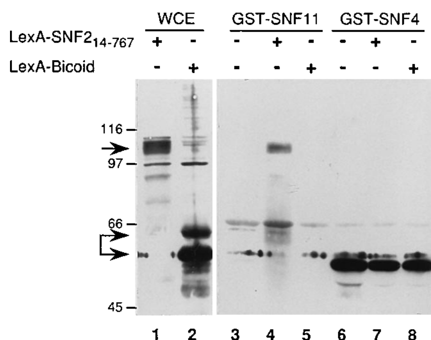

FIG. 5. GST-SNF11 binds to LexA-SNF2_{14-767}. Whole cell extracts (WCE) were prepared from MCY3507 expressing $LexA_{87}$-SNF2_{14-767} or $LexA_{87}$-Bicoid (pSH11-1) (12) as indicated. Total proteins (10 μg) are shown in lanes 1 and 2. Protein extracts (600 μg) were added to glutathione-agarose beads with bound GST-SNF11 (lanes 4, 5) or GST-SNF4 (gift of R. Jiang) (lanes 7 and 8). No yeast protein extract was added to the beads in lanes 3 and 6. Binding assays were carried out as described in Materials and Methods. Proteins were detected by Western blotting using anti-LexA and the chemiluminescence method. Arrows indicate LexA fusion proteins. The prominent band at 55 kDa in lanes 6 to 8 is an *E. coli* protein.

SNF2_{14-767} in vitro. These biochemical data support genetic evidence for interaction of SNF11 and SNF2 in vivo.

SNF11 copurifies and coimmunoprecipitates with the SNF-SWI complex. To assess the possibility that SNF11 interacts with, or is a component of, the SNF-SWI complex, we prepared an antibody to GST-SNF11. This antibody detected a polypeptide with an apparent molecular mass of 23 kDa in extracts from cells expressing SNF11 from the multicopy plasmid 1-22-3 but not in extracts from a *snf11*Δ mutant (data not shown). Immunoblot analysis of a partially purified fraction of the SNF-SWI complex revealed a polypeptide of identical molecular mass that cross-reacted with the SNF11 antibody. This polypeptide copurified with SNF6 and SWI3 in several column chromatography experiments (Fig. 6).

We next tested whether SNF11 is tightly associated with the SNF-SWI complex. Previous studies showed that immune precipitates formed with SNF-SWI complex and anti-SNF6 antibodies on protein A-Sepharose beads can be washed extensively with buffer containing 600 mM potassium acetate and 0.2% NP-40 without loss of SNF-SWI complex components; all members of the complex (except the antigen) can then be eluted quantitatively with 5 M urea (3). In a similar experiment, we formed immune precipitates with the peak fraction from the TSK-heparin column (Fig. 6) and either an anti-SNF6 or anti-SNF11 antibody conjugated to protein A-Sepharose beads. Both SNF6 and SNF11 were quantitatively precipitated in each case (Fig. 7A and B). The immune precipitates were then incubated with buffer containing 600 mM potassium acetate and 0.2% NP-40. SNF11 remained associated even after

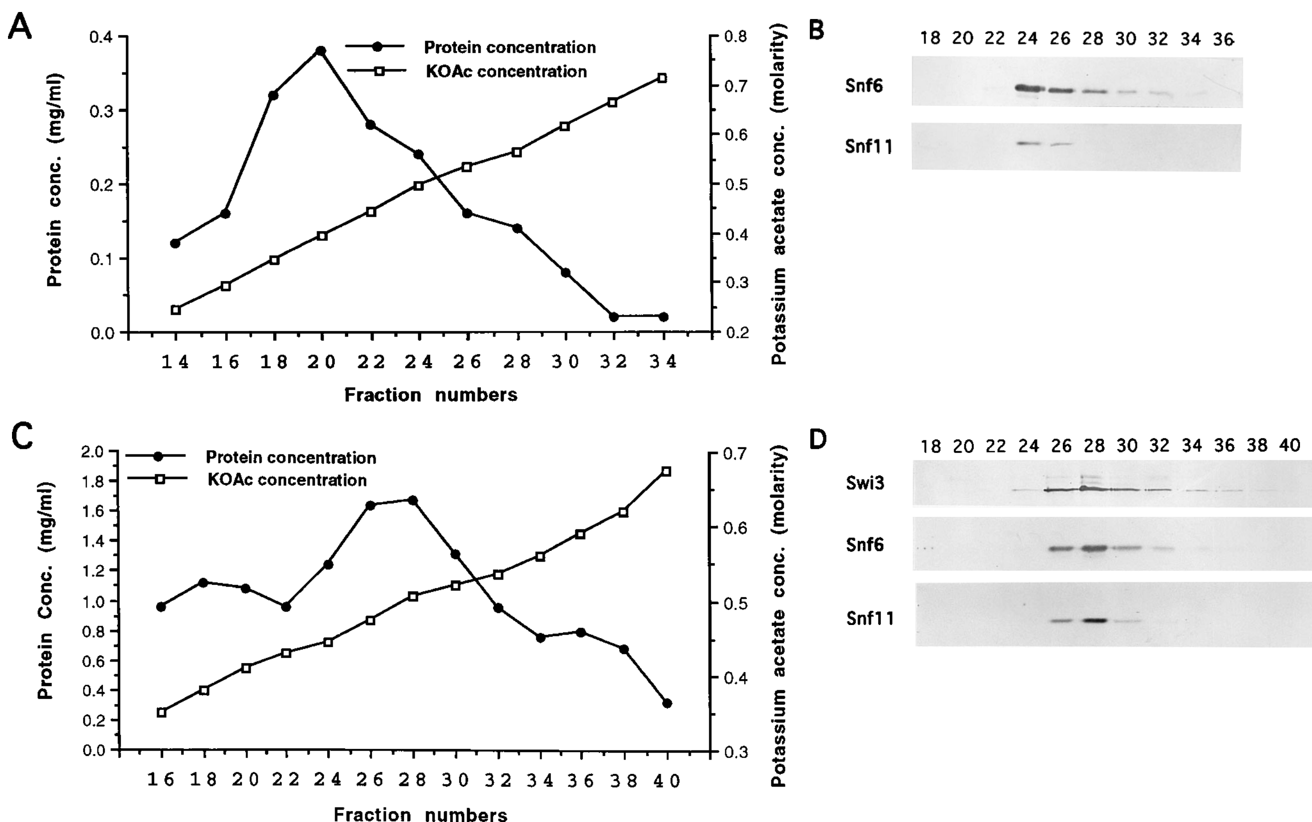

FIG. 6. SNF11 copurifies with SNF6 and SWI3. Whole cell extracts were fractionated on Bio-Rex 70, DEAE-Sephacel, and hydroxylapatite (see Materials and Methods). Peak fractions were further resolved by high-pressure liquid chromatography on DEAE-Sephacel (A) and TSK-heparin (C). Protein concentration is indicated by circles, and potassium acetate (KOAc) concentration is indicated by squares. (A) Peak fractions from hydroxylapatite were resolved on DEAE-Sephacel. Adsorbed proteins were eluted in buffer A with a linear gradient of 200 to 1,000 mM potassium acetate. (B) Fractions (2.5 μg) were separated in an SDS–10% acrylamide gel and immunoblotted with antisera against SNF6 and SNF11. Similar results were obtained with an SWI3 antiserum (not shown). (C) Peak fractions from DEAE-Sephacel were further resolved on TSK-heparin. Adsorbed proteins were eluted in buffer B with a linear gradient of 200 to 800 mM potassium acetate. (D) Fractions were analyzed as for panel B. No SNF11 protein was detected in the flowthrough from either column. In addition, SNF11 copurified with the SNF-SWI complex on the first DEAE-Sephacel and hydroxylapatite columns (not shown).

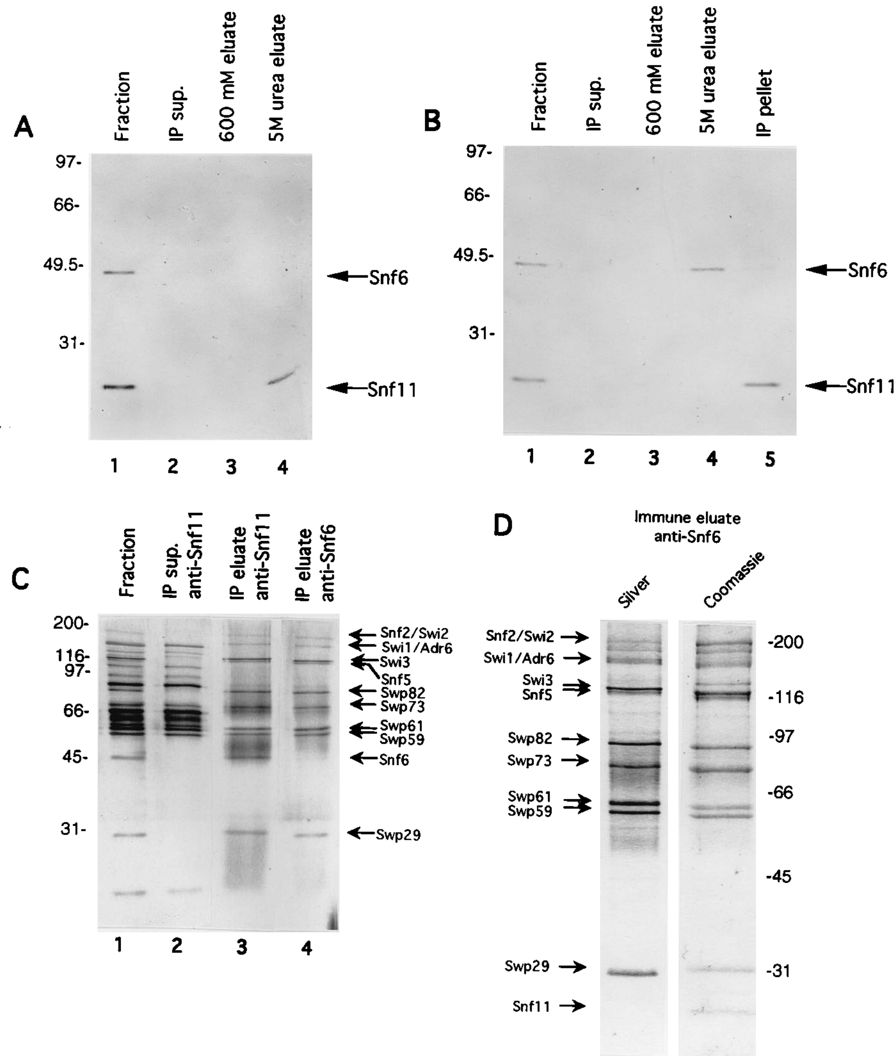

FIG. 7. SNF11 coimmunoprecipitates with the SNF-SWI complex. (A to C) Immune complexes were prepared with the peak TSK-heparin fraction and an anti-SNF6 or anti-SNF11 antibody coupled to protein A-Sepharose. Immune complexes were washed, subjected to elution with buffer A containing 600 mM potassium acetate and 0.2% NP-40, washed further, and subjected to elution with 5 M urea (see Materials and Methods). Samples were separated on SDS–9% acrylamide gels and either immunoblotted with anti-SNF6 and anti-SNF11 antibodies or stained with silver. (A) Immunoblot analysis of immune complexes formed with anti-SNF6. Lanes: 1, peak TSK-heparin fraction (2.5 μg); 2, supernatant of the immunoprecipitation (2.5 μg); 3, half of the 600 mM potassium acetate–0.2% NP-40 eluate; 4, half of the 5 M urea eluate. (B) Immunoblot analysis of immune complexes formed with anti-SNF11. Lanes: 1 to 4, same as for panel A; 5, half of the remaining pellet, after boiling in SDS loading buffer. (C) Analysis of the anti-SNF6 and SNF11 immunoprecipitations by SDS-PAGE and silver staining. Lanes: 1, peak TSK-heparin fraction (2.5 μg); 2, supernatant of the anti-SNF11 immunoprecipitation (2.5 μg); 3 and 4, half of the 5 M urea eluates of the anti-SNF11 and anti-SNF6 immunoprecipitations, as indicated. (D) Staining of SNF-SWI complex components with silver or Coomassie blue dye. The SNF-SWI complex was purified in large scale (see Materials and Methods). Proteins recovered from the 5 M urea eluate of the anti-SNF6 antibody column were separated on an SDS–9% acrylamide gel and revealed by staining with silver (lane 1; 1 μg of protein) or Coomassie blue dye (lane 2; 5 μg of protein).

extensive washing and was eluted quantitatively from the anti-SNF6 immunoprecipitation with 5 M urea (Fig. 7A). Likewise, SNF6 was eluted quantitatively from the anti-SNF11 immunoprecipitation with 5 M urea (Fig. 7B). These results demonstrate that SNF11 is a tightly associated member of the SNF-SWI complex.

To verify that the SNF11 antiserum specifically recognized SNF11 and immunoprecipitated only the SNF-SWI complex, the immunoprecipitations were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) analysis and stained with silver (Fig. 7C). Anti-SNF11 antibodies precipitated only the SNF-SWI complex; all other members of the complex were selectively removed from the TSK-heparin fraction (lane 2) and recovered in the 5 M urea eluate (lane 3). Thus, SNF11 antibodies immunodepleted SNF6 and all other SNF-SWI complex components, indicating that all SNF-SWI complexes in the TSK-heparin fraction contain SNF11.

The apparent lack of the SNF11 polypeptide in the 5 M urea eluate from the anti-SNF6 immune precipitate results from the protein's inability to bind silver under the staining conditions used. In a similar analysis of a large-scale preparation of the SNF-SWI complex, SNF11 was detected by staining with Coomassie blue dye but not with silver (Fig. 7D). These results confirm that SNF11 is a stoichiometric component of the SNF-SWI complex.

Genetic analysis of *SNF11* function. To assess the function of SNF11, we introduced the mutations *snf11Δ1*::*HIS3* and *snf11Δ2*::*HIS3* (Fig. 1) into the chromosomal locus of a diploid.

Upon sporulation of these heterozygous diploids, all tetrads (total of 25) yielded four viable spores. The mutant segregants grew on all carbon sources tested (glucose, galactose, sucrose, raffinose, and glycerol), grew at high (37°C) and low (16°C) temperatures, showed wild-type regulation of invertase expression, and did not require inositol. In addition, a homozygous diploid sporulated. Finally, disruption of *SNF11* in a strain carrying a chromosomal *HO-lacZ* fusion (BY86) did not cause a Swi$^-$ phenotype (data not shown).

The presence of a functional homolog of SNF11 could prevent detection of a Snf$^-$ phenotype in the *snf11*Δ mutants. We therefore deleted the SNF2 domain that interacts with SNF11. A deletion of domain 1, designated *SNF2ΔD1* (Fig. 4), was introduced into the genomic locus. The mutants showed normal carbon source utilization and invertase expression. In addition, plasmids YEP24-SNF2ΔD1 and YEP24-SNF2Δ5 complemented a *snf2* mutation. It remains possible that this deletion does not disrupt association of SNF11, or a homolog, with the SNF-SWI complex if there is an additional site of interaction.

DISCUSSION

We report the identification of a new integral component of the yeast SNF-SWI complex, a 19-kDa protein designated SNF11. The presence of SNF11 in purified preparations of the SNF-SWI complex previously went unrecognized because the protein is small and does not stain with silver. Here we identified SNF11, by a genetic approach, as a protein that interacts with SNF2 in the two-hybrid system.

Our conclusion that SNF11 is a component of the SNF-SWI complex is based on both genetic and biochemical evidence. First, SNF11 interacts specifically with a defined region of SNF2 in the two-hybrid system. Second, SNF11 functions as a transcriptional activator in genetic tests. Third, bacterially produced SNF11 protein binds to SNF2 in vitro. Fourth, the yeast SNF11 protein, identified by a specific antibody, copurifies with the SNF-SWI complex through several chromatographic steps. Fifth, anti-SNF11 specifically immunoprecipitates members of the SNF-SWI complex, and conversely, anti-SNF6 immunoprecipitates SNF11. Furthermore, SNF11 was shown to be tightly associated with the immunoprecipitated complex, and SNF11 appears to be a stoichiometric member of the complex, as judged by immunodepletion studies. Finally, SNF11 protein can be detected in purified SNF-SWI complex by staining with Coomassie blue.

What is the function of SNF11? We present evidence that SNF11 can mediate transcriptional activation of target gene expression, consistent with its intimate association with the SNF-SWI complex. Surprisingly, deletion of *SNF11* did not cause the Snf$^-$ phenotype characteristic of mutations in related *SNF* and *SWI* genes. The designation *SNF11* seems appropriate nonetheless in light of the clear relationship of SNF11 to the SNF-SWI complex. It is possible that an unidentified SNF11 homolog provides function in the *snf11*Δ mutant, but all cross-hybridizing clones recovered in our two-hybrid screen were derived from the *SNF11* locus, and we detected no genomic homolog by Southern blot hybridization (unpublished results). Moreover, deletion of the SNF2 sequence that interacts with SNF11 did not cause a Snf$^-$ phenotype, although we cannot exclude the possibility that SNF11, or a homolog, still associates with the SNF-SWI complex by interacting with another component. In view of the tight association of SNF11 with the SNF-SWI complex and the conservation from yeasts to humans of the interacting SNF2 region, we think it highly probable that SNF11 contributes to some function of the SNF-SWI complex. Most likely, mutation of *SNF11* affects a phenotype that we have not yet assayed.

One possibility is that SNF11 targets the SNF-SWI complex to specific transcriptional activators or chromosomal loci. In that case, we simply did not assay any phenotypes associated with those activators or loci. Another possibility is that SNF11 is required for optimal SNF-SWI complex function at all affected promoters, but our assays were not sufficiently sensitive to detect an effect. Finally, it is possible that SNF11 is required for SNF-SWI complex function only under particular conditions, for example, during certain environmental stresses. Further study of SNF11 may provide insight into the physiological roles of the SNF-SWI complex in transcriptional control.

Evidence suggests that homologs of SNF11 will be found in higher eukaryotes. SNF11 binds to a ~40-residue region of SNF2, called domain 1 (43), that is conserved in the *Drosophila* brm and human BRG1 and hbrm proteins. Moreover, we showed that SNF11 interacts strongly with the hbrm domain 1 in the two-hybrid system. Interestingly, brm, BRG1, and hbrm are SNF2 homologs that have been directly implicated in transcriptional activation (18, 29, 43), and a protein recognized by BRG1 antibody is present in the hSNF-SWI complex (20). In contrast, domain 1 is not conserved in several members of the SNF2 family that are not known to function as activators (STH1/NPS1 [25, 44], MOT1 [8], or hSNF2L [33]). Thus, this region may prove to be a signature sequence for true functional homologs of SNF2. Its conservation suggests that the interacting SNF11 protein is conserved as well. It will be of interest to determine whether the SNF-SWI complex of higher eukaryotes contains a counterpart of SNF11.

ACKNOWLEDGMENTS

We thank R. Brent, E. Golemis, and C. Peterson for plasmids and antibody, L. Riles for lambda clone grids, B. Laurent and E. J. A. Hubbard for helpful advice, and P. Lesage for comments on the manuscript.

This work was supported by Public Health Service grant GM47259 from the National Institutes of Health to M.C.

REFERENCES

1. **Abrams, E., L. Neigeborn, and M. Carlson.** 1986. Molecular analysis of *SNF2* and *SNF5*, genes required for expression of glucose-repressible genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6:**3643–3651.
2. **Bartel, P. L., C.-T. Chien, R. Sternglanz, and S. Fields.** 1993. Elimination of false positives that arise in using the two-hybrid system. BioTechniques **14:**920–924.
3. **Cairns, B. R., Y.-J. Kim, M. H. Sayre, B. C. Laurent, and R. D. Kornberg.** 1994. A multisubunit complex containing the *SWI1/ADR6*, *SWI2/SNF2*, *SWI3*, *SNF5*, and *SNF6* gene products isolated from yeast. Proc. Natl. Acad. Sci. USA **91:**1950–1954.
4. **Carlson, M., and B. C. Laurent.** 1994. The SNF/SWI family of global transcriptional activators. Curr. Opin. Cell Biol. **6:**396–402.
5. **Celenza, J. L., and M. Carlson.** 1986. A yeast gene that is essential for release from glucose repression encodes a protein kinase. Science **233:**1175–1180.
6. **Chien, C.-T., P. L. Bartel, R. Sternglanz, and S. Fields.** 1991. The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. Proc. Natl. Acad. Sci. USA **88:**9578–9582.
7. **Côté, J., J. Quinn, J. L. Workman, and C. L. Peterson.** 1994. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SNF/SWI complex. Science **265:**53–60.
8. **Davis, J. L., R. Kunisawa, and J. Thorner.** 1992. A presumptive helicase (*MOT1* gene product) affects gene expression and is required for viability in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**1879–1892.
9. **Elfring, L. K., R. Deuring, C. M. McCallum, C. L. Peterson, and J. W. Tamkun.** 1994. Identification and characterization of *Drosophila* relatives of the yeast transcriptional activator *SNF2/SWI2*. Mol. Cell. Biol. **14:**2225–2234.
10. **Estruch, F., and M. Carlson.** 1990. *SNF6* encodes a nuclear protein that is required for expression of many genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**2544–2553.
11. **Fields, S., and O. Song.** 1989. A novel genetic system to detect protein-protein interactions. Nature (London) **340:**245–246.

11a. **Hanes, S. D.** Personal communication.
12. **Hanes, S. D., and R. Brent.** 1989. DNA specificity of the bicoid activator protein is determined by homeodomain recognition helix residue 9. Cell **57:**1275–1283.
13. **Happel, A. M., M. S. Swanson, and F. Winston.** 1991. The *SNF2*, *SNF5*, and *SNF6* genes are required for Ty transcription in *Saccharomyces cerevisiae*. Genetics **128:**69–77.
14. **Hawkins, A. R., H. K. Lamb, and C. F. Roberts.** 1992. Structure of the *Aspergillus nidulans qut* repressor-encoding gene: implications for the regulation of transcription initiation. Gene **110:**109–114.
15. **Hirschhorn, J. N., S. A. Brown, C. D. Clark, and F. Winston.** 1992. Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. Genes Dev. **6:**2288–2298.
16. **Hubbard, E. J. A., R. Jiang, and M. Carlson.** 1994. Dosage-dependent modulation of glucose repression by MSN3 (STD1) in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:**1972–1978.
17. **Imbalzano, A. N., H. Kwon, M. R. Green, and R. E. Kingston.** 1994. Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature (London) **370:**481–485.
18. **Khavari, P. A., C. L. Peterson, J. W. Tamkun, D. B. Mendel, and G. R. Crabtree.** 1993. BRG1 contains a conserved domain of the SNF2/SWI2 family necessary for normal mitotic growth and transcription. Nature (London) **366:**170–174.
19. **Kruger, W., and I. Herskowitz.** 1991. A negative regulator of *HO* transcription, SIN1 (SPT2), is a nonspecific DNA-binding protein related to HMG1. Mol. Cell. Biol. **11:**4135–4146.
20. **Kwon, H., A. N. Imbalzano, P. A. Khavari, R. E. Kingston, and M. R. Green.** 1994. Nucleosome disruption and enhancement of activator binding by a human SWI/SNF complex. Nature (London) **370:**477–481.
21. **Laurent, B. C., and M. Carlson.** 1992. Yeast SNF2/SWI2, SNF5, and SNF6 proteins function coordinately with the gene-specific transcriptional activators GAL4 and Bicoid. Genes Dev. **6:**1707–1715.
22. **Laurent, B. C., I. Treich, and M. Carlson.** 1993. The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. Genes Dev. **7:**583–591.
23. **Laurent, B. C., M. A. Treitel, and M. Carlson.** 1990. The SNF5 protein of *Saccharomyces cerevisiae* is a glutamine- and proline-rich transcriptional activator that affects expression of a broad spectrum of genes. Mol. Cell. Biol. **10:**5616–5625.
24. **Laurent, B. C., M. A. Treitel, and M. Carlson.** 1991. Functional interdependence of the yeast SNF2, SNF5, and SNF6 proteins in transcriptional activation. Proc. Natl. Acad. Sci. USA **88:**2687–2691.
25. **Laurent, B. C., X. Yang, and M. Carlson.** 1992. An essential *Saccharomyces cerevisiae* gene homologous to *SNF2* encodes a helicase-related protein in a new family. Mol. Cell. Biol. **12:**1893–1902.
26. **Lesage, P., X. Yang, and M. Carlson.** 1994. Analysis of the SIP3 protein identified in a two-hybrid screen for interaction with the SNF1 protein kinase. Nucleic Acids Res. **22:**597–603.
27. **Li, L., S. J. Elledge, C. A. Peterson, E. S. Bales, and R. J. Legerski.** 1994. Specific association between the human DNA repair proteins XPA and ERCC1. Proc. Natl. Acad. Sci. USA **91:**5012–5016.
28. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Muchardt, C., and M. Yaniv.** 1993. A human homologue of *Saccharomyces cerevisiae SNF2/SWI2* and *Drosophila brm* genes potentiates transcriptional activation by the glucocorticoid receptor. EMBO J. **12:**4279–4290.
30. **Neigeborn, L., and M. Carlson.** 1984. Genes affecting the regulation of *SUC2* gene expression by glucose repression in *Saccharomyces cerevisiae*. Genetics **108:**845–858.
31. **Neigeborn, L., J. L. Celenza, and M. Carlson.** 1987. *SSN20* is an essential gene with mutant alleles that suppress defects in *SUC2* transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:**672–678.
32. **O'Hara, P. J., H. Horowitz, G. Eichinger, and E. T. Young.** 1988. The yeast *ADR6* gene encodes homopolymeric amino acid sequences and a potential metal-binding domain. Nucleic Acids Res. **16:**10153–10169.
33. **Okabe, I., L. C. Bailey, O. Attree, S. Srinivasan, J. M. Perkel, B. C. Laurent, M. Carlson, D. L. Nelson, and R. L. Nussbaum.** 1992. Cloning of human and bovine homologs of SNF2/SWI2: a global activator of transcription in yeast *S. cerevisiae*. Nucleic Acids Res. **20:**4649–4655.
34. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
35. **Peterson, C. L., A. Dingwall, and M. P. Scott.** 1994. Five SWI/SNF gene products are components of a large multi-subunit complex required for transcriptional enhancement. Proc. Natl. Acad. Sci. USA **91:**2905–2908.
36. **Peterson, C. L., and I. Herskowitz.** 1992. Characterization of the yeast *SWI1*, *SWI2*, and *SWI3* genes, which encode a global activator of transcription. Cell **68:**573–583.
37. **Prelich, G., and F. Winston.** 1993. Mutations that suppress the deletion of an upstream activating sequence in yeast: involvement of a protein kinase and histone H3 in repressing transcription *in vivo*. Genetics **135:**665–676.
38. **Rose, M. D., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics, a laboratory course manual. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
39. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
40. **Smith, D. B., and K. S. Johnson.** 1988. Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione *S*-transferase. Gene **67:**31–40.
41. **Stern, M. J., R. E. Jensen, and I. Herskowitz.** 1984. Five *SWI* genes are required for expression of the *HO* gene in yeast. J. Mol. Biol. **178:**853–868.
42. **Sternberg, P. W., M. J. Stern, I. Clark, and I. Herskowitz.** 1987. Activation of the yeast *HO* gene by release from multiple negative controls. Cell **48:**567–577.
43. **Tamkun, J. W., R. Deuring, M. P. Scott, M. Kissinger, A. M. Pattatucci, T. C. Kaufman, and J. A. Kennison.** 1992. brahma: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell **68:**561–572.
44. **Tsuchiya, E., M. Uno, A. Kiguchi, K. Masuoka, Y. Kanemori, S. Okabe, and T. Mikayawa.** 1992. The *Saccharomyces cerevisiae NPS1* gene, a novel *CDC* gene which encodes a 160 kDa nuclear protein involved in G_2 phase control. EMBO J. **11:**4017–4026.
45. **Winston, F., and M. Carlson.** 1992. Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet. **8:**387–391.
46. **Yoshinaga, S. K., C. L. Peterson, I. Herskowitz, and K. R. Yamamoto.** 1992. Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. Science **258:**1598–1604.