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,4 AND MARIAN CARLSON1,3,4*

Institute of Cancer Research1 and Departments of Microbiology 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

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...
candidates for functional homologs of SNF2. The brahma (brm) protein of D. melanogaster and the human brm 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 without a DNA-dependent ATPase activity was purified by using an antibody to BRG1 (20). These homologs also share with SNF2 a C-terminal bro-modomain 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. Eachenica col strains were XLI-Blue (Stratagene) and BA1 (the leu6 thi4 thr7-101 his3A2 strain). Two-hybrid screen and analysis of clones. S. cerevisiae CTY10-5d carrying an integrated lexAop-GAL1-lacZ reporter was sequentially transformed with pLexA-768 to 881, pLexA-hbrm 165-215, and pLexA-hbrm 165-215 fusions to yeast genomic DNA fragments (6).

Plasmids. pLexA-768 to 881 (previously called pLexA-SNF2 768 to 881 (22)) encodes the N-terminal 87-residue fragment of LexA fused to residue 14 of SNF2; SNF2 coding sequence is interrupted by an amber mutation at codon 768. Like pLexA-768 to 881, pLexA-SNF2 768 to 881 also contains the EcoRI fragment of pLexA-768 to 881 cloned into pSH2-12 and therefore lacks SNF2 sequence distal to codon 834. pLexA-SNF2 768 to 881 contains the same EcoRI 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 (20 residues) fused to the indicated sequences. pLexA-SNF2 768 to 881, -1 to 289, -1 to 616, -239 to 767, -277 to 767, and -293 to 260 contain BamHI-SalI PC fragments generated by amplification of pLN138-4 (1), using primers OL33 and OL49, OL33 and OL50, OL54 and OL52, OL54 and OL52, OL54 and OL52 and OL53 and OL52 (Table 1), respectively. All plasmids expressed proteins of the expected sizes. To generate YEp24-SNF2-D1 and YEp24-SNF2A5, the KpnI fragment of pLN136-4 was subcloned into M13mp18, subjected to site-directed mutagenesis with OL61 and OL62, and used to transform strain CTY10-5d carrying pGEX-KS2 (40). To construct plpLexA-SWI3, we first cloned the BamHI-SalI fragment of pLN136-4 carrying the SNF2-1 region into pEG202. pLexA-SWI3 was then cloned into pGEX-KS2 (Stratagene) to generate YEp24-SNF2A5 containing the BamHI-SalI fragment amplified from YEp24-SNF2A5 with OL53 and OL50. pLexA-SWI3 was then transformed into strain CTY10-5d carrying pGEX-KS2 (Stratagene), and the fusion protein was purified on glutathione-agarose beads as described.

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

Purification of GST fusion proteins and binding assay. Overnight cultures of E. coli XLI-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 NaHPO4, 4 mM NaH2PO4, 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 (36). Cultures of MCY3507 carrying LexA fusion plasmids were grown to
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 7.5), 0.3 M NaCl–3 mM EDTA–5 mM N-acetylmethylphenylsulfonyl 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 containing 100 mM potassium acetate (100), was applied to a TSK-heparin column, and eluted in buffer B with a linear gradient of 200 to 1,000 mM potassium acetate. The peak of the SNF-SWI complex eluted at 480 mM potassium acetate.

**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-Sephaloc, and hydroxyapatite) were performed as described previously (3). Peak fractions from hydroxyapatite were further resolved on DEAE-Sephaloc. Ad- sorbed 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 mM dexamethasone, 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-Sephaloc. Peak fractions were pooled, dialyzed against buffer B (identical to buffer A except that 20 mM N-acetylmethylphenylsulfonyl fluoride 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 480 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 immunoblotting enhancement agent (Enhance; New England Biolabs), 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 LexA87-SNF214-767, thereby activating expression of a lexAop-GAL1-lacZ reporter. From 213,000 transformants, we recovered 31 plasmids that caused blue color in combination with LexA87-SNF214-767 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 LexA87-SNF214-767, GAD-SWI3245-825 increased β-galactosidase expression 10-fold (Table 3). Sequence analysis of the remaining clones indicated

**TABLE 2. Oligonucleotides used**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>OL33</td>
<td>-CCCCGGATCCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL43</td>
<td>-CCCCGGATCCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL45</td>
<td>-GGGGGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL46</td>
<td>-AAGGGATCCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL47</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL50</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL51</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL52</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL53</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL54</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL55</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL56</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL61</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL62</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL64</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
<tr>
<td>OL65</td>
<td>-CCCCGGATCCTTATGACATAGATGACACGCG-3</td>
</tr>
</tbody>
</table>

* Oligonucleotides were synthesized with an Applied Biosystems 394 synthesizer. Restriction sites are underlined.

**TABLE 3. Interaction of SNF214-767 with SNF11 and SWI3245-825 in the two-hybrid system**

<table>
<thead>
<tr>
<th>DNA-binding protein</th>
<th>Activation protein</th>
<th>β-Galactosidase activity (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LexA87-SNF214-767</td>
<td>GAD</td>
<td>0.4</td>
</tr>
<tr>
<td>LexA87-SNF214-767</td>
<td>SNF11</td>
<td>0.4</td>
</tr>
<tr>
<td>LexA87-SNF214-767</td>
<td>SNF2</td>
<td>0.4</td>
</tr>
<tr>
<td>LexA87-SNF214-767</td>
<td>SNF6</td>
<td>0.4</td>
</tr>
<tr>
<td>LexA87-SNF214-767</td>
<td>SNF214-767</td>
<td>250</td>
</tr>
<tr>
<td>LexA87-SNF214-767</td>
<td>GAD-SWI3245-825</td>
<td>0.4</td>
</tr>
<tr>
<td>LexA87-SNF214-767</td>
<td>GAD-SWI3245-825</td>
<td>4</td>
</tr>
</tbody>
</table>

* Proteins were expressed from pLexA87-SNF214-767, pSh2-1 (LexA87) (12), pGAD3 (6), 3-28-2, 1-22-3, and pGAD-SNF11. Transformants of CRY 10-5d 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 <1.4%.

Downloaded from http://mcb.asm.org/ on March 28, 2021 by guest
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 PvuII and SpeI 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 codes for 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<sup>Δ7</sup>-SNF2<sub>14-767</sub> 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<sub>Δ7</sub>-SNF2<sub>14-767</sub> 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<sub>Δ7</sub>-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<sub>Δ7</sub>-SNF2<sub>14-767</sub> did not activate target gene expression.

**TABLE 4. Interactions of GAD-SNF11 in the two-hybrid system**

<table>
<thead>
<tr>
<th>DNA-binding protein</th>
<th>β-Galactosidase activities for activation protein:</th>
<th>Fold increase in activation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GAD</td>
<td>GAD-SNF11</td>
</tr>
<tr>
<td>LexA&lt;sub&gt;Δ7&lt;/sub&gt;</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>LexA-SNF2</td>
<td>38</td>
<td>164</td>
</tr>
<tr>
<td>LexA-SNF2K798R</td>
<td>2.7</td>
<td>148</td>
</tr>
<tr>
<td>LexA-SWI3</td>
<td>4.6</td>
<td>20</td>
</tr>
<tr>
<td>LexA-SNF5</td>
<td>86</td>
<td>86</td>
</tr>
<tr>
<td>LexA-SNF6</td>
<td>505</td>
<td>670</td>
</tr>
<tr>
<td>LexA-Bicoid</td>
<td>108</td>
<td>84</td>
</tr>
<tr>
<td>LexA-GAL4</td>
<td>1,090</td>
<td>920</td>
</tr>
</tbody>
</table>

<sup>a</sup>Proteins are fused to LexA<sub>Δ7</sub>, except for LexA-SWI3. Proteins were expressed from pSH2-1, pLexA-SNF2 (24), pLexA-SNF2K798R (22), pLexA-SWI3, pLexA-SNF5 (23), pLexA-SNF6 (21), pSH1-1 (LexA-Bicoid) (12), pSH1-7-4 (LexA-GAL4) (11a), pACTII (GAD) (27), and pGAD-SNF11. Transforms 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%.
All LexA fusion were expressed at comparable levels, as judged by Western blotting, except that LexA-SNF21-289, -SNF2239-289, and hbrm165-215 were much less abundant when coexpressed with GAD-SNF11 than when coexpressed with GAD. Values for LexA-SNF hybrids alone were within twofold of those observed when GAD-SWI3245-825 also interacted with the N terminus of SNF2 in our two-hybrid system. 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-SWI3245-825 stimulated activation by LexA-SNF214-767 15-fold and also interacted with other fusions containing residues 277 to 616. Because GAD-SWI3245-825 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.

Relationship of SNF11 and SWI3. To determine whether SNF11 interacts with SNF2 protein, we sought evidence for interaction of SNF11 with other proteins of the complex. SWI3 was a likely candidate because GAD-SWI3245-825 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).

**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).
SNF214-767 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 *snf11Δ* mutant (data not shown). Immunoblots 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. 5.** GST-SNF11 binds to LexA-SNF214-767. Whole cell extracts (WCE) were prepared from MCY307 expressing LexA87-SNF214-767 or LexA87-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.

**FIG. 6.** SNF11 copurifies with SNF6 and SWI3. Whole cell extracts were fractionated on Bio-Rex 70, DEAE-Sepharose, and hydroxyapatite (see Materials and Methods). Peak fractions were further resolved by high-pressure liquid chromatography on DEAE-Sepharose (A) and TSK-heparin (C). Protein concentration is indicated by circles, and potassium acetate (KOAc) concentration is indicated by squares. (A) Peak fractions from hydroxyapatite were resolved on DEAE-Sepharose. 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-Sepharose 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-Sepharose and hydroxyapatite columns (not shown).
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 immuno-
precipitation with 5 M urea (Fig. 7B). These results demon-
strate 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-polyacryl-
amide 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 immunoprecipitation results from
the protein’s inability to bind silver under the staining condi-
tions 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 tetrad (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 snf11A 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-SNF2ΔD5 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 snf11A 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**

11. Hanes, S. D. Personal communication.