Sfh1p, a Component of a Novel Chromatin-R remodeling Complex, Is Required for Cell Cycle Progression

YIXUE CAO,1 BRADLEY R. CAIRNS,2† ROGER D. KORNBERG,2 and BREHON C. LAURENT1*

Department of Microbiology and Immunology and Morse Institute of Molecular Biology and Genetics, State University of New York, Brooklyn, New York 11203,1 and Department of Structural Biology, Stanford University School of Medicine, Stanford, California 943052

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Several eukaryotic multiprotein complexes, including the Saccharomyces cerevisiae Snf/Swi complex, remodel chromatin for transcription. In contrast to the Snf/Swi proteins, Sfh1p, a new Snf5p paralog, is essential for viability. The evolutionarily conserved domain of Sfh1p is sufficient for normal function, and Sfh1p interacts functionally and physically with an essential Snf2p paralog in a novel nucleosome-remodeling complex called RSC (for remodels the structure of chromatin). A temperature-sensitive sfh1 allele arrests cells in the G2/M phase of the cell cycle, and the Sfh1 protein is specifically phosphorylated in the G1 phase. Together, these results demonstrate a link between chromatin remodeling and progression through the cell division cycle, providing genetic clues to possible targets for RSC function.

Transcription by eukaryotic RNA polymerase II is a dynamic process controlled by the interplay of gene-specific activator proteins, general transcription factors, and chromatin. Nucleosomes, the repeated nucleoprotein units of chromatin, repress transcription for many promoters (for reviews, see references 23 and 37). For transcription to occur, chromatin structure is altered, presumably by remodeling or displacing nucleosomes by replication-independent mechanisms (reviewed in reference 20). Several eukaryotic multiprotein complexes have been implicated in this process, including the Saccharomyces cerevisiae Snf/Swi complex (for reviews, see references 13, 35, 59, and 80). Purified yeast Snf/Swi, comprised of Snf2p/Swi2p, Snf5p, Snf6p, Swi1p, Swi3p, Snf11p, Tfg3/TA F30p, Swp73p, and three other, uncharacterized polypeptides (7–9, 14, 57, 68), rearranges chromatin structure to allow enhanced binding of activators to nucleosomal sites in vitro (14). Homologs of several of the Snf/Swi proteins have been identified in eukaryotes, and Snf/Swi complexes with related biochemical functions have been purified from human cells (32, 40, 79). Two related complexes have been identified in Drosophila melanogaster, brm (16) and NURF (72), which contain the Snf2p/Swi2p homologs brm and Iswi, respectively. Recently, the yeast Snf/Swi proteins were suggested to be members of the R NA polymerase II holoenzyme and mediator complexes (79), in contrast to several reports (8, 9, 14, 75). Despite the existence of several Snf/Swi paralogs, which are homologous proteins that result from a gene duplication, there is as yet no evidence for additional Snf/Swi-related complexes in yeast.

The yeast SNF and SWI genes were first identified as mutants defective in the expression of the SUCL2 (snf) and HO (swi) genes (HO encodes an endonuclease required for mating-type switching) (54, 63). Genetic studies implicated these genes in the transcription of several differently regulated promoters (1, 19, 28, 42, 56, 58). The Snf/Swi proteins were also shown to assist activation by several sequence-specific transcriptional activators expressed in S. cerevisiae (41, 58, 81).

Genetic evidence provided the first clue that the Snf/Swi proteins function in transcriptional activation by repressing repressor by chromatin. Mutations in genes encoding histones and other chromatin assembly factors suppress the growth and transcriptional defects of snf/swi mutants (5, 30, 38, 39, 55, 61, 64). The chromatin structure at the SUC2 promoter is also perturbed in snf2 and snf5 mutants at sites near the TATA box (30, 48). Biochemical evidence for a direct role in nucleosome remodeling was provided by experiments in which purified yeast and human Snf/Swi complexes were shown to disrupt histone-DNA contacts in vitro in an ATP-dependent manner (14, 32, 40, 79).

The Snf2p/Swi2p subunit of the Snf/Swi complex contains a nucleic acid-dependent ATPase domain conserved in prokaryotes and eukaryotes (for a review, see reference 13). Members of the Snf2 protein family participate in a variety of nuclear processes, including transcription, chromosome segregation, and DNA recombination and repair. The fundamental role of Snf2p and a subset of Snf2p homologs in chromatin restructuring is indicated by the fact that each of the six nucleosome-remodeling complexes described so far, i.e., yeast Snf/Swi and RSC (for remodels the structure of chromatin) (a new yeast complex), Drosophila brm and NURF, and two human SWI/SNF complexes, includes one of the known or putative ATPases Snf2p, Sth1p, brm, Iswi, BRG1, or hbrm (10, 16, 34, 43, 53, 67, 71, 75).

With the exception of the DNA-dependent catalytic activity of Snf2p-related proteins, little is known about the biochemical or physiological functions of other Snf/Swi or Snf/Swi-related proteins. A nalysis of the yeast Snf5 protein revealed a functionally important domain that is conserved in eukaryotes (11). Furthermore, Snf5p or Snf5p homologs are members of the core of conserved proteins found in each of the Snf/Swi and related complexes so far characterized (8, 14, 16, 75). The presence of a single Snf5p paralog called Sfh1p (for Snf5 homolog) prompted our inquiry into its function. We hypothesized that Sfh1p performs an important physiological role in the cell and cooperates with other known Snf/Swi paralogs in a new protein complex. Snf5p shows significant sequence identity to human Ini1/hSNF5 (33, 52), D. melanogaster snr1 (16),
and Caenorhabditis elegans, Schizosaccharomyces pombe, and S. cerevisiae. Insights into Snf5p function is provided by experiments with the human and D. melanogaster homologs. Ini1p/SNF5 interacts with the human immunodeficiency virus in vivo (30) and also binds to two human Snf2p relatives, BRG1 and hbrm (33, 52). snr1a is a component of a large Snf/Swi-related complex in Drosophila which also contains brm, and it is important for transcription of homoeotic and other genes during development (16).

Here we present genetic and biochemical evidence demonstrating that the essential Snf1p protein cooperates with an essential Snf2p paralog as a member of a novel ATP-dependent nucleosome-remodeling complex, RSC, that is distinct from Snf/Swi. Mutation in SFH1 arrests cells in the cell cycle, indicating that Sfh1p is required for cell cycle progression. We show further that Sfh1p is a phosphoprotein and that Sfh1p phosphorylation is regulated during the cell cycle. Our results establish a connection between chromatin remodelling and the cell division cycle and provide insight into the physiological role of RSC.

MATERIALS AND METHODS

Strains and genetic methods. The yeast strains used are listed in Table 1. Rich (yeast extract-peptone-dextrose [YPED]) and synthetic complete (SC) or SC-araffinose media contained 2% sugars (62). Standard genetic methods were followed (62). The Escherichia coli strain used was XL1-Blue (Stratagene) and the plasmid pGEX-4T-1 (Pharmacia).

Cloning of the S. cerevisiae SFH1 gene. To clone SFH1, oligonucleotides 5′-GGGATCCGTCATACATTCCATTGTTG-3′ and 5′-GGGATCCGTCATACATTCCATTTGTTG-3′ were used to prime the PCR amplification of a 1.3-kb BamH1-Sall fragment of the SFH1 coding region from yeast genomic DNA (288C). Plasmid pCMY52-Sfh1 was screened by yeast genomic DNA library in pYEp24 (12) by colony hybridization with the amplified DNA fragment.

Plasmids. Leu5 hybrid plasmids are derivatives of pSH2-1 (27) and express, from the constitutive ADH1 promoter, the amine-86 terminal 89 residues of the LexA protein fused to portions of Snf5p, Snf1p, or Snf1p. LexA-Sfh1, encoding LexA fused to the entire coding region of SFH1, including amino acids 1 to 426, was cloned by PCR using the SFH1 gene by the PCR amplification of the 5′-3′ genomic DNA (288C), primed by 5′-GACATAGCCTTCCATGTTCCACAAACCAG-3′ and 5′-GACATAGCCTTCCATGTTCCACAAACCAG-3′. The resulting plasmid complemented the lethality of an sfh1 mutation. LexA-Sfh1_1396 was constructed by cloning the 0.6-kb BamH1-BclI fragment (Klenow fragment-treated) fragment of pLexA-Sfh1 into pSH2-1, digested previously with BamH1 and Sall (ends blunted). pLexA-Sfh1_1396 contains the 0.7-kb BclI (Klenow fragment-treated)-Sall fragment of pLexA-Sfh1 in pSH2-1, digested with BamH1 (Klenow fragment treated) and Sall. pLexA-Sfh1_242 contains the 0.7-kb BamH1-BglI (Klenow fragment-treated)-Sall fragment of pLexA-Sfh1 in pSH2-1. pLexA-Sfh1_242 is expressed LexA fused to the entire SFH1 coding region, including amino acids 1 through 905. pLexA-Sfh1_1330 (previously called pLexA-SSH1 [1-1330] (44), encodes LexA residues 1 to 87 fused to residues 1 to 1335 of Snf1p. pLexA-Sfh1_1330 was created by ligating the 2-kb EcoRI-AgeI fragment of pLexA-Sfh1_1330, the 3.4-kb AgeI-Small fragment of pBLS50 (44), and pEG202 (a gift of E. Golemis and R. Brent), previously digested with EcoRI and BamHI (BamHI ends filled in), and pLexA residues 1 to 220 fused to the entire SFH1 coding region, including amino acids 1 to 1335. Both plasmids pLexA-Sfh1_1330 and pLexA-Sfh1_1330 complemented the lethality of an sfh1 mutation. All LexA fusion plasmids expressed hybrid proteins of the expected sizes at comparable levels, as judged by immunoblotting with anti-LexA polyclonal antibodies (data not shown).

pVP16-Sfh1 was constructed by ligating the 1.3-kb BamHI-Sall fragment (Sall ends Klenow fragment treated) of pLexA-Sfh1 and the BamH1-EcoRI fragment (EcoRI ends Klenow fragment treated) of pVP16 (73), resulting in fusion of the nuclease-localized acidic activation domain of VP16 to the entire coding region of SFH1. pVP16-Sfh1N contains the 0.6-kb BamH1-BclI (ends filled in) fragment of pLexA-Sfh1, encoding amino acids 1 through 189, in pVP16 digested previously with BamH1 and EcoRI (Klenow fragment treated). pVP16-Sfh1NC was created by removing the 0.6-kb BamH1-BclI fragment of pLexA-Sfh1, filling in the ends, and religating. This plasmid results in a fusion of VP16 to residues 189 to 426 of Sfh1p. pVP16-Sfh1NI contains the 0.7-kb BamH1-BglI (Klenow fragment-treated) fragment of pVP16-Sfh1 in pVP16. pVP16-Sfh1NI complemented the sh1-1::His3 mutation.

Disruption of the SFH1 chromosomal locus. pY′C1 was constructed in two steps. In the first step, the 2.4-kb SalI-HindII fragment of pCS28 was cloned into pPL11 (a gift of P. Leesage), a pC19 derivative containing the BglII fragment of LEU2 cloned into the BamH1 site, creating pY′C1. In the second step, the 1.8-kb SacI-BglIII fragment (Klenow fragment-treated) fragment of pY′C1 was cloned into pY′CS2, digested with SacI-BglII, creating pY′C2. pY′C1 results in the replacement of the SFH1 HindII-XbaI fragment of pY′CS2 (encoding amino acids 12 through 410) with a SacI-XbaI LEU2 fragment. The 7.0-kb SacI-BglIII fragment of pY′C2 was used to transform diploid strains BLY 29 and BLY 65.

To construct pY′C2, we first cloned the 1.7-kb XbaI fragment of pY′CS2 in pBULUH. A BamH1 fragment harboring the yeast HIS3 gene was then inserted at the unique BglII site within the coding sequence of SFH1 to create pY′C2. This results in the insertion of the HIS3 gene after SFH1 codon 242, extending the reading frame for eight codons before a stop codon is reached. Diploid strains BLY 18 and BLY 29 were transformed with the 3.5-kb XbaI fragment of pY′C2. Southern blot analysis confirmed that one of the two chromosomal homologs of the SFH1 locus had been replaced by the two mutations.

Antibody production and immunoblotting. pGST-Sfh1_1330 expresses a GST-Sfh1 fusion protein constructed by inserting the 1.1-kb EcoRI-Sall fragment of pLexA-Sfh1 (encoding amino acids 57 to 426) in pGEX-4T-1 (Pharmacia). E. coli cells transformed with pGST-Sfh1_1330, were induced for 1 h with 1 mM isopropyl β-D-thiogalactopyranoside (IPTG) and harvested. Glutathione S-transferase (GST)-Sfh1p was purified as described previously (4) and used to immunize rabbits (29). Antibodies against GST were removed by passing whole sera over glutathione-Sepharose. Antibodies against Sfh1p were collected from the unbound material by incubation with nitrocellulose strips con-
In vitro binding analysis with GST fusion proteins. Bacterial lysates from *E. coli* expressing GST-Sfh1, GST-Snf51-193 (expressed from a plasmid constructed by cloning the product of a PCR amplification of pJW34 [1] with primers 5OL-01 and 5OL-04 into pGEX-3X [Pharmacia]), or GST proteins were prepared and purified on glutathione-agarose beads as described for the GST-Sfh157-426 fusion protein. Beads were washed with 1 ml of binding buffer (20 mM HEPES [pH 7.7], 75 mM KCl, 0.1 mM EDTA, 2.5 mM MgCl₂, 1% skim milk, 1 mM dithiothreitol, 0.05% Nonidet P-40). Sfh1p carried on pDJ91 was transcribed and translated.
briefly, and fixed with 3.7% formaldehyde in phosphate-buffered saline (100 mM 37°C YEPD medium. At several time points, aliquots were removed, sonicated and large-budded cells were determined microscopically. 37°C YEPD medium to concentrations of approximately 4 10^6 cells/ml. At various time intervals, approximately 2 10^6 cells were removed, sonicated briefly, and fixed in 70% ethanol at room temperature for 12 h. After two washes in 50 mM Tris-HCl (pH 7.8), fixed cells were resuspended in 1 ml of the same buffer containing 2 mg of RNase A per ml and incubated at 37°C for 12 h on a rotator. Cells were pelleted, resuspended in 0.5 ml of 55 mM HCl containing 5 mg of pepsin (Sigma) per ml, and incubated at 37°C for 30 min. Cells were washed once in 1 ml of T M N buffer (200 mM Tris-HCl [pH 7.5], 211 mM NaCl, and 78 mM M gCl_2) and then resuspended in T M N buffer containing 50 μg of propidium iodide (Sigma) per ml. The fluorescence intensities of stained cells were measured after 10 to 30 min on a Becton Dickinson 900 F A C S can machine and analyzed with L Y S I S II software.

**Cell synchronization.** Mid-log-phase cultures of BLY 46 cells were arrested in G_1, or G_2/M phase by incubation in YEPD medium containing 5 μg of α-factor per ml, 0.1 M hydroxyurea, or 15 μg of nocodazole per ml, respectively, for 150 min at 30°C. Synchronous release from the α-factor block was accomplished by washing cells twice with H_2O and then returning them to fresh YEPD growth medium. Aliquots of cells were lysed, and lysates were analyzed by immunoblotting.

**Identification of SFH1 and alignment of Sfh1p-related proteins.** The National Center for Biotechnology Information BLAST electronic mail server was used to retrieve the nucleotide sequence of Y S C L 8 4 3 (SFH1) from the EMBL data library. Sequences related to the predicted Sfh1p in the Nonredundant GenBank translations, PDB, Swiss-Prot, SPupdate, and PIR databases were identified by using the BLASTP program (2). Multiple protein sequences were aligned by using the clustal algorithm of the MEGA LIGN program (DNAStar, Inc.).

**Nucleotide sequence accession number.** The SFH1 sequence was entered with the sequence of S. cerevisiae chromosome XI into the EMBL data library under accession no. U 2 0 6 1 8 and S 5 3 9 9 9. The GenBank accession numbers for c. 27 7 6 9 2 and R O T E 5 3, the putative Sfh1p homologs in S. pombe and C. elegans, are 1 1 7 5 3 7 1 and C E R O T E 5 4 , respectively.

**RESULTS**

**SFH1 is a unique and essential SNF5 paralog.** The Snf5p protein sequence is significantly similar to polypeptides encoded by open reading frames in human (33), Drosophila (16), C. elegans (E M B L accession number Z 3 2 6 6 8 3 ), and S. pombe (E M B L accession number Z 5 0 1 4 2 ) cells. These SFH1 homologs are candidates for functional homologs. Sequence identity for the proteins in this Snf5p family is highest for Snf5p amino acids 455 through 676, which lie entirely within the functional domain of Snf5p (Fig. 1A and B) (11). In pairwise comparisons, this conserved region of Snf5p is 35 to 46% identical to the corresponding regions of the Snf5p family proteins. When the Snf5p sequence was compared to those of proteins in the S. cerevisiae Genome Database (Stanford University), a single open reading frame, L 8 5 4 3 . 3 , with significant similarity to the conserved region of Snf5p (BLASTP P value, 3.7 10^{-2}) was identified, and the corresponding gene was cloned. The L 8 5 4 3 . 3 gene, designated here SFH1 (for Snf5p homolog), is predicted to encode a 48,747-dalton protein of 426 amino acids (Fig. 1C). A phylogenetic analysis of the Snf5p homologs indicates the distinctiveness of Sfh1p (Fig. 1D). The human, D. melanogaster, and C. elegans proteins form a cluster, as do the S. cerevisiae Snf5p and S. pombe proteins, presumably reflecting a common function, and Sfh1p is part of neither cluster. Instead, Sfh1p appears to have diverged from the other homologs before the two clusters branched from each other.

To determine the phenotypic consequence of loss of the SFH1 gene function, we replaced one of the two chromosomal wild-type SFH1 alleles in diploid strains of the S288C and W 3 0 3 genetic backgrounds with the disruption allele created by inserting H I S 3 at codon 242 (Fig. 1C). Leu^{+} transformants of both genetic backgrounds were sporulated and subjected to tetrad analysis. From 14 dissected ascii, only two viable Leu^{+} spore clones were recovered in each case. Invariable spores germinated and divided on average twice, indicating that the SFH1 gene is essential for the mitotic growth of yeast cells. When His^{+} transformants of the S288C genetic background were sporulated and dissected, viability again segregated 2:2 and only His^{−} spore clones were obtained from each of 14 ascii. However, four viable spore clones were recov-

![Image](http://mcb.asm.org/)

**FIG. 2.** SFH1 is essential for mitotic growth of yeast cells. (A) The sfh1-1 allele confers a temperature-sensitive phenotype on W 3 0 3 cells. Growth of isogenic SFH1 wild-type (BLY 7 3 ) and sfh1-1 temperature-sensitive (BLY 7 4 ) strains at 25 and 37°C is shown. (B) sfh1-1 encodes a truncated Sfh1 protein of the expected size. Extracts prepared from sfh1-1 (BLY 7 4 ) and SFH1 (BLY 7 3 ) cells were immunoblotted with an anti-Sfh1p polyclonal antibody. Size standards are indicated in kilodaltons.

with [35S]methionine in vitro by using the TNT coupled transcription-translation kit according to the procedures described by the manufacturer (Promega). Twenty microliters of reaction mixture in reticulocyte lysates was then mixed with 1.5 ml of binding buffer and GST, GST-SnF5_{1-193}, or GST-Sfh1p affinity resin. After rocking at 4°C for 2 h, the beads were washed five times with 1 ml of M T P B S (150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4, and 0.1% Triton X-100, pH 7.3) and mixed with sample buffer. Bound proteins were released by boiling and analyzed by sodium dodecyl sulfate (SDS)-8% polyacrylamide gel electrophoresis followed by autoradiography.

**Purification of RSC complex.** RSC was purified from whole-cell extracts with the following chromatography steps: Bio-Rex 70, DEAE-Sepharose, hydroxyapatite, M monQ, TSK-heparin, and M monO S (10). The peak fraction from M monQ is approximately 50% pure, as estimated from Coomassie blue-stained gels. Immune complexes of RSC with anti-Sfh1p antibodies were prepared with the peak M monQ fraction and anti-Sfh1p antibodies conjugated to protein A-Sepharose. Conjugation was performed as described previously for anti-Snfp6 (8). Immune complexes (50-μl total volume) were formed in buffer A (20 mM Tris-acetate [pH 7.5], 10% glycerol, 0.1 M dithiothreitol, 1 mM EDTA, 0.1% Nondet P-40) containing 100 mM potassium acetate with peak M monO S (20 μg; 50% pure) and 20 μl of anti-Sfh1p-protein A-Sepharose beads. Samples were rotated for 4 h at 4°C, and immune complexes were collected by centrifugation. Pellets were washed twice with 1 ml of buffer A containing 200 mM potassium acetate at 4°C, recovered by centrifugation, and eluted with two 25-μl aliquots of 5 M urea.

**Budding morphology.** To determine the budding index, cultures of logarhythmically growing BLY 74 (sfh1-1S) cells and wild-type cells (for comparison) were split, diluted to a density of 4 10^4 cells/ml, and maintained in prewarmed 25 or 37°C YEPD medium. At several time points, aliquots were removed, sonicated briefly, and fixed with 3.7% formaldehyde in phosphate-buffered saline (100 mM NaCl, 80 mM Na_2HPO_4, 20 mM NaH_2PO_4, pH 7.3), and the numbers of single, small-budded, and large-budded cells were determined microscopically.

**DNA flow cytometry.** Early- to mid-log-phase cultures of the temperature-sensitive strain BLY 74 and the isogenic wild-type strain BLY 73 grown in YEPD medium with 2% glucose at 25°C were split and diluted into prewarmed 25 or 37°C YEPD medium to concentrations of approximately 4 10^6 cells/ml. At
The additional 54 amino acids, 42 of which are part of the conserved domain of Sfh1p (amino acids 201 to 404) was investigated by comparing N-terminal (amino acids 1 to 189) and C-terminal (amino acids 189 to 426) halves of Sfh1p expressed from dissected tetrads of a cross between the W303 sfh1-1ts strain and an S288C SFH1 wild-type strain. We confirmed that the sfh1-1 insertion allele encodes a truncated Sfh1 protein which migrates with an apparent mass of 31 kDa (Fig. 2B), slightly larger than the mass of 27 kDa predicted. The wild-type Sfh1p migrates as a 54-kDa protein species, which is also slightly larger than predicted. The lower immunoreactivity for Sfh1p than for Sfh1p may be due to the fact that Sfh1p contains only one-half of the amino acids of Sfh1p used as immunogen or, alternatively, to the fact that Sfh1p is unstable.

To assess whether SFH1 and SNF5 function in redundant pathways, a snf5Δ2 strain (BLY 3) was transformed with either pYC5H, a high-copy-number 2μm plasmid expressing SFH1 from its own promoter, or a complementing hybrid plasmid, pLexA-Sfh1, expressing SFH1 from the constitutive ADH1 promoter, and tested for the ability to grow on SC-rafinose plates. Neither plasmid complemented the snf5Δ mutation for growth on rafinose. In the converse experiment, when an sfh1-1Δ1::EU2/SFH1 heterozygous diploid carrying a multi-copy 2μm plasmid expressing SNF5, pW34 (1), was sporulated and dissected, only two viable Leu+ spores were recovered. pW34 also failed to suppress the temperature-sensitive defects of the sfh1-1::HIS3 mutation (data not shown). These results confirm that SFH1 and SNF5 are not functionally redundant.

The highly conserved domain of Sfh1p is sufficient for normal function. The functional significance of the evolutionarily conserved domain of Sfh1p (amino acids 201 to 404) was investigated by comparing N-terminal (amino acids 1 to 189) and C-terminal (amino acids 189 to 426) halves of Sfh1p expressed as LexA fusion proteins from high-copy-number 2μm plasmids for their ability to complement the sfh1-1Δ1 mutation for viability. Only two viable Leu+ spores were recovered from each ascus following sporulation and tetrad dissection of an sfh1-1Δ1::EU2/SFH1 diploid transformed with pLexA-Sfh11-242. In contrast, four viable spores were obtained from diploids transformed with pLexA-Sfh11-189-426, all viable Leu+ strains carried the plasmid, and growth of these strains was indistinguishable from that of an isogenic wild-type strain at 25 and 37°C (data not shown). These results demonstrate that the highly conserved domain of Sfh1p is sufficient for normal wild-type function. Although the sfh1-1 N-terminal 189 amino acids fail to complement sfh1-1Δ1, sfh1-1Δ1 (amino acids 1 to 242) was shown (see above) to be partially functional, suggesting that the additional 54 amino acids, 42 of which are part of the conserved domain, are necessary for Sfh1p function.

The S. cerevisiae Snf5p and human In11 proteins activate transcription of target genes when fused to heterologous DNA-binding domains and expressed in yeast (33, 42, 52). We found that a DNA-bound LexA fusion protein containing the full-length Sfh1p activated transcription of two different target genes, LexA-GAL1-lacZ and LexA-CYC1-lacZ, in vivo to levels comparable to those of a LexA-Snf5p fusion protein (Table 2). Furthermore, two N-terminal fusion proteins activated transcription as well as the full-length Sfh1p, while a functional C-terminal fusion protein did not (Table 2), implying that Sfh1p activates through a cryptic activation domain. Significantly, Sfh1p activation is Snf/Swi dependent when tested with a single lexA operator upstream of the reporter gene but is independent of Snf/Swi when tested with several lexA operators (Table 3). Levels of the LexA-Sfh1p fusion protein in snf mutants were comparable to those in the wild type, as revealed by immunoblotting (data not shown).

### Table 2. LexA-Sfh1p activates transcription in vivo

<table>
<thead>
<tr>
<th>Protein</th>
<th>lexA-GAL1-lacZ</th>
<th>lexA-CYC1-lacZ</th>
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<tr>
<td></td>
<td>operators</td>
<td>operators</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>LexA-Snh1p</td>
<td>&lt;1</td>
<td>16</td>
</tr>
<tr>
<td>LexA-Snh2p</td>
<td>&lt;1</td>
<td>86</td>
</tr>
<tr>
<td>LexA-Snh5p</td>
<td>&lt;1</td>
<td>4</td>
</tr>
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</table>

* A cultivation by LexA-Sfh1p and LexA-Snf5p fusion proteins was assessed with lexA operators at both the GAL1 (zero, one, or six overlapping lexA operators replacing UASG1) and CYC1 (zero or one lexA operator; UASG1 and UASG2, deleted) promoters, as described in Materials and Methods. Values for LexA-Snh2p and LexA-Snh5p in snf5 and snf2 mutants, respectively, are from reference 41.

* Values are averages for at least four transformations. Standard errors were less than 10%.

### Table 3. Transcriptional activation by LexA-Sfh1p in snf mutants is dependent on the number of lexA operators

<table>
<thead>
<tr>
<th>Protein</th>
<th>snf2</th>
<th>snf5</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0 operators</td>
<td>1 operators</td>
</tr>
<tr>
<td>LexA-Sfh1p</td>
<td>&lt;1</td>
<td>16</td>
</tr>
<tr>
<td>LexA-Snh2p</td>
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<td>86</td>
</tr>
<tr>
<td>LexA-Snh5p</td>
<td>&lt;1</td>
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mutants (data not shown), consistent with the view that Sfh1p and Sth1p function together in a unit.

We reasoned next that if the Sfh1p and Sth1p proteins worked together, the two proteins would interact directly or indirectly. This model was tested in the two-hybrid genetic interaction system in which the DNA-binding partner contained Sth1p fused to the LexA DNA-binding domain and the activating partner contained Sfh1p residues fused to the VP16 activation domain. In combination with LexA-Sth1p, VP16-Sfh1p increased $\beta$-galactosidase expression sevenfold (Fig. 3B), suggesting that the Sth1p and Sfh1p proteins interact in vivo. To determine which Sfh1p sequences mediate interaction with Sth1p, we expressed partial Sfh1p sequences as VP16 chimeric proteins and tested for interaction with LexA-Sth1p. A VP16 fusion protein containing the amino-terminal 189 amino acids of Sfh1p (VP16-Sfh1N1) did not interact with Sth1p, although a fusion protein containing the carboxy-terminal half of Sfh1p, amino acids 189 to 426 (VP16-Sfh1C), interacted with Sth1p nearly as strongly as the full-length Sfh1p (Fig. 3B). Significantly, amino acids 219 to 242 are part of the first of two 45-amino-acid imperfect direct repeats conserved in all proteins in the Snf5p family (Fig. 1C).

To determine whether the interactions between Sth1p and Sfh1p detected in vivo by two-hybrid assays were direct or instead resulted from an interaction mediated by other proteins, we used an in vitro affinity binding assay. We tested a recombinant GST-Sfh1p protein immobilized on agarose beads for the ability to bind specifically to in vitro-translated $^{35}$S-labelled Sth1p from reticulocyte lysates (Fig. 3C). After washing, the proteins bound to the beads were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. Sth1p bound specifically to GST-Sfh1p (Fig. 3C, lane 6) but
not to control proteins GST and GST-Snf5p, which contains an N-terminal portion of Snf5p (Fig. 3C, lanes 3 and 4). GST-Sfh1p also failed to bind to the in vitro-translated luciferase control (Fig. 3C, lane 5). Coomassie blue staining of the acrylamide gels confirmed that all of the reaction mixtures contained approximately equal amounts of GST or GST fusion proteins (Fig. 3C, lanes 7 to 9). These biochemical data support the genetic evidence for direct interaction of Sth1p and Sfh1p in vivo, although it is possible that evolutionarily conserved proteins in the reticulocyte lysate are required for the interaction.

**Sfh1p is a component of purified RSC complex.** Independently, a complex containing Sfh1p and 14 other proteins called RSC (for remodels the structure of chromatin) has been purified to homogeneity from yeast cells (10). RSC has DNA-dependent ATPase and ATPase-dependent nucleosome destabilizing activities. Our genetic results predict that Sfh1p interacts with, or is a member of, this complex. To test this, fractions of partially purified RSC complex eluted from a Mono Q column were immunoblotted with affinity-purified Sfh1p polyclonal antibody. Sfh1p cochromatographed with two other members of RSC, Sth1p and Rsc6p (Fig. 4A).

To determine whether the Sfh1 protein is present in the purified RSC complex, proteins from whole-cell extracts and from a preparation highly enriched for RSC (20% pure) and either anti-Sfh1p antibodies (lanes 3 and 4) or control antibodies (anti-Snf6p) (lanes 5 and 6) conjugated to protein A-Sepharose. Immunoprecipitates were washed extensively, and pellets and eluates were examined by immunoblotting with anti-Sfh1p, anti-Rsc6p (10), and anti-Sfh1p antibodies. Sfh1p, Rsc6p, and Sfh1p were effectively precipitated by anti-Sfh1p antibodies (Fig. 4C, lanes 3 and 4), whereas the anti-Snf6p control antiserum was incapable of immunoprecipitation (compare lanes 3 and 4 in Fig. 4C). These results demonstrate that Sfh1p is a subunit of the RSC complex and further suggest that Snf6p, a subunit of Snf/Swi, is not a member of RSC.

**Mutation in SFH1 causes a cell cycle arrest in G2/M.** To gain insight into the role of RSC in vivo, the physiological function of Sfh1p was further characterized by studying the sfh1-1 temperature-sensitive mutant. Logarithmically growing sfh1-1ts mutant cells were diluted into prewarmed 25 or 37°C YEPD medium and examined microscopically at several time points. sfh1-1ts cells raised to the restrictive temperature (37°C) exhibited a dramatic increase in the number of large-budded cells and ceased cell division 8 h following the shift. sfh1-1ts cells grown at 37°C are larger than cells grown at 25°C, with daughter cells nearly as large as mother cells. The arrest was strongly biased: approximately 86% of the sfh1-1ts cells arrested as large-budded cells, characteristic of the G2/M phase (Fig. 5A). In contrast, only 43% of the sfh1-1ts cells grown permissively (25°C) or 45% of the isogenic wild-type cells grown at 25 or 37°C were large budded (Fig. 5A).
cytometry confirmed a perturbation of the cell cycle distribution. sfh1-1ts cells showed a dramatic increase in the proportion of cells with a 2N DNA content, whereas the DNA content of sfh1-1ts mutants grown to mid-log phase at 25°C was similar to that of the isogenic wild-type strain at 25 or 37°C (Fig. 5B). Moreover, the increase in the number of sfh1-1ts cells grown at 37°C with a G2/M DNA content was proportional to the accumulation of budded cells, indicating that SFH1 is required for progression of cells through the G2/M transition of the yeast cell cycle. DAPI (4′,6-diamidino-2-phenylindole) and tubulin staining revealed that most of the large-budded sfh1-1ts cells shifted to 37°C had nearly completed nuclear division, as evidenced by nuclei that were bilobed and extended across the bud neck or by short spindles spanning separated nuclei, characteristic of late G2/M phase (Fig. 5C and data not shown). We also noticed an unusual, multiple-bud morphology in approximately 10% of the arrested sfh1-1ts mutants (data not shown).

Sfh1p phosphorylation is regulated during the cell cycle. Antibodies specific for Sfh1p detected two protein species with apparent masses of 54 and 56 kDa in whole-cell protein extracts prepared from logarithmically growing asynchronized cells (Fig. 6), suggesting a posttranslational modification of the protein. Examination of the amino acid sequence of Sfh1p revealed two potential phosphorylation sites for the cyclin-dependent kinase, Cdc28p (SPYR and TPER at amino acids 182 and 306, respectively), one consensus phosphorylation site for cyclic AMP (cAMP)-dependent kinase (KRET at amino acid 403), and several sites each for protein kinase C and casein kinase II. Although there is a single potential CDC28 phosphorylation site in Ini1 (SPEK at amino acid 289), no other consensus phosphorylation sites for CDC28 or cAMP-dependent kinase were found in the other Snf5p family proteins. Lambda protein phosphatase treatment of protein extracts shifted the migration of the 56-kDa species to that of the 54-kDa protein (Fig. 6A, lane 2), while control incubation with phosphatase and phosphatase inhibitors had no effect on the mobility of the two protein bands (Fig. 6A, lane 3). This result strongly implicates phosphorylation in the mobility shift of the 56-kDa Sfh1p species.

The uniform cell cycle arrest resulting from loss of SFH1 function prompted us to test the phosphorylation pattern of Sfh1p at different stages of the cell cycle. Wild-type cells were synchronized by treatment with the mating pheromone α-factor. G1-arrested cells were then washed free of the pheromone, allowing synchronous passage through the S phase and mitosis.

![Image](http://mcb.asm.org/)

**FIG. 5.** Altered cell cycle distribution and morphology in sfh1-1ts mutant cells. (A) Budding morphology of sfh1-1ts mutant cells. The distributions of single, small-budded, and large-budded cells are compared for sfh1-1ts (BLY74) cells and SFH1 (BLY73) wild-type cells grown at 25 and 37°C. (B) Fluorescence-activated cell sorting analysis of sfh1-1ts and wild-type cells grown at 25 or 37°C. Strains containing wild-type or temperature-sensitive alleles of SFH1 were analyzed by fluorescence-activated cell sorting 8 h after being shifted from 25 to 37°C. The number of cells, depicted on the vertical axis, is plotted versus the fluorescence intensity of emitted light on the horizontal axis. (C) Aberrant morphology of sfh1-1ts mutant cells. The sfh1-1ts strain (BLY74) grown in YEPD at 25 or 37°C was examined by Nomarski differential interference contrast microscopy (Nom) and by epifluorescence microscopy after staining of the DNA with DAPI. Nomarski optics and DAPI staining of isogenic wild-type strain BLY73 grown at 25 or 37°C were indistinguishable from those of BLY74 grown at 25°C (data not shown).
The phosphorylated form of Sfh1p is designated Sfh1-P. Lane A, lysate from asynchronously growing cells (A) and at each time point asynchronous log-phase cells. (C) Cell budding morphologies were determined microscopically for asynchronously growing cells (A) and for the hydroxyurea (HU)- and nocodazole (N)-treated cells shown in panel D.

**DISCUSSION**

**Evolutionary conservation of Snf5p.** The conservation of sequences within the functional domain of Snf5p among eukaryotes (11) prompted the present study of the function of the yeast Snf5p paralog Sfh1p. Despite the sequence homology, the similarity between members of the Snf5 family varies considerably. For example, Sfh1p shows greater similarity to human Ini1 (BLAST value, 7.2 x 10^{-25}) than to yeast Snf5p (BLAST P value, 7.2 x 10^{-9}). The sequence similarities among the more highly related human, Drosophila, and C. elegans proteins extend throughout their entire lengths, implying that these regions correspond to additional, and possibly shared, functional domains. Thus, like members of the Snf2/ Swi2 family, Snf5p family proteins might perform similar functions in a variety of cellular processes.

Our results indicate that the 200-amino-acid conserved domain of Sfh1p shared by Snf5p homologs is sufficient for Sfh1p function, and they strongly suggest that at least part of the domain is required for function. In vivo protein binding assays show that the same conserved sequences are required for binding to Sfh1p, thereby suggesting that the capacity to bind Sfh1p is an essential function of Sfh1p. Moreover, the corresponding region of the human Ini1/Snf5 is necessary for protein-protein interactions with BRG1 and hbrm (16, 33, 52), indicating functional conservation of this domain. Four of the six Snf5p homologs are associated with high-molecular-weight multiprotein complexes (8, 16, 33, 57), three of which have been shown to remodel nucleosomes in vitro by using the energy from ATP hydrolysis (10, 14, 32, 40). We infer that the Snf5p family proteins are integral components of several distinct multimeric factors that restruc-
turing activities are functional and cells are viable. In the absence of the RSC component Sth1p or Sfh1p, however, cells are inviable.

The role of Snf/Swi in chromatin remodeling to facilitate transcription factor binding is supported by genetic and biochemical evidence (14, 30). Although Sfh1p tethered to DNA activates transcription from two different target promoters in vivo, suggesting that RSC shares with Snf/Swi the ability to reorganize nucleosomes in transcription, other results temper this interpretation. First, the conserved C-terminal functional domain of Sfh1p lacks the ability to activate. Second, under some conditions, activation by Sfh1p is dependent on Snf5p and Snf2p, which are believed to be neither part of nor associated with RSC. One interpretation is that overexpressed Sfh1p forces a nonphysiological association with Snf/Swi, probably through weakly conserved N-terminal sequences. Hence, Sfh1p becomes a transcriptional activator dependent on Snf/Swi function. Alternatively, Sfh1p is a weak transcriptional activator that activates synergistically with multiple DNA-binding sites. Several essential cellular processes require the temporal and disporal regulation of chromatin structure that occurs as cells progress through the cell division cycle. In addition to transcription by RNA polymerases I, II, and III, these include DNA replication, DNA repair and recombination, epigenetic regulation, and retroelement integration (reviewed in reference 15). Any of these might require multiprotein complexes with properties similar to those of Snf/Swi or RSC. The specific for nucleosome restructuring may be conferred by non-"conserved sequences in Sfh1p, by other Snf/Swi paralogs, or by other, unrelated components of these complexes.

Sfh1p and Sth1p are required for progression through G2/M: possible targets. At least three models can be proposed to explain the G2/M arrest phenotype caused by the sfh1-1 mutation. The first is that Sfh1p plays a direct role in modulating cell cycle progression. In this model, the Sfh1p cell cycle function is not shared by RSC. The second model is that decreased levels of the Sth1 protein in sfh1 mutants prevent cells from progressing through G2/M; Sfh1p thereby plays an indirect role in regulating the cell cycle. A preliminary result argues against this model: Sfh1p expressed from a high-copy number 2μm plasmid failed to suppress the temperature-sensitive growth arrest caused by the sfh1-1 allele (data not shown). A third possibility, which we favor, is that RSC is required for progression through the G2/M transition of the cell division cycle. According to this model, mutation of critical RSC components impairs RSC function or results in decreased levels of RSC, either of which causes a cell cycle arrest phenotype. We show here that Sfh1p is tightly associated with RSC. A analysis of the DNA content in sfh1-1ts cells shows an accumulation of cells in the G2/M phase at the restrictive temperature. Similar studies of an sh1-3ts strain also demonstrated an accumulation of cells with a 2N DNA content at the restrictive temperature (17), consistent with STH1 deletion experiments (17, 70). These results indicate that two key subunits of the RSC chromatin-remodeling complex, Sfh1p and Sh1p, are required for progression through the mitotic cell division cycle. This provides the first genetic clue into possible cell cycle targets of Sfh1p and Sh1p and further distinguishes RSC from the Snf/Swi complex. In this regard, it is interesting that the human Snf2p/Sfh1p homologs, BRG1 and hbrm, interact with the retinoblastoma protein family of proteins and cooperate with these proteins in regulating cell cycle progression (18, 66). It is therefore possible that other Snf/Swi-related complexes also function in the cell cycle. A rigorous test of this model will require experiments that address whether RSC activity is altered in sh1 cell cycle mutants and whether RSC activity fluctuates during the cell cycle. If RSC function is unaltered in sh1 cell cycle mutants and is not periodic in the cell cycle, the first model is supported.

The simplest explanation for a cellular G2/M arrest according to the third model is that RSC activity is crucial for transcription of a distinct gene or set of genes that functions at the G2/M transition of the cell cycle, such as the B-type cyclins. The ability of the yeast cell to progress through the mitotic cell division cycle requires the coordinate and highly regulated transcription of several genes (36). Temperature-dependent conformational changes in the mutant Sfh1p or Sh1p proteins might impair productive interactions with gene-specific transcription factors necessary for cell cycle progression. Importantly, several yeast TAF15s (TATA-box-binding protein-associated factors) have been shown recently to be required for progression through the cell cycle (3, 50, 74). The dynamic phosphorylation of Sfh1p may represent a direct connection to its requirement for progression through mitosis. Protein phosphorylation plays a major role in regulating the periodicity of cell cycle functions. The G2-specific phosphorylation of Sfh1p implicates kinases that are active at this stage. A possibility is that Sfh1p is phosphorylated by a Cdc28p kinase. Sfh1p is dephosphorylated in the S phase and mitosis, and SFH1 function is required in mitosis, suggesting that phosphorylation negatively regulates Sfh1p function. If so, dephosphorylation of Sfh1p by a phosphatase active in S phase or mitosis may be required to render Sfh1p functional. For example, dephosphorylation of the S. pombe cdc2 protein at G2/M by the cdc25 phosphatase activates its function, permitting cells to progress through mitosis (22, 60). Significantly, hbrm, BRG1, and CHD1, three mammalian proteins highly related by sequence to Sh1p, are excluded from condensed chromosomes during mitosis (51, 65), and in the case of hbrm and BRG1, exclusion correlates with early mitotic phosphorylation. A similar mechanism, although opposite in modification (dephosphorylation), may regulate the activities of Sh1p, Sh1p, or RSC. Mitotic exclusion of transcription factors has been proposed as a mechanism for transcriptional regulation (47). Discovery of the kinase(s) and phosphatase(s) responsible for regulating Sh1p activity will be informative in further characterizing the cell cycle regulation of RSC.

Alternatively, it is possible that the arrest observed in sh1 and sh1 temperature-sensitive strains results from activation of any one of several independent G2/M-phase checkpoint pathways in S. cerevisiae. One monitors the integrity of DNA at entry into mitosis (the RAD9-dependent pathway) (76), and another monitors the integrity of the spindle apparatus at exit from mitosis (31, 46). A third monitors bud formation (45). A link to any of these checkpoints can be tested by genetic analysis of interactions with previously identified mutations affecting these checkpoints. Significantly, mutation of lodestar, a Snf2/Sh1p homolog in Drosophila, leads to chromatic tangling and fragmentation at anaphase (21, 77), defects that trigger mitotic checkpoints in yeast. The morphology of sh1-1ts and sh1-3ts cells at the restrictive temperature is compatible with a model in which RSC function is required for chromosome dynamics during the cell cycle.

The convergence of genetic studies on Sh1p and biochemical studies on RSC identified Sfh1p as a subunit of RSC and establishes a molecular link between nucleosome remodeling and the cell division cycle. Although specific cellular targets of RSC have yet to be identified, the phenotypes resulting from the inactivation of two key RSC subunits suggest cell cycle factors as a class of target. Genetic analysis of additional sh1 and sh1 conditional alleles will provide further insight into the
mechanism of RSC chromatin-remodeling activity and its reg-
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