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

YIXUE CAO,¹ BRADLEY R. CAIRNS,^{2†} ROGER D. KORNBERG,² AND BREHON C. LAURENT^{1*}

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

Received 27 December 1996/Returned for modification 6 February 1997/Accepted 13 March 1997

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-restructuring 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 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/TAF30p, 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, 75). 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 RNA 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 *SUC2* (*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 relieving repression 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. Analysis 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),

* Corresponding author. Mailing address: Morse Institute of Molecular Biology and Genetics, Box 44, 450 Clarkson Ave., Brooklyn, NY 11203. Phone: (718) 270-3755. Fax: (718) 270-2656. E-mail: laurent@hscbklyn.edu.

† Present address: Department of Genetics, Harvard Medical School, Boston, Mass.

TABLE 1. *S. cerevisiae* strains used

Strain ^a	Genotype
BLY1	<i>MATα his3-Δ200 lys2-801 ura3-52 SUC2</i>
BLY3	<i>MATα snf5-Δ2 his3-Δ200 ura3-52 ade2-101</i>
BLY4	<i>MATα snf2-141 his3-Δ200 ura3-52</i>
BLY65	<i>MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63</i>
W303	<i>MATα ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1</i>
BLY18	<i>MATα his3-Δ200/his3-Δ200 ura3-52/ura3-52 ADE2/ade2-101 LYS2/lys2-801</i>
CTY10-5d	<i>MATα ade2-101 his3Δ200 ura3-52 leu2-3,112 trp1-901 gal4 gal80 URA3::lexAo-lacZ</i>
BLY46	<i>MATα ade2-101 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</i>
BLY68	<i>MATα sfh1-Δ1::LEU2/SFH1 his3-Δ200/his3-Δ200 ura3-52/ura3-52 leu2Δ1/leu2Δ1 trp1Δ63/trp1Δ63</i>
BLY69	<i>MATα sfh1-Δ1::LEU2/SFH1 ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1</i>
BLY70	<i>MATα sfh1-1::HIS3/SFH1 his3-Δ200/his3-Δ200 ura3-52/ura3-52 ade2-101/ADE2 lys2-801/LYS2</i>
BLY71	<i>MATα sfh1-1::HIS3/SFH1 ade2-1/ade2-1 can1-100/can1-100 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/trp1-1 ura3-1/ura3-1</i>
BLY73	<i>MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</i>
BLY74	<i>MATα sfh1-1::HIS3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1</i>

^a Strains BLY1, BLY3, BLY4, BLY65, BLY68, and BLY70 have the S288C genetic background, and BLY46, BLY69, BLY71, BLY73, and BLY74 are derived from W303. BLY1, BLY3, and BLY4 are the same as strains MCY829, MCY2099, and MCY2156, respectively, from M. Carlson. BLY65 is FY250 $\times$ FY251 (FY250 and FY251 were obtained from F. Winston). W303 was obtained from R. Rothstein. CTY10-5d was constructed by R. Sternglanz.

and *Caenorhabditis elegans*, *Schizosaccharomyces pombe*, and *S. cerevisiae* proteins. Insight into Snf5p function is provided by experiments with the human and *D. melanogaster* homologs. Ini1/hSNF5 interacts with the human immunodeficiency virus integrase and stimulates its activity in vitro (33) and also binds to two human Snf2p relatives, BRG1 and hbrm (33, 52). snr1 is a component of a large Snf/Swi-related complex in *Drosophila* which also contains brm, and it is important for transcription of homeotic and other genes during development (16).

Here we present genetic and biochemical evidence demonstrating that the essential Sfh1 protein cooperates with an essential Snf2p paralog as a member of a novel ATP-dependent nucleosome-restructuring 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 remodeling 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 [YEPD]) and synthetic complete (SC) or SC-rafinoase media contained 2% sugars (62). Standard genetic methods were followed (62). The *Escherichia coli* strains used were XL1-Blue (Stratagene) and GM48 (*F⁻ thr leu thi lacY galK galT ara fhuA tsx dam dcm supE44*).

Cloning of the *S. cerevisiae* *SFH1* gene. To clone *SFH1*, oligonucleotides ySNF5h-03 (5'-GCGGATCCCCGATGTCGACCAAAACC-AG-3') and ySNF5h-04 (5'-ACGCGTCGACTACATTCTCATTGTGGT-3') were used to prime the PCR amplification of a 1.3-kb *Bam*HI-*Sal*I fragment of the *SFH1* coding region from yeast genomic DNA (S288C). Plasmid pYC5H was recovered by screening a yeast genomic DNA library cloned in YEp24 (12) by colony hybridization with the amplified DNA fragment.

Plasmids. LexA hybrid plasmids are derivatives of pSH2-1 (27) and express, from the constitutive *ADHI* promoter, the amino-terminal 87 residues of the LexA protein fused to portions of Sfh1p, Snf5p, or Sth1p. pLexA-Sfh1, encoding LexA fused to the entire coding region of *SFH1*, including amino acids 1 to 426, was created by cloning the *SFH1* gene by PCR amplification of yeast genomic DNA (S288C), primed by ySNF5h-03 and ySNF5h-04, in pSH2-1. The resulting plasmid complemented the lethality of an *sfh1* mutation. pLexA-Sfh1₁₋₁₈₉ was constructed by cloning the 0.6-kb *Bam*HI-*Bcl*I (Klenow fragment-treated) fragment of pLexA-Sfh1 into pSH2-1, previously digested with *Bam*HI and *Sal*I (ends blunted). pLexA-Sfh1₁₈₉₋₄₂₆ contains the 0.7-kb *Bcl*I (Klenow fragment-treated)-*Sal*I fragment of pLexA-Sfh1 in pSH2-1, digested with *Bam*HI (Klenow fragment treated) and *Sal*I. pLexA-Sfh1₁₋₂₄₂ contains the 0.7-kb *Bam*HI-*Bgl*II (Klenow fragment-treated) fragment of pVP16-Sfh1 in pSH2-1. pLexA-Snf5 (11) expresses LexA fused to the entire *SNF5* coding region, including amino

acids 1 through 905. pLexA-Sth1₁₋₁₃₃₅ [previously called pLexA-STH1(1-1330)] (44), encodes LexA residues 1 to 87 fused to residues 1 to 1335 of Sth1p. pLexA₂₀₂-Sth1₁₋₁₃₅₉ was created by ligating the 2-kb *Eco*RI-*Age*I fragment of pLexA-Sth1₁₋₁₃₃₅, the 3.4-kb *Age*I-*Sma*I fragment of pBL50 (44), and pEG202 (a gift of E. Golemis and R. Brent), previously digested with *Eco*RI and *Bam*HI (*Bam*HI ends filled in), and encodes LexA residues 1 to 220 fused to the entire *STH1* coding region, including amino acids 1 to 1359. Both pLexA₂₀₂-Sth1₁₋₁₃₅₉ and pLexA-Sth1₁₋₁₃₃₅ complemented the lethality of an *sth1* 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 *Bam*HI-*Sal*I fragment (*Sal*I ends Klenow fragment treated) of pLexA-Sfh1 and the *Bam*HI-*Eco*RI fragment (*Eco*RI ends Klenow fragment treated) of pVP16 (73), resulting in fusion of the nucleus-localized acidic activation domain of VP16 to the entire coding region of Sfh1p. pVP16-Sfh1N contains the 0.6-kb *Bam*HI-*Bcl*I (ends filled in) fragment of pLexA-Sfh1, encoding amino acids 1 through 189, in pVP16 digested previously with *Bam*HI and *Eco*RI (Klenow fragment treated). pVP16-Sfh1C was created by removing the 0.6-kb *Bam*HI-*Bcl*I 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-Sfh1N2 contains the 0.7-kb *Bam*HI-*Bgl*II (Klenow fragment-treated) fragment of pVP16-Sfh1 in pVP16. pVP16-Sfh1 complemented the *sfh1-1::HIS3* mutation.

pDJ91 (17) is the *Eco*RI-*Nof*I fragment of pLexA₂₀₂-Sth1₁₋₁₃₅₉ cloned into pYES2 (Invitrogen). *STH1* in this orientation can be transcribed from the T7 RNA promoter by T7 RNA polymerase. pGST-Sfh1 contains the 1.3-kb *Sma*I-*Sal*I fragment of pLexA-Sfh1 in pGEX-4T-1 (Pharmacia).

Disruption of the *SFH1* chromosomal locus. pYC1 was constructed in two steps. In the first step, the 2.4-kb *Xba*I-*Hind*III fragment of pYC5H was cloned into pPL1.1 (a gift of P. Lesage), a pUC19 derivative containing the *Bgl*II fragment of *LEU2* cloned into the *Bam*HI site, creating pYC500. In the second step, the 1.8-kb *Sac*I-*Hind*III (Klenow fragment-treated) fragment of pYC5H was cloned into pYC500, digested with *Sac*I-*Sma*I, creating pYC1. pYC1 results in the replacement of the *SFH1 Hind*III-*Xba*I fragment of pYC5H (encoding amino acids 12 through 410) with a *Sma*I-*Xba*I *LEU2* fragment. The 7.0-kb *Sac*I-*Hind*III fragment of pYC1 was used to transform diploid strains BLY29 and BLY65.

To construct pYC2, we first cloned the 1.7-kb *Xba*I fragment of pYC5H in pBLU Δ H. A *Bam*HI fragment harboring the yeast *HIS3* gene was then inserted at the unique *Bgl*II site within the coding sequence of *SFH1* to create pYC2. 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 BLY18 and BLY29 were transformed with the 3.5-kb *Xba*I fragment of pYC2. 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-Sfh1₅₇₋₄₂₆ expresses a GST-Sfh1 fusion protein constructed by inserting the 1.1-kb *Eco*RI-*Sal*I fragment of pLexA-Sfh1 (encoding amino acids 57 to 426) in pGEX-4T-1 (Pharmacia). *E. coli* cells transformed with pGST-Sfh1₅₇₋₄₂₆ 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 GST-bound nitrocellulose membranes. Antibodies against Sfh1p were collected from the unbound material by incubation with nitrocellulose strips con-

A

190 455 676 905
1 Snf5p (*S. cerevisiae*)

142 184 377 384
1 Ini1 (human)

126 168 370 370
1 SNR1 (*Drosophila*)

137 179 373 529
1 R07E5.3 (*C. elegans*)

62 140 385 632
1 C2F7.08C (*S. pombe*)

108 201 404 426
1 Sth1p (*S. cerevisiae*)

[illegible]

C

MSHQNQLIPQAYISFNHNRLTNEDDGIPFTMAQQRTHKRAKVVNYAEY 50

△

DNDLDFEFNMNGSNFNADTHYKDNASHENTPALNGVTMDGSEYNVLE 100

NNMGADSIISNNKYDAGSNMVVESLGLSNNNASNGPSNKAQADIGNA 150

VL PDLQDQHHPFNITLRYPKIRDTFINGKVVSPYRLNTDQETKANANSGE 200

.....

Rep1 ▽

[AIMIPITLDEIHHGHTIKDQLFWNYNDSDISPEEFASYICKDLMTSATL 250

Rep2

QTQIANIIEKQLDLENTAAEIMSDLHVIIINTCNLQDR[FEDFNQWNL 300

NKDSLTPERFATSVQDLGLTRFPIPLISQSLHETILIKKKDWVDGHLIQ 350

.....

DHPVNDAAFGLSGIRLDIDELGSNWCPRVEILTKEEIQKREIEKERNLR 400

[RLKRFIDRLSRGRRRLDDLETTMRM 426

△

D

Ini1
SNR1
RO7E5.3
Snf5p
C2F7.08C
Sfh1p

FIG. 2. *SFH1* is essential for mitotic growth of yeast cells. (A) The *sfh1-1* allele confers a temperature-sensitive phenotype on W303 cells. Growth of isogenic *SFH1* wild-type (BLY73) and *sfh1-1* temperature-sensitive (BLY74) 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* (BLY74) and *SFH1* (BLY73) 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-Snf5₁₋₁₉₃, or GST-Sfh1 affinity resin. After rocking at 4°C for 2 h, the beads were washed five times with 1 ml of MTPBS (150 mM NaCl, 16 mM Na₂HPO₄, 4 mM NaH₂PO₄ [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-Sephacel, hydroxylapatite, Mono Q, TSK-heparin, and Mono S (10). The peak fraction from Mono Q is approximately 50% pure, as estimated from Coomassie blue-stained gels. Immune complexes of RSC with anti-Sfh1p antibodies were prepared with the peak Mono Q fraction and anti-Sfh1p antibodies conjugated to protein A-Sepharose. Conjugation was performed as described previously for anti-Snf5p (8). Immune complexes (50-μl total volume) were formed in buffer A (20 mM Tris-acetate [pH 7.5], 10% glycerol, 0.1 mM dithiothreitol, 1 mM EDTA, 0.1% Nonidet P-40) containing 100 mM potassium acetate with peak 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 logarithmically growing BLY74 (*sfh1-1ts*) cells and wild-type cells (for comparison) were split, diluted to a density of 4×10^6 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 Na₂HPO₄, 20 mM NaH₂PO₄, 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 BLY74 and the isogenic wild-type strain BLY73 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

various time intervals, approximately 2×10^7 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 nutator. 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 TMN buffer (200 mM Tris-HCl [pH 7.5], 211 mM NaCl, and 78 mM MgCl₂) and then resuspended in TMN buffer containing 55 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 FACSscan machine and analyzed with LYSIS II software.

Cell synchronization. Mid-log-phase cultures of BLY46 cells were arrested in G₁, S, or G₂/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 H₂O 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 YSCL8543 (*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 MEGALIGN program (DNASTAR, Inc.).

Nucleotide sequence accession number. The *SFH1* sequence was entered with the sequence of *S. cerevisiae* chromosome XII into the EMBL data library under accession no. U20618 and S53399. The GenBank accession numbers for C2F7.08C and RO7E5.3, the putative *SNF5* homologs in *S. pombe* and *C. elegans*, are 1175371 and CERO7E54, respectively.

RESULTS

***SFH1* is a unique and essential *SNF5* paralog.** The Snf5 protein sequence is significantly similar to polypeptides encoded by open reading frames in human (33), *Drosophila* (16), *C. elegans* (EMBL accession number Z32683), and *S. pombe* (EMBL accession number Z50142) cells. These *SNF5* 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, L8543.3, with significant similarity to the conserved region of Snf5p (BLAST *P* value, 3.7×10^{-21}) was identified, and the corresponding gene was cloned. The L8543.3 gene, designated here *SFH1* (for *SNF5* 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 W303 genetic backgrounds with *LEU2* or with the disruption allele created by inserting *HIS3* at codon 242 (Fig. 1C). *Leu*⁺ transformants of both genetic backgrounds were sporulated and subjected to tetrad analysis. From 14 dissected asci, only two viable *Leu*⁺ spore clones were recovered in each case. In viable 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 asci. However, four viable spore clones were recovered

ered from dissected tetrads from the W303 background: two His⁻ spore clones which grew as well as wild-type cells and two His⁺ spores which grew very slowly. The His⁺ strains were thermosensitive and failed to grow at 37°C (Fig. 2A). These results indicate that in the W303 strain, the amino-terminal 242 amino acids of Sfh1p are partially functional at the permissive temperature (25°C) but fail to provide *SFH1* function at the restrictive temperature (37°C). Subsequently, we have recovered viable His⁺ temperature-sensitive spore clones 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 Sfh1-1p than for Sfh1p may be due to the fact that Sfh1-1p contains only one-half of the amino acids of Sfh1p used as immunogen or, alternatively, to the fact that Sfh1-1p is unstable.

To assess whether *SFH1* and *SNF5* function in redundant pathways, a *snf5-Δ2* strain (BLY3) 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 *ADHI* promoter, and tested for the ability to grow on SC-raffinose plates. Neither plasmid complemented the *snf5* mutation for growth on raffinose. In the converse experiment, when an *sfh1-Δ1::LEU2/SFH1* heterozygous diploid carrying a multi-copy 2μm plasmid expressing *SNF5*, pJW34 (1), was sporulated and dissected, only two viable Leu⁻ spores were recovered. pJW34 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-Δ1* mutation for viability. Only two viable Leu⁻ spores were recovered from each ascus following sporulation and tetrad dissection of an *sfh1-Δ1::LEU2/SFH1* diploid strain transformed with pLexA-Sfh1₁₋₁₈₉. In contrast, four viable spores were obtained from diploids transformed with pLexA-Sfh1₁₈₉₋₄₂₆. 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 Sfh1p N-terminal 189 amino acids fail to complement *sfh1-Δ1*, Sfh1-1p (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 In1l 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. Signifi-

TABLE 2. LexA-Sfh1p activates transcription in vivo^a

Protein	β-Galactosidase activity (U) ^b				
	<i>lexA-GAL1-lacZ</i>			<i>lexA-CYC1-lacZ</i>	
	0 operators	1 operator	6 operators	0 operators	1 operator
LexA-Snf5p	<1	455	783	<1	309
LexA-Sfh1p	<1	199	710	<1	344
LexA-Sfh1 ₁₋₁₈₉	<1	166	1,317	ND ^c	ND
LexA-Sfh1 ₁₋₂₄₂	<1	424	478	ND	ND
LexA-Sfh1 ₁₈₉₋₄₂₆	<1	<1	<1	ND	ND
LexA	<1	<1	<1	<1	<1

^a Activation 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 UAS_C) and *CYC1* (zero or one *lexA* operator; UAS_{C1} and UAS_{C2} deleted) promoters, as described in Materials and Methods.

^b The values reported are the averages for at least four transformations performed in duplicate with <10% error.

^c ND, not determined.

cantly, 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).

Sfh1p functions with Sth1p, an essential Snf2p-related protein. Several lines of genetic evidence support the existence of a functional interaction between Sfh1p and Sth1p, a member of the Snf2p/Swi2p family of DNA-dependent ATPases that is also essential for viability in yeast. First, we noted that Sth1p levels in an *sfh1-1ts* strain were dramatically reduced, even at the semi permissive temperature, compared to those in isogenic *SFH1* cells, as judged by immunoblot analysis (Fig. 3A). In contrast, comparable levels of Snf5p were detected in the two strains by using an anti-Snf5p antibody. This result is consistent with the model that Sth1p and Sfh1p are components of the same protein complex and suggests further that Snf5p is not shared between this complex and Snf/Swi. Second, if the *SFH1* and *STH1* genes function in the same pathway or encode products that associate in a complex, we predicted that the phenotype of a strain bearing conditional mutations in both genes should be no more abnormal than that of cells bearing mutations in either gene. *sfh1-1ts sth1-3ts* double mutants (17) were viable and temperature sensitive for growth, a phenotype indistinguishable from that of the single *sfh1-1ts* or *sth1-3ts*

TABLE 3. Transcriptional activation by LexA-Sfh1p in *snf* mutants is dependent on the number of *lexA* operators^a

Protein	β-Galactosidase activity (U) ^b					
	<i>snf2</i>			<i>snf5</i>		
	0 operators	1 operator	6 operators	0 operators	1 operator	6 operators
LexA-Sfh1p	<1	16	376	<1	12	630
LexA-Snf2p	<1	86	380	<1	2	8
LexA-Snf5p	<1	4	8	<1	247	762

^a Activation by LexA-Sfh1p, LexA-Snf2p, and LexA-Snf5p fusion proteins was assessed with *lexA* operators at the *GAL1* (zero, one, or six overlapping *lexA* operators) promoter in *snf2* or *snf5* mutants, as described in Materials and Methods. Values for LexA-Snf2p and LexA-Snf5p in *snf5* and *snf2* mutants, respectively, are from reference 41.

^b Values are averages for at least four transformants. Standard errors were <10%.

FIG. 3. Sfh1p functions with the Snf2p paralog Sth1p. (A) Sth1p levels are decreased in *sfh1-1* cells. Immunoblot analysis of *sfh1-1* (BLY74) and isogenic *SFH1* wild-type (BLY73) cells grown at 30°C with anti-Sth1p or anti-Snf5p antibodies is shown. (B) Sfh1p and Sth1p interact in vivo in the two-hybrid assay. Proteins were expressed from pSH2-1 (LexA₈₇) (27), pVP16 (75), or derivatives. The CTY10-5d reporter strain containing an integrated *lexA0-GAL1-lacZ* target gene was doubly transformed with the plasmids. β -Galactosidase activity is expressed in Miller units (49). Values are averages for at least four independent transformants. Standard errors were <10%. (C) Direct binding of Sfh1p to Sth1p in vitro. Partially purified GST-Sfh1, GST-Snf5₁₋₁₉₃, and GST (500 ng of each) were immobilized on glutathione-agarose beads, and reticulocyte lysates containing in vitro-translated ³⁵S-labelled luciferase (Luc) (lane 1) or Sth1p (lane 2) were allowed to bind. Bound proteins were released and electrophoresed on an SDS-8% polyacrylamide gel, which was followed by autoradiography (lanes 3 to 6). The amount of ³⁵S-labelled Sth1p or luciferase in lanes 1 and 2 was 10% of that used for the binding assays (lanes 3 to 6). GST, GST-Snf5₁₋₁₉₃, or GST-Sfh1 proteins were separated by electrophoresis on SDS-10% polyacrylamide gels (lanes 7 to 9) and stained with Coomassie blue. Size standards are indicated in kilodaltons.

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 β -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), inter-

acted with Sth1p nearly as strongly as the full-length Sfh1p (Fig. 3B). Significantly, an amino-terminal derivative containing an additional 54 amino acids, amino acids 1 to 242 (VP16-Sfh1N2), also interacted with Sth1p, although more weakly than the carboxy-terminal derivative. These findings indicate that Sfh1p and Sth1p interact in vivo and implicate amino acids 201 to 242 of the conserved domain in this protein-protein interaction. 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 ³⁵S-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

FIG. 4. Sfh1p is a component of purified RSC complex. (A) Immunoblot analysis of Mono Q fractionation. Fractions (4 μ g) were separated on an SDS-9% acrylamide gel and immunoblotted with anti-Sth1p, -Rsc6p, or -Sfh1p antibodies. (B) Immunoblot analysis of the peak Mono Q fraction (RSC) and whole-cell extracts prepared from wild-type (*SFH1*) and *sfh1-1* cells. Purified RSC complex proteins (0.3 μ g) and total yeast cell proteins (30 μ g) were separated on an SDS-9% acrylamide gel and immunoblotted with anti-Sfh1p antiserum. (C) Immunoblot analysis of anti-Sfh1p immunoprecipitates (IP). Immune complexes were prepared from the peak Mono Q fraction (20 μ g; 50% pure) and either anti-Sfh1p antibodies (lanes 3 and 4) or control antibodies (anti-Snf6p) (lanes 5 and 6) conjugated to protein A-Sepharose. Immune complexes were washed extensively with buffer A containing 200 mM potassium acetate and eluted with 5 M urea (see Materials and Methods). Samples were separated on an SDS-10% acrylamide gel and immunoblotted with anti-Sth1p, -Rsc6p, or -Sfh1p antibodies. Sup., supernatant.

not to control proteins GST and GST-Snf5₁₋₁₉₃, 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 Sth1p 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 (~50% pure) were immunoblotted with anti-Sfh1p antiserum. Polypeptides with identical molecular masses (54 kDa) were detected in wild-type cell extracts and in purified RSC but were absent in *sfh1-1ts* cell extracts (Fig. 4B). A slower-migrating, 56-kDa form of Sfh1p present in wild-type cell extracts is barely detected in purified RSC, raising the possibility that either the 54-kDa species is the predominant form of the protein in RSC or that modification was lost during the purification.

If Sfh1p is a tightly associating component of RSC, anti-

Sfh1p antibodies should immunoprecipitate other RSC component proteins. To test this, immune precipitates were formed with the peak Mono Q fraction and anti-Sfh1p antiserum conjugated to protein A-Sepharose beads. Immune precipitates were washed extensively, and pellets and eluates were examined by immunoblotting with anti-Sth1p, anti-Rsc6p (10), and anti-Sfh1p antibodies. Sth1p, 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 immunoprecipitating RSC components (Fig. 4C, lanes 5 and 6). The anti-Sfh1p antibody did not entirely deplete the peak Mono Q fraction of these proteins, as approximately half of these proteins remained in the supernatant following 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).

Examination of the DNA content of *sfh1-1ts* mutants by flow

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).

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 asynchronous 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.

FIG. 6. Sfh1p is differentially phosphorylated during the cell cycle. (A) Sfh1p is a phosphoprotein. Whole-cell extracts from asynchronous log-phase cells were incubated at 30°C for 0.5 h in the presence (lanes 2 and 3) or absence (lane 1) of 400 U of lambda protein phosphatase (λ -PP) (New England Biolabs). Phosphatase inhibitors (Inhib) were added in lane 3. The phosphorylated form of Sfh1p is designated Sfh1-P. Extracts were analyzed by immunoblotting with anti-Sfh1p antibodies. (B) Sfh1p is dephosphorylated during S phase and mitosis. Cells were arrested in G₁ by treatment with mating pheromone, washed to allow release from the arrest, and lysed at the times indicated (0 to 165 min). Lysates were analyzed by immunoblot analysis with anti-Sfh1p and anti-Snf5p antibodies. The phosphorylated form of Sfh1p is designated Sfh1-P. Lane A, lysate from asynchronous log-phase cells. (C) Cell budding morphologies were determined microscopically for asynchronously growing cells (A) and at each time point following α -factor arrest (as described for panel B; 0 to 165 min). (D) The phosphorylated form of Sfh1p is not detected in cells arrested in the S or G₂/M phase of the cell cycle. Protein extracts prepared from asynchronous cells (lane A) and cells treated with hydroxyurea (lane HU) or nocodazole (lane N) were analyzed by immunoblotting with anti-Sfh1p antibodies. Sfh1-P, phosphorylated form of Sfh1p. (E) Cell budding morphologies were determined microscopically for the asynchronously growing cells (A) and for the hydroxyurea (HU)- and nocodazole (N)-treated cells shown in panel D.

Immunoblot analysis showed the presence of the phosphorylated Sfh1p band in the G₁ phase (Fig. 6B and C, 0 to 30 min). Upon entry into the S phase and mitosis, however, Sfh1p is dephosphorylated, as evidenced by the disappearance of the 56-kDa band and an increase in the intensity of the faster-migrating 54-kDa Sfh1p species (Fig. 6B and C, 60 to 105 min). Phosphorylated Sfh1p is detected again 150 min following release from α -factor arrest. In contrast, neither the form nor the level of Snf5p changes during the cell cycle, as revealed by immunoblot analysis with an anti-Snf5p antibody. To confirm that phosphorylation of Sfh1p is periodic in the cell cycle, the Sfh1p phosphorylation pattern was examined in cells arrested in both the S and G₂/M phases by treatment with hydroxyurea or nocodazole, respectively (Fig. 6D and E). The phosphorylated Sfh1p band detected in G₁-arrested and asynchronously grown cells (Fig. 6B and D) is absent in S- or G₂/M-arrested cells (Fig. 6D), in agreement with the α -factor release experiments which showed that this band is lost as synchronized cells leave G₁ (Fig. 6B). These findings demonstrate that Sfh1p phosphorylation is regulated during the cell cycle and suggest a correlation between dephosphorylation of Sfh1p and entry into the G₂/M transition.

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 protein family varies considerably. For example, Sfh1p shows greater similarity to human Ini1 (BLAST *P* value, 2.7×10^{-25}) than to yeast Snf5p (BLAST *P* value, 7.2×10^{-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 Sth1p, thereby suggesting that the capacity to bind Sth1p is an essential function of Sfh1p. Moreover, the corresponding region of the human Ini1/hSNF5 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 restructure chromatin.

Sfh1p functions with Sth1p in a novel nucleosome-remodeling complex. We demonstrate by several lines of genetic and biochemical evidence that Sfh1p functions with the essential Snf2p paralog, Sth1p, and is a component of RSC, an ATP-dependent nucleosome-destabilizing complex comprised of 15 polypeptides, including Sth1p (10). Our data, in agreement with those of Cairns et al. (10), suggest that several of the members of the Snf/Swi complex are not shared with RSC. The importance of this novel nucleosome-remodeling complex can be appreciated by a direct comparison to Snf/Swi: in the absence of Snf/Swi, RSC and possibly other chromatin-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 disruption 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 specificity 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: Sth1p expressed from a high-copy-number 2 μ m 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. 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 *sth1-3ts* strain also demonstrated an accumulation of cells with a 2N DNA content at the restrictive temperature (17), consistent with *STH1* depletion experiments (17, 70). These results indicate that two key subunits of the RSC chromatin-remodeling complex, Sfh1p and Sth1p, are required for progression through the mitotic cell division cycle. This provides the first genetic clue into possible cell cycle targets of Sfh1p and Sth1p and further distinguishes RSC from the Snf/Swi complex. In this regard, it is interesting that the human Snf2p/Sth1p 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 *sfh1* cell cycle mutants and whether RSC

activity fluctuates during the cell cycle. If RSC function is unaltered in *sfh1* 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 Sth1p proteins might impair productive interactions with gene-specific transcription factors necessary for cell cycle progression. Importantly, several yeast TAF_{II}s (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 G₁-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 Sth1p, 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 Sfh1p, Sth1p, 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 Sfh1p activity will be informative in further characterizing the cell cycle regulation of RSC.

Alternatively, it is possible that the arrest observed in *sfh1* and *sth1* 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/Sth1p homolog in *Drosophila*, leads to chromatid tangling and fragmentation at anaphase (21, 77), defects that trigger mitotic checkpoints in yeast. The morphology of *sfh1-1ts* and *sth1-3ts* 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 Sfh1p 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 *sfh1* and *sth1* conditional alleles will provide further insight into the

mechanism of RSC chromatin-remodeling activity and its regulation in the cell.

ACKNOWLEDGMENTS

We thank Ben Benton for valuable help with the fluorescence-activated cell sorter analysis, Yaacov Anziska for technical assistance, Aaron Mitchell for encouragement, Steve Kurtz and Michelle Treitel for scientific and editorial assistance, Irem Nasir and Jian Du for helpful discussions, Jian Du for reagents, Marian Carlson and Isabelle Treich for comments on the manuscript, and all colleagues for providing yeast strains and plasmids.

This work was funded by grants from the American Cancer Society (NP-871) and the March of Dimes Basil O'Connor Starter Scholar Research Award (5-FY95-1066) to B.C.L. and by National Institutes of Health (NIH) grant GM36659 and a grant from the March of Dimes to R.D.K. B.R.C. was a Fellow of the Leukemia Society of America.

REFERENCES

- 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.
- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**:403-410.
- Apone, L. M., C. A. Virbasius, J. C. Reese, and M. R. Green. 1996. Yeast TAF_{II}90 is required for cell-cycle progression through G₂/M but not for general transcription activation. *Genes Dev.* **10**:2368-2380.
- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1995. Current protocols in molecular biology. John Wiley and Sons, Inc., Boston, Mass.
- Bortvin, A., and F. Winston. 1996. Evidence that Spt6p controls chromatin structure by a direct interaction with histones. *Science* **272**:1473-1476.
- Brent, R., and M. Ptashne. 1985. A eukaryotic transcriptional activator bearing the DNA specificity of a prokaryotic repressor. *Cell* **43**:729-736.
- Cairns, B. R., N. L. Henry, and R. D. Kornberg. 1996. TFG3/TAF30/ANC1, an integral component of the yeast SWI/SNF complex and a homolog of the leukemogenic proteins ENL and AF-9. *Mol. Cell. Biol.* **16**:3308-3316.
- 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.
- Cairns, B. R., R. S. Levinson, K. R. Yamamoto, and R. D. Kornberg. 1996. Essential role of Swp73 in the function of yeast Snf/Swi complex. *Genes Dev.* **10**:2131-2144.
- Cairns, B. R., Y. Lorch, Y. Li, M. Zhang, L. Lacomis, H. Erdjument-Bromage, P. Tempst, J. Du, B. Laurent, and R. D. Kornberg. 1996. RSC, an essential, abundant chromatin-remodeling complex. *Cell* **87**:1249-1260.
- Cao, Y., and B. C. Laurent. 1996. Unpublished data.
- Carlson, M., and D. Botstein. 1982. Two differentially regulated mRNAs with different 5' ends encode secreted and intracellular forms of yeast invertase. *Cell* **28**:145-154.
- Carlson, M., and B. C. Laurent. 1994. The SNF/SWI family of global transcriptional activators. *Curr. Opin. Cell Biol.* **6**:396-402.
- Côté, J., J. Quinn, J. L. Workman, and C. L. Peterson. 1994. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265**:53-60.
- Curcio, M. J., and R. H. Morse. 1996. Tying together integration and chromatin. *Trends Genet.* **11**:436-438.
- Dingwall, A. K., S. J. Beek, C. M. McCallum, J. W. Tamkun, G. V. Kalpana, S. P. Goff, and M. P. Scott. 1995. The *Drosophila* snr1 and brm proteins are related to yeast SWI/SNF proteins and are components of a large protein complex. *Mol. Biol. Cell.* **6**:777-791.
- Du, J., and B. C. Laurent. 1996. Unpublished data.
- Dunaief, J. L., B. E. Strober, S. Guha, P. A. Khavari, K. Alin, J. Luban, M. Begemann, G. R. Crabtree, and S. P. Goff. 1994. The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. *Cell* **79**:119-130.
- 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.
- Felsenfeld, G. 1996. Chromatin unfolds. *Cell* **86**:13-19.
- Girdham, C. H., and D. M. Glover. 1991. Chromosome tangling and breakage at anaphase result from mutations in *lodestar*, a *Drosophila* gene encoding a putative nucleoside triphosphate-binding protein. *Genes Dev.* **5**:1786-1799.
- Gould, K. L., and P. Nurse. 1989. Tyrosine phosphorylation of the fission yeast *cdc2⁺* protein kinase regulates entry into mitosis. *Nature* **342**:39-45.
- Grunstein, M. 1990. Nucleosomes: regulators of transcription. *Trends Genet.* **6**:395-400.
- Guarente, L. 1983. Yeast promoters and *lacZ* fusions designed to study expression of cloned genes in yeast. *Methods Enzymol.* **101**:181-191.
- Guarente, L., and M. Ptashne. 1981. Fusion of *Escherichia coli lacZ* to the cytochrome c gene of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **78**:2199-2203.
- Hanes, S., and R. Brent. (Massachusetts General Hospital, Harvard Medical School). 1991. Personal communication.
- 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.
- 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.
- Harlow, E., and D. Lane. 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- 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.
- Hoyt, M. A., L. Totis, and B. T. Roberts. 1991. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* **66**:507-517.
- Imbalzano, A. N., H. Kwon, M. R. Green, and R. E. Kingston. 1994. Facilitated binding of TATA-binding protein to nucleosomal DNA. *Nature* **370**:481-485.
- Kalpana, G. V., S. Marmon, W. Wang, G. R. Crabtree, and S. P. Goff. 1994. Binding and stimulation of HIV-1 integrase by a human homolog of yeast transcription factor SNF5. *Science* **266**:2002-2006.
- Khavari, P. A., C. L. Peterson, J. W. Tamkun, D. B. Mendel, and G. R. Crabtree. 1993. BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. *Nature* **366**:170-174.
- Kingston, R. E., C. A. Bunker, and A. N. Imbalzano. 1996. Repression and activation by multiprotein complexes that alter chromatin structure. *Genes Dev.* **10**:905-920.
- Koch, C., and K. Nasmyth. 1994. Cell cycle regulated transcription in yeast. *Curr. Opin. Cell Biol.* **6**:451-459.
- Kornberg, R. D., and Y. Lorch. 1992. Chromatin structure and transcription. *Annu. Rev. Cell Biol.* **8**:563-587.
- 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.
- Kruger, W., C. L. Peterson, A. Sil, C. Coburn, G. Arents, E. N. Moudrianakis, and I. Herskowitz. 1995. Amino acid substitutions in the structured domains of histones H3 and H4 partially relieve the requirement of the yeast SWI/SNF complex for transcription. *Genes Dev.* **9**:2770-2779.
- 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* **370**:477-481.
- 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.
- 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.
- 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.
- 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.
- Lew, D. J., and S. I. Reed. 1995. A cell cycle checkpoint monitors cell morphogenesis in budding yeast. *J. Cell Biol.* **129**:739-749.
- Li, R., and A. Murray. 1991. Feedback control of mitosis in budding yeast. *Cell* **66**:519-531.
- Martinez-Balbas, M. A., A. Dey, S. K. Rabindran, K. Ozato, and C. Wu. 1995. Displacement of sequence-specific transcription factors from mitotic chromatin. *Cell* **83**:29-38.
- Matalana, E., L. Franco, and J. E. Perez-Ortin. 1992. Chromatin structure of the yeast *SUC2* promoter in regulatory mutants. *Mol. Gen. Genet.* **231**:395-400.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Moghtaderi, Z., Y. Bai, D. Poon, P. A. Weil, and K. Struhl. 1996. TBP-associated factors are not generally required for transcriptional activation in yeast. *Nature* **383**:188-191.
- Muchardt, C., J.-C. Reyes, B. Bourachot, E. Legouy, and M. Yaniv. 1996. The hbrm and BRG-1 proteins, components of the human SNF/SWI complex, are phosphorylated and excluded from the condensed chromosomes during mitosis. *EMBO J.* **15**:3394-3402.
- Muchardt, C., C. Sardet, B. Bourachot, C. Onufryk, and M. Yaniv. 1995. A human protein with homology to *Saccharomyces cerevisiae* SNF5 interacts with the potential helicase hbrm. *Nucleic Acids Res.* **23**:1127-1132.
- 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.
54. Neigeborn, L., and M. Carlson. 1984. Genes affecting the regulation of *SUC2* gene expression by glucose repression in *Saccharomyces cerevisiae*. Genetics **108**:845–858.
 55. 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.
 56. 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.
 57. Peterson, C. L., A. Dingwall, and M. P. Scott. 1994. Five *SWI/SNF* gene products are components of a large multisubunit complex required for transcriptional enhancement. Proc. Natl. Acad. Sci. USA **91**:2905–2908.
 58. 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.
 59. Peterson, C. L., and J. W. Tamkun. 1995. The SWI-SNF complex: a chromatin remodeling machine? Trends Biochem. Sci. **20**:143–146.
 60. Pondaven, P., L. Meijer, and D. Beach. 1990. Activation of M-phase-specific histone H1 kinase by modification of the phosphorylation of its p34^{cdc2} and cyclin components. Genes Dev. **4**:9–17.
 61. 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.
 62. Rose, M. D., F. Winston, and P. Hieter. 1990. Methods in yeast genetics, a laboratory course manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
 63. 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.
 64. 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.
 65. Stokes, D. G., and R. P. Perry. 1995. DNA-binding and chromatin localization properties of CHD1. Mol. Cell. Biol. **15**:2745–2753.
 66. Strober, B. E., J. L. Dunaief, S. Guha, and S. P. Goff. 1996. Functional interaction between the hBRM/hBRG1 transcriptional activators and the pRB family of proteins. Mol. Cell. Biol. **16**:1576–1583.
 67. Tamkun, J. W., R. Deuring, M. P. Scott, M. Kissinger, A. M. Pattatucci, T. C. Kaufman, and J. A. Kennison. 1992. *brhma*: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell **68**:561–572.
 68. Treich, I., B. R. Cairns, T. De Los Santos, E. Brewster, and M. Carlson. 1995. SNF11, a new component of the yeast SNF-SWI complex that interacts with a conserved region of SNF2. Mol. Cell. Biol. **15**:4240–4248.
 69. Treitel, M. A., and M. Carlson. 1995. Repression by SSN6-TUP1 is directed by MIG1, a repressor/activator protein. Proc. Natl. Acad. Sci. USA **92**:3132–3136.
 70. 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 G₂ phase control. EMBO J. **11**:4017–4026.
 71. Tsukiyama, T., C. Daniel, J. Tamkun, and C. Wu. 1995. *ISW1*, a member of the *SWI2/SNF2* ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. Cell **83**:1021–1026.
 72. Tsukiyama, T., and C. Wu. 1995. Purification and properties of an ATP-dependent nucleosome remodeling factor. Cell **83**:1011–1020.
 73. Vojtek, A. B., S. M. Hollenberg, and J. A. Cooper. 1993. Mammalian Ras interacts directly with the serine/threonine kinase Raf. Cell **74**:205–214.
 74. Walker, S. S., J. C. Reese, L. M. Apone, and M. R. Green. 1996. Transcription activation in cells lacking TAF_{11s}. Nature **383**:185–188.
 75. Wang, W., Y. Xue, S. Zhou, A. Kuo, B. R. Cairns, and G. R. Crabtree. 1996. Diversity and specialization of mammalian SWI/SNF complexes. Genes Dev. **10**:2117–2130.
 76. Weinert, T. A., and L. H. Hartwell. 1988. The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. Science **241**:317–322.
 77. Weinmann, R. 1992. The basic RNA polymerase II transcriptional machinery. Gene Expression **2**:81–91.
 78. West, R. W., Jr., R. R. Yocum, and M. Ptashne. 1984. *Saccharomyces cerevisiae* *GAL1-GAL10* divergent promoter region: location and function of the upstream activating sequence UAS_G. Mol. Cell. Biol. **4**:2467–2478.
 79. Wilson, C. J., D. M. Chao, A. N. Imbalzano, G. R. Schnitzler, R. E. Kingston, and R. A. Young. 1996. RNA polymerase II holoenzyme contains SWI/SNF regulators involved in chromatin remodeling. Cell **84**:235–244.
 80. Winston, F., and M. Carlson. 1992. Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet. **8**:387–391.
 81. 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.