MOLECULAR AND CELLULAR BIOLOGY, Apr. 1997, p. 1768–1775 Vol. 17, No. 4
0270-7306/97/$04.00+0

Interaction of a Swi3 Homolog with Sth1 Provides Evidence for a Swi/Snf-Related Complex with an Essential Function in *Saccharomyces cerevisiae*

ISABELLE TREICH[1] AND MARIAN CARLSON[1,2]*

Department of Genetics and Development and Institute of Cancer Research[1] and Department of Microbiology,[2] Columbia University College of Physicians and Surgeons, New York, New York 10032

Received 8 October 1996/Returned for modification 7 November 1996/Accepted 20 December 1996

The *Saccharomyces cerevisiae* Swi/Snf complex has a role in remodeling chromatin structure to facilitate transcriptional activation. The complex has 11 components, including Swi1/Adr6, Swi2/Snf2, Swi3, Snf5, Snf6, Snf11, Swp73/Snf12, and Tfg3. Mammalian homologs of these proteins have been shown to form multiple Swi/Snf-related complexes. Here we characterize an *S. cerevisiae* Swi3 homolog (Swh3) and present evidence that it associates in a complex with a Snf2 homolog, Sth1. We identified Swh3 as a protein that interacts with the N terminus of Snf2 in the two-hybrid system. Swh3 and Swi3 are functionally distinct, and overexpression of one does not compensate for loss of the other. Swh3 is essential for viability and does not activate transcription of reporters. The Snf2 sequence that interacts with Swh3 was mapped to a region conserved in Sth1. We show that Swh3 and Sth1 fusion proteins interact in the two-hybrid system and coimmunoprecipitate from yeast cell extracts. We also map interactions between Swh3 and Sth1 and examine the role of a leucine zipper motif in self-association of Swh3. These findings, together with previous analysis of Sth1, indicate that Swh3 and Sth1 are associated in a complex that is functionally distinct from the Swi/Snf complex and essential for viability.

Transcriptional control involves a complex interplay between gene-specific regulators, chromatin, and the general transcription apparatus. The Swi/Snf complex has an important role in this process in the yeast *Saccharomyces cerevisiae* and is required for transcriptional activation of diversely regulated promoters (40, 59). The complex has 11 components, including Swi1/Adr6, Snf2/Swi2, Swi3, Snf5, Snf6, Snf11, Swp73/Snf12, and Tfg3/TAF30/Anc1 (5–7, 11, 33, 39, 49). Genetic and biochemical evidence implicates the Swi/Snf complex in remodeling chromatin structure to facilitate binding of transcription factors (for reviews, see references 22, 41, and 59). Biochemical studies have shown that the Snf2 protein has a DNA-dependent ATPase activity (6, 11, 26) and that the Swi/Snf complex alters nucleosomal structure and stimulates binding of GAL4 derivatives to nucleosomes in an ATP-dependent manner (11). The Swi/Snf complex also binds DNA (42) and catalyzes disruption of histones from GAL4-bound nucleosomes (38). Recently, Swi/Snf proteins were reported to be associated with RNA polymerase II holoenzyme (58).

The Swi/Snf complex has been widely conserved in eukaryotes. Putative functional homologs of several of its components have been identified in a range of organisms (for a review, see reference 8). The closest Snf2 homologs are *Drosophila* brahma (brm) protein and the human hbrm and BRG1 proteins (21, 35, 48). Snf5-related proteins have been identified in *Drosophila melanogaster* (snr1) (12), humans (hSnf5/Ini1/BAF47) (19, 34, 56), and *Caenorhabditis elegans* (12). Tfg3 is similar to ENL and AF9, two proteins that are involved in acute human leukemia (5). Finally, a Swp73 homolog (BAF60) and two Swi3 homologs (BAF155 and BAF170) have been isolated from a large human complex that contains BRG1 and BAF47 (56). Moreover, biochemical studies suggest that mammalian Swi/Snf-like complexes are functional counterparts of the yeast Swi/Snf complex. Human Swi/Snf complexes disrupt nucleosome structure and facilitate binding of transcription factors (16, 23).

Higher eukaryotes contain multiple Swi/Snf-related complexes, perhaps with distinct functions. In mammals, distinct Swi/Snf-like complexes have been separated chromatographically (23, 56). In *Drosophila*, in addition to a complex containing brm and snr1 (12), a Swi/Snf-related complex termed NURF (nucleosome-remodeling factor) has been characterized. One of the four components, ISWI, is highly homologous to Snf2 in the ATPase domain (13, 52); however, the ATPase activity of NURF is modestly stimulated by nucleosomal DNA and is not significantly stimulated by naked DNA (53). NURF appears to be functionally distinct from Swi/Snf.

In yeast, there has been no evidence for multiple forms of the Swi/Snf complex or Swi/Snf-related complexes. Sequence homologs of some Swi/Snf components have been identified (5, 7, 8, 24, 52), but these homologs have not been shown to reside in Swi/Snf-related complexes. Here we present evidence that a homolog of Swi3 is associated with a homolog of Snf2 in such a complex.

Previously, we undertook a two-hybrid screen (15) to identify proteins that interact with Snf2 in vivo (49). As bait, we used a LexA fusion to the N-terminal region of Snf2 preceding the ATPase domain (amino acids 14 to 767). A library of fusions between the GAL4 activation domain (GAD) and *S. cerevisiae* genomic sequences was tested for interaction. We recovered 31 positive plasmids, among which 12 expressed Snf11 and 1 expressed a GAD fusion to Swi3 at residue 245 (49). Here we report the characterization of a new interacting protein, designated Swh3, that is a homolog of Swi3. We show that the *SWH3* gene is essential and functionally distinct from *SWI3*. In the two-hybrid system, Swh3 interacts with a small region of Snf2 that is conserved in Sth1, a Snf2 homolog. We show that Swh3 and Sth1 interact in the two-hybrid system and

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

TABLE 1. *S. cerevisiae* strains used

Strain[a]	Genotype
MCY3647	*MAT*α *his3*Δ*200 lys2-801 leu2-3,112 ura3-52*
MCY3106	*MAT***a**/*MAT*α *his3*Δ*200 /his3*Δ*200 lys2-801/+ +/leu2-3,112 ura3-52 /ura3-52*
MCY3840	*MAT***a**/*MAT*α *swh3*Δ*1::HIS3/+his3*Δ*200/his3*Δ*200 lys2-801/+ +/leu2-3,112 ura3-52/ura3-52*
MCY3860	*MAT*α *swh3*Δ*1::HIS3 his3*Δ*200 lys2-801 ura3-52* (pGAL1::SWH3)
MCY829	*MAT*α *his3*Δ*200 lys2-801 ura3-52*
FY250[b]	*MAT*α *his3*Δ*200 ura3-52 leu2*Δ*1 trp1*Δ*63*
CTY10-5d[c]	*MAT***a** *ade2-101 his3*Δ*200 leu2-3,112 trp1-901 gal4 gal80 URA3::lexAop-lacZ*
NOY398[d]	*MAT***a**/*MAT*α *rpa135::LEU2/+ his3-11/his3-11 trp1-1/trp1-1 leu2-3,112/leu2-3,112 ura3-1/ura3-1 ad2-1/ade2-1 can1-100/can1-100*

[a] All MCY strains have the S288C genetic background.
[b] Obtained from F. Winston.
[c] Constructed by R. Sternglanz.
[d] Obtained from M. Nomura (61).

coimmunoprecipitate from cell extracts. These and other findings indicate that Swh3 and Sth1 are components of a Swi/Snf-related complex with an essential function.

MATERIALS AND METHODS

Yeast strains and genetic methods. *S. cerevisiae* strains are listed in Table 1. Standard genetic methods were followed. Media used were yeast extract-peptone (YEP) containing 2% glucose or selective synthetic complete (SC) medium containing 2% glucose (43) except where otherwise noted. Growth on different carbon sources was scored under anaerobic conditions (14). The *Escherichia coli* strain was XL1-Blue (Stratagene).

Plasmids. pLexA$_{87}$-SNF2$_{14-767}$ (26) encodes the N-terminal 87 residues of LexA fused to residue 14 of SNF2; the *SNF2* coding sequence is interrupted by an amber mutation at codon 768.

All PCRs on *SWH3* were done with FY250 genomic DNA except where noted. Primers are listed in Table 2. To construct LexA and GAD fusions to Swh3, a PCR fragment was generated with OL93 and OL94 and cloned in pGEM-T (Promega). The *Bam*HI-*Sal*I fragment was isolated from this plasmid and cloned in pEG202 (gift of E. Golemis and R. Brent) and in pACTII (31) to obtain pLexA-SWH3 and pGAD-SWH3, respectively. The resulting plasmids complemented *swh3*Δ*1::HIS3* for growth. pGAL1::SWH3 contains the same fragment cloned in pBM272 (a pBM150 derivative with a *Hin*dIII site added near the *Bam*HI site [18]) and expresses *SWH3* under the control of the *GAL1* promoter. pGAD-SWH3ΔC and pGAD-SWH3ΔZC contain *Bam*HI-*Sal*I PCR fragments generated by amplification of the pGEM-T-SWH3 subclone, using primers OL93 plus OL104 and OL93 plus OL103, respectively.

To construct pRS426-HA-SWH3 and pRS315-HA-SWH3, we first cloned into pUC19 (60) a *Bam*HI-*Sal*I fragment generated by amplification of *SWH3* with OL99 and OL94, which created a *Not*I site after the ATG of *SWH3*. A *Not*I fragment isolated from pGTEP1 (54) encoding a triple hemagglutinin (HA) tag was then introduced, and the orientation of the insert was determined by sequence. An *Eco*RI-*Sma*I PCR fragment containing the *SWH3* promoter was generated with OL100 and OL101 and cloned in this plasmid. The resulting *Eco*RI-*Sal*I fragment was subcloned into pRS426 (10) to give pRS426-HA-SWH3, which expresses HA-Swh3 from the *SWH3* promoter. pRS315-HA-SWH3 contains the *Pst*I-*Sal*I fragment from pRS426-HA-SWH3 cloned in pRS315 (45). The resulting plasmids complemented *swh3*Δ*1::HIS3* for growth.

pLexA-STH1$_{1-470}$, pLexA-STH1$_{1-338}$, and pLexA-STH1$_{264-470}$ contain *Bam*HI-*Sal*I PCR fragments generated by amplification of pBL51 (29), using primers OL95 plus OL96, OL95 plus OL97, and OL98 plus OL96, respectively.

Disruption of the *SWH3* gene. A PCR fragment was generated from pUC-HIS3 (44) with OL89 and OL90, as described previously (46), and used to transform the diploid strain MCY3106 to histidine independence. A transformant carrying the *swh3*Δ*1::HIS3* allele, deleted for codons 1 to 543, was identified by performing PCRs on genomic DNA with OL91 and OL92.

β-Galactosidase assays. β-Galactosidase activity was assayed either quantitatively in permeabilized cells (43) or by using filter lifts from cells patched on plates as described previously (30). For quantitative assays, transformants were grown to mid-log phase in SC-His-Leu medium to select for plasmids. β-Galactosidase activity is expressed in Miller units.

Immunoprecipitation assay. Cultures were grown to mid-log phase in selective SC medium. Protein lysates were prepared as described previously (9) in immunoprecipitation (IP) buffer (20 mM HEPES [pH 7.8], 20% glycerol, 1 mM EDTA, 0.1 M potassium acetate, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 μg each of aprotinin, leupeptin, and pepstatin per ml) containing 500 mM NaCl. Protein concentrations were determined with the Bio-Rad protein assay kit. Crude extract (250 or 500 μg) was diluted to 500 μl with IP buffer, and 2 μl of monoclonal HA antibody (12CA5; Babco) was added. After incubation for 2 h at 4°C, protein A-Sepharose beads (Sigma) were added. The mixture was incubated for 1 h at 4°C. The absorbed immune complexes were collected by centrifugation at 4,000 × *g* and washed twice with IP buffer containing 0.2% Nonidet P-40 (NP-40) and twice with IP buffer containing 1% NP-40 and 300 mM NaCl or four times with IP buffer containing 0.2% NP-40 and 500 mM NaCl. Pellets were resuspended in 80 μl of sample buffer and boiled for 5 min. Proteins from the supernatant (60 μl) were electrophoresed in a sodium dodecyl sulfate–8% polyacrylamide gel and immunoblotted. Primary antibodies were anti-LexA (gift of C. Denis, University of New Hampshire, Durham) and

TABLE 2. Oligonucleotides used

Name	Sequence[a]
OL89	5′-GAAGGGTAGAGCAGAGAAGCAAAGAGTAGATAGAAGGCCAGGATAGGATCCGCTGCACGGTCCTG-3′
OL90	5′-TTACAATGACCACGGTTTGTACACCTGGGGCTGCAATTTTGCAATGGATCCGCCTCGTTCAGAATGACACG-3′
OL91	5′-ATGTCAGCCGTTAGGTCATTGACTTCCTTCTC-3′
OL92	5′-GCAAACGACAACCACAACCCCTTCTACATCCG-3′
OL93	5′-CCCCGGATCCGTATGAGCGACACTGAAAAGGATAAGGATGTCCC-3′
OL94	5′-CCCCGTCGACGCAAACGACAACCACAACCCCTTCTACATCCG-3′
OL95	5′-GGGGGGATCCGAATGCTTCAGGAACAATCTG-3′
OL96	5′-GGGGGTCGACTTATAAAGTGCCGCCAACAAG-3′
OL97	5′-CCCCGTCGACTTACTGCCTTTCTTTGATGAAATC-3′
OL98	5′-CCCCGGATCCGAAATGTTGCCAGCCAGGCAC-3′
OL99	5′-CCCCGGATCCGTATGAGCGGCCGCAGCGACACTGAAAAGGATAAGGATGTCCC-3′
OL100	5′-AGAGCAATACTTTGAACAGTCTTACCAAGACC-3′
OL101	5′-CCCCCCGGGTATCCTGGCCTTCTATCTACTCTTTGCTTCTC-3′
OL102	5′-CCCCGGATCCAAAAAGCCGTAAGAGGTC-3′
OL103	5′-CCCCCTCGAGTTACTTGCTCTCTAATTTCTCC-3′
OL104	5′-GGGGCTCGAGTTACGAGTCTACTTGCTCCGAG-3′

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

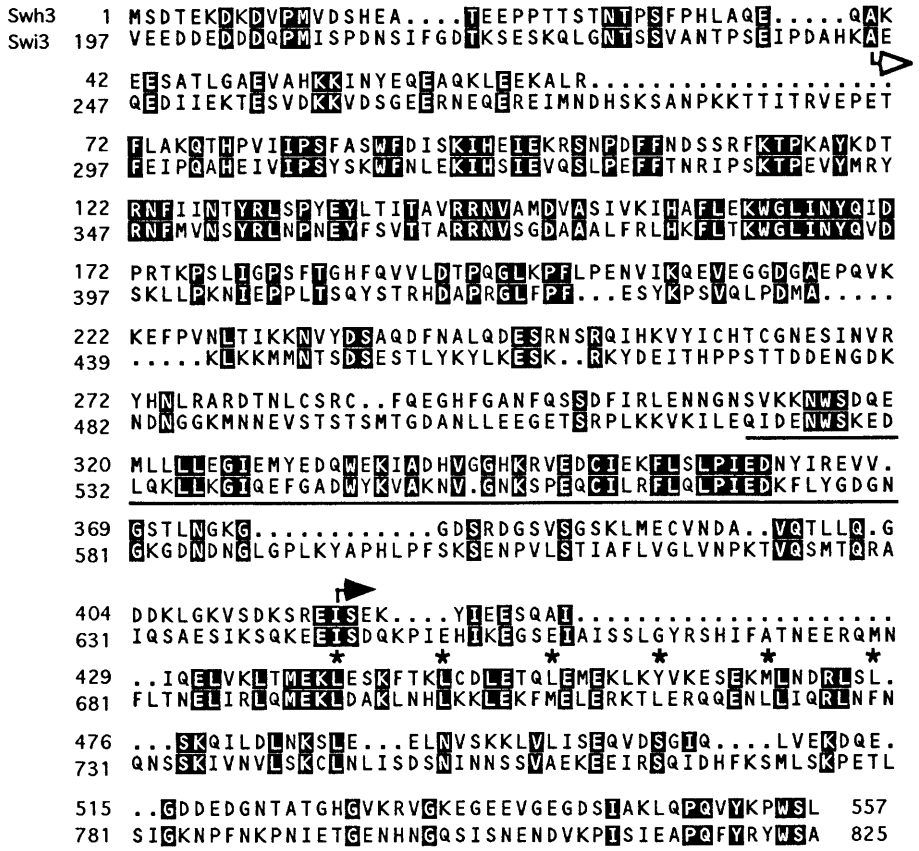

FIG. 1. Sequence comparison of Swh3 and Swi3. Predicted amino acid sequences were aligned by the Genetics Computer Group sequence analysis software package (University of Wisconsin). Identical residues are shown in reverse contrast. The filled and open arrowheads indicate the GAD-Swh3 and GAD-Swi3 fusion points, respectively, for the clones recovered from the two-hybrid screen. Asterisks mark the leucine zipper motif. The SANT domain is underlined.

monoclonal HA antibodies. Antibodies were detected by enhanced chemiluminescence with ECL reagents (Amersham International).

RESULTS

A Swi3 homolog interacts with the N terminus of Snf2. A two-hybrid screen for proteins that interact with the N terminus of Snf2 (49) yielded one plasmid that encodes a GAD fusion to open reading frame YFR037c (accession number P32591). The predicted 63-kDa protein is homologous to Swi3 (30% identity and 52% similarity) but lacks N-terminal residues (Fig. 1). We called this gene *SWH3*, for SWI3 homolog, and the plasmid was designated pGAD-SWH3$_{417}$, as the fusion occurs at codon 417. A potential leucine zipper motif, located near the C terminus of Swh3, and a SANT domain (1) are conserved in Swi3 (Fig. 1). The SANT domain has similarity to DNA-binding domains, but no DNA-binding activity was detected for the corresponding region of the mammalian Swi3 homolog BAF170 (56).

***SWH3* is essential and functionally distinct from *SWI3*.** To disrupt *SWH3* function, we introduced the mutation *swh3Δ1::HIS3*, deleted for codons 1 to 543, into the chromosomal locus of a diploid (see Materials and Methods). A His$^+$ disruptant (MCY3840) was subjected to tetrad analysis. In each of 16 tetrads, only two spores formed clones and all viable spore clones were His$^-$. Subsequent studies showed that the inviable spores germinate (see below). We confirmed that *swh3Δ1* was responsible for the lethality by transforming the disrupted diploid with a *URA3* centromeric plasmid expressing *SWH3* from the *GAL1* promoter (pGAL1::SWH3). Tetrad analysis of a transformant yielded His$^+$ Ura$^+$ haploid segregants when spores were allowed to germinate on medium containing galactose. One of the *swh3Δ1::HIS3* segregants carrying pGAL1::SWH3 was grown in galactose and then shifted to glucose. Growth was arrested after six to eight generations, and the arrested cells did not resume growth when shifted back to galactose. Thus, *SWH3* is essential for mitotic growth.

Despite their different mutant phenotypes, the sequence homology suggests that *SWI3* and *SWH3* might nonetheless have redundant functions. We therefore examined whether increased dosage of one gene can suppress the phenotype caused by deletion of the other. We first tested if *SWI3* in multicopy can suppress the lethality of *swh3Δ1*. The diploid strain MCY3840 (*SWH3*/*swh3Δ1::HIS3*) was transformed with a multicopy *SWI3* plasmid (BD7) (40), and two Ura$^+$ transformants were subjected to tetrad analysis. Each of 21 tetrads gave only two viable spores; all were His$^-$ and 36 of 42 were Ura$^+$, indicating that the plasmid had not been lost. To test if increased dosage of *SWH3* can compensate for the loss of *SWI3*, we transformed CY73 (*swi3*Δ) with a multicopy plasmid carrying a functional *SWH3* gene (pRS426-HA-SWH3). Growth of Ura$^+$ transformants on sucrose, galactose, or raffinose (Snf/Swi phenotype) was not improved relative to vector controls. Taken together, these experiments suggest that Swi3 and Swh3 are functionally distinct.

LexA-Swh3 does not significantly activate transcription of *GAL1-lacZ* or *CYC1-lacZ* reporters. Several members of the

TABLE 3. Assay for transcriptional activation by LexA-Swh3[a]

No. of *lexA* operators	β-Galactosidase activity (U)			
	LexA	LexA-Swh3	LexA-Snf2	LexA-Sth1
0	0.1	0.2	0.7	0.2
2	0.6	5.1	120	12

[a] Proteins were expressed from pLexA-SWH3, pLexA-SNF2 (28), pLexA-STH1 (29), and pEG202 (gift of E. Golemis and R. Brent). Reporter plasmids pΔSS and pSV168 are derived from pLGΔ312S and carry a *CYC1-lacZ* fusion with zero or two *lexA* operators, respectively, replacing the *CYC1* UASs (17, 55). MCY829 transformants were grown to mid-log phase in SC-His-Ura to select for plasmids. Values are averages of β-galactosidase activities for at least three transformants or two for control assays. Standard errors were <7%.

Swi/Snf complex, including Swi3, activate transcription when tethered to a *lexAop-GAL1-lacZ* reporter via fusion to LexA (5, 7, 25, 27, 28, 49). To test whether Swh3 activates in this assay, we expressed a functional LexA-Swh3 protein in strains carrying a *GAL1-lacZ* reporter with zero or four *lexA* operators replacing the *GAL* upstream activating sequence (UAS) (pLR1Δ1 [57] or p1840 [3]). LexA-Swh3 did not significantly stimulate expression of β-galactosidase (data not shown). We next tested both LexA-Swh3 and LexA-Snf2 for activation of a *lexAop-CYC1-lacZ* reporter. LexA-Swh3 was 25-fold less effective than LexA-Snf2 (Table 3).

We also considered the notion that LexA-Swh3 might repress transcription. However, LexA-Swh3 did not repress expression of a reporter with four *lexA* operators 5′ to the UAS of a *CYC1-lacZ* fusion (pJK1621 [20]) (data not shown).

Role of the leucine zipper in self-association of Swh3. Because LexA-Swh3 does not activate transcription, we were able to test whether the putative leucine zipper mediates self-association of Swh3. We constructed various GAD-Swh3 fusions and assayed two-hybrid interactions with LexA-Swh3 (Fig. 2). LexA-Swh3 interacted with both GAD-Swh3 and GAD-Swh3ΔC but failed to interact with GAD-Swh3ΔZC, a fusion lacking the leucine zipper. Subsequent experiments confirmed that GAD-Swh3ΔZC is produced and interacts with LexA-Sth1 in this assay (data not shown). These findings indicate that Swh3 can self-associate to form dimers, or possibly multimers, and that the leucine zipper motif is required. The leucine zipper may not be sufficient, however, because GAD-$Swh3_{417}$ did not interact with LexA-Swh3.

We also tested these four GAD fusions for ability to provide

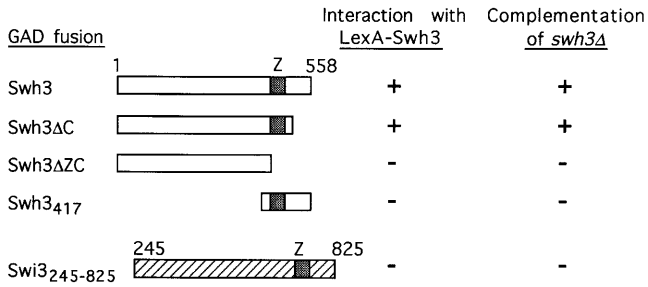

FIG. 2. Two-hybrid assays for interaction of Swh3 with itself and with Swi3. Transformants of CTY10-5d expressing LexA-Swh3 and the indicated GAD fusion were tested for β-galactosidase expression by filter lift assay. Interacting proteins (+) turned blue in less than 3 h. Noninteracting proteins (−) remained white after overnight incubation. CTY10-5d expressing LexA plus any GAD-Swh3 derivative or GAD-$Swi3_{245-825}$ or LexA-Swh3 plus GAD remained white after overnight incubation (data not shown). GAD fusion proteins were also tested for complementation of *swh3Δ1* strain as described in the text. Z, leucine zipper motif (shaded bars).

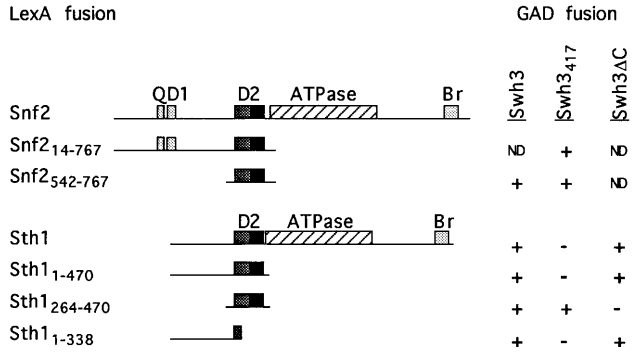

FIG. 3. Interaction of Sth1 and Snf2 with Swh3 sequences. A schematic representation of Snf2 and Sth1 is shown. Q, Gln-Gln-Ala repeat; D1 and D2, conserved domains in Snf2, brm, BRG1, and hbrm (the black box corresponds to D2 as defined by Tamkun et al. [48], but the homology extends farther, as indicated); ATPase, region with seven motifs that are conserved in nucleic acid-dependent ATPases; Br, bromodomain. Snf2 and Sth1 sequences are fused to LexA or to the LexA DNA-binding domain. GAD-Swh3 fusions are shown in Fig. 2. Transformants of CTY10-5d expressing the indicated pair of fusion proteins were tested for β-galactosidase expression by filter lift assay. Interacting proteins (+) turned blue in less than 3 h. Noninteracting proteins (−) remained white after overnight incubation. Control strains expressing LexA fusions in combination with GAD or GAD fusions in combination with LexA were white. In addition, LexA-Snf1 or LexA-lamin in combination with GAD-Swh3 were white. LexA-Snf2 activates transcription on its own, and therefore interactions with GAD-Swh3 fusions were not tested. ND, not determined.

Swh3 function. A *swh3Δ1* mutant carrying plasmid pRS426-HA-SWH3 (*URA3* marked) was transformed with each of the *LEU2*-marked GAD fusion plasmids. Transformants expressing GAD-Swh3 and GAD-Swh3ΔC were able to lose the *URA3* plasmid, whereas those expressing GAD-Swh3ΔZC or GAD-$Swh3_{417}$ were not (Fig. 2). Thus, GAD-Swh3ΔC complements *swh3Δ1*.

The sequence homology between Swh3 and Swi3 raised the possibility that the two proteins interact, perhaps forming heterodimers. Wang et al. isolated mammalian complexes containing two different Swi3 homologs (56). We therefore tested for interaction between LexA-Swh3 and GAD-$Swi3_{245-825}$. This Swi3 fusion protein contains most of Swi3, including the leucine zipper, and interacts with Snf2 in the two-hybrid system (49). However, no interaction with LexA-Swh3 was detected (Fig. 2). Interaction of LexA-Swi3 with GAD-Swh3 cannot be assessed because LexA-Swi3 activates transcription.

Swh3 interacts with the N terminus of Sth1. Although GAD-$Swh3_{417}$ interacts with LexA-$Snf2_{14-767}$ in the two-hybrid system, it seemed unlikely that the native Swh3 protein interacts with Snf2 in vivo. First, no interaction was detected between GAD-Swh3 and a full-length, nonactivating derivative of Snf2, LexA-Snf2-K798R, which carries a mutation in the ATP-binding site (26); β-galactosidase activities for LexA-Snf2-K798R in combination with GAD-Swh3 and GAD were 1.6 and 2.8 U, respectively (averages for four transformants of CTY10.5d). Second, several lines of evidence distinguish Swh3 from components of the Swi/Snf complex: Swh3 is essential for viability, LexA-Swh3 does not activate transcription, and Swh3 does not copurify with the Swi/Snf complex (4). We reasoned that Swh3 should interact with a protein that has homology to the N terminus of Snf2. The only *S. cerevisiae* protein with such homology is Sth1, a Snf2 homolog (Fig. 3). The N-terminal homology encompasses a region designated domain 2, which is also conserved in *Drosophila* (48) and human (21, 35) Snf2 homologs (Fig. 4). Further experiments showed that domain 2 of Snf2 interacts with GAD-Swh3 and GAD-$Swh3_{417}$ (Fig. 3). Thus, Sth1 was a likely candidate for interaction with Swh3.

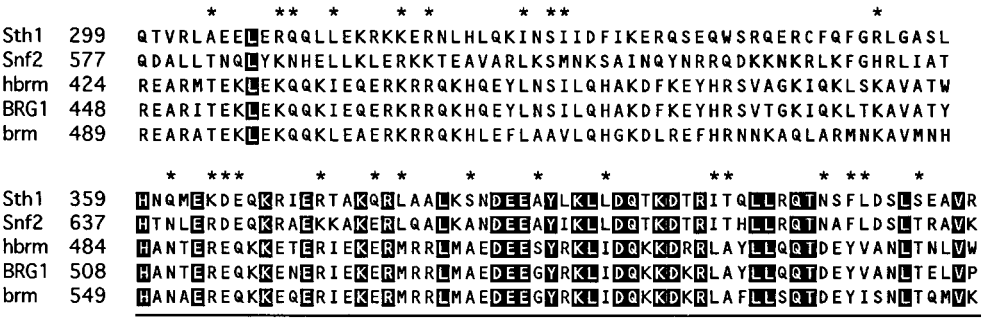

FIG. 4. Conservation of domain 2 in yeast, human, and *Drosophila* homologs of Sth1. Numbers indicate the N-terminal residues shown. Residues identical in the five sequences are shown in reverse contrast. Residues conserved in all five sequences are marked by asterisks (conservative substitutions are S-T-A-G, D-E, N-Q, N-D, Q-E, H-R-K, M-I-L-V, and F-Y). Domain 2 as originally defined (48) is underlined, but the conserved sequence extends farther. Sequences (references): Sth1 (29), Snf2 (28), hbrm (35), BRG1 (21), brm (48).

Previous studies of Sth1 also supported this idea. Sth1 is a close sequence homolog of Snf2; besides domain 2, Sth1 contains an ATPase domain which is functionally interchangeable with that of Snf2 (29) and a C-terminal bromodomain. Despite their high degree of similarity, however, the two proteins are functionally distinct, and overexpression of one does not substitute for the other (29). Moreover, like Swh3, Sth1 is essential for viability and a LexA-Sth1 fusion protein does not activate or repress transcription (29) (Table 3). The function of Sth1 is not known, but the protein is localized to the nucleus (29) and depletion of the protein caused arrest at the G_2-to-M transition (51).

To assess their relationship, we first tested Sth1 and Swh3 for two-hybrid interaction. Strains expressing both LexA-Sth1 and GAD-Swh3 activated β-galactosidase expression from a *lexAop-GAL1-lacZ* reporter (Fig. 3). In contrast, LexA-Sth1 did not interact with GAD-Swi3$_{245-825}$, which was recovered by its ability to interact with LexA-Snf2$_{14-767}$ (49) (data not shown). As predicted, GAD-Swh3 interacted with LexA-Sth1$_{264-470}$, containing domain 2 (Fig. 3). Interestingly, GAD-Swh3 also interacted with LexA-Sth1$_{1-338}$, suggesting a second point of contact between Swh3 and the Sth1 N terminus.

To define the Swh3 sequences that interact with Sth1, we tested different fusions for interaction (Fig. 3). GAD-Swh3$_{417}$ interacted with LexA-Sth1$_{264-470}$, containing only domain 2, but GAD-Swh3ΔC did not, suggesting that the extreme C terminus of Swh3 interacts with domain 2. Moreover, GAD-Swh3ΔC interacted with fusions containing Sth1 sequences N terminal to domain 2, supporting the idea of a second point of contact. The failure to detect interaction of GAD-Swh3$_{417}$ with LexA-Sth1 or LexA-Sth1$_{1-470}$ is also consistent with the model that the native Swh3 interacts with two regions of Sth1. GAD-Swh3$_{417}$ cannot form heterodimers with the native Swh3 (Fig. 2); we suggest that GAD-Swh3$_{417}$, which binds only to domain 2, does not effectively compete in this assay with homodimers of Swh3 that bind to Sth1 at two sites. These data, together with the ability of GAD-Swh3ΔC to complement *swh3*Δ (Fig. 2), suggest that interaction of Swh3 with Sth1 at the site N terminal to domain 2 is sufficient for function in vivo. Interaction with domain 2 may contribute to stability of the complex.

HA-Swh3 and LexA-Sth1 coimmunoprecipitate. To obtain biochemical evidence that Swh3 and Sth1 interact in vivo, we carried out coimmunoprecipitation experiments. Wild-type cells were cotransformed with multicopy plasmids expressing HA-Swh3 from the *SWH3* promoter and LexA-Sth1 from the *ADH1* promoter (29), which complement deletions of the cognate genes. The HA-Swh3 protein was immunoprecipitated from cell extracts with monoclonal HA antibody, and the precipitate was analyzed by Western blotting for the presence of LexA-Sth1 by using LexA antibody (Fig. 5A and B). LexA-Sth1 coimmunoprecipitated with HA-Swh3, and precipitation of LexA-Sth1 required the HA-Swh3 protein. Coimmunoprecipitation was also observed when HA-Swh3 was expressed from a centromere-containing plasmid (Fig. 5C to E). Control experiments with LexA-Mig1 (50) and HA-Glc7 (47) fusions indicated that the observed coprecipitation cannot be attributed primarily to the LexA and HA moieties. These results, together with the two-hybrid evidence, indicate that Swh3 and Sth1 are physically associated.

In this experiment, we also tested for coimmunoprecipitation of LexA-Snf2 with HA-Swh3; none was detected, but LexA-Snf2 was expressed at lower levels than LexA-Sth1. These data do not exclude that a fraction of the Snf2 and Swh3 proteins are associated in the cell; however, no two-hybrid interaction between Snf2-K798R and Swh3 was detected, and the genetic evidence strongly suggests that their primary functions are distinct.

Growth arrest upon depletion of Swh3. Previously, Tsuchiya et al. (51) reported that depletion of Sth1 (called Nps1) causes a cell cycle arrest phenotype. In this study, diploid cells homozygous for a deletion of *STH1* and carrying a plasmid expressing the gene from the *GAL1* promoter were grown in YEP-galactose medium and then shifted to YEP-glucose to shut off the *GAL1* promoter. Growth was arrested after one or two rounds of cell division, and >85% of the cells arrested with large buds, most with a single nucleus and DNA content corresponding to G_2/M phase. Some arrested cells rereplicated DNA without passage through mitosis (51).

We carried out a similar experiment to compare the effects of depletion of Swh3. Two *swh3Δ1::HIS3* segregants carrying pGAL1::SWH3 were grown to mid-log phase in YEP-galactose. Cells were then inoculated at low density in YEP-galactose or YEP-glucose. Growth in glucose continued for six to eight generations before arrest. The arrested cells were larger than those grown in galactose, as was also reported for Sth1-depleted cells; however, the cells exhibited diverse morphologies. While a substantial fraction (49%) had large buds, many cells (39%) had multiple buds. In addition, cells were frequently elongated rather than round. These phenotypes are unlikely to result from nutrient limitation because growth arrested when cultures were at an optical density at 600 nm of 0.2 to 0.3. The diverse arrest phenotypes observed in this experiment, compared to the results of Tsuchiya et al. (51), possibly result from the greater number of cell divisions occurring before arrest, which presumably reflects the rate of depletion of

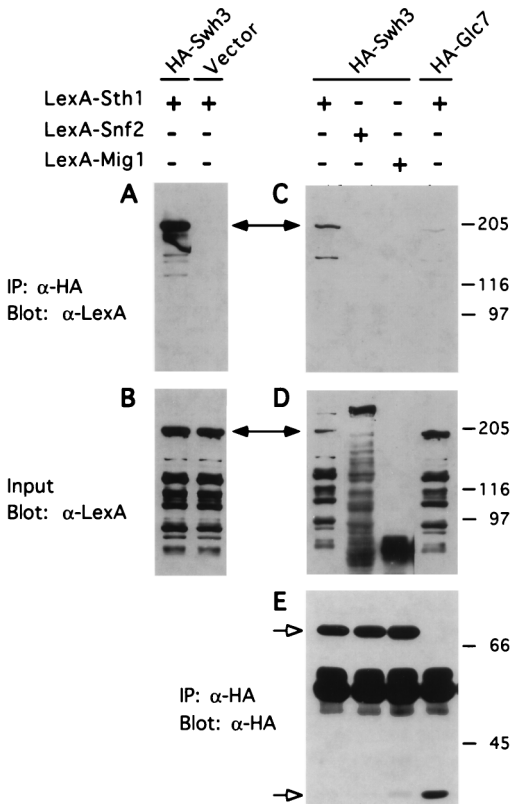

FIG. 5. Coimmunoprecipitation of LexA-Sth1 and HA-Swh3. Protein extracts were prepared from wild-type strain MCY3647 expressing the indicated fusion proteins. LexA-Sth1 (29), LexA-Snf2 (28), and LexA-Mig1 (50) were expressed from the *ADH1* promoter on multicopy plasmids. HA-Swh3 was expressed from its native promoter on a multicopy (A and B) or centromeric (C to E) plasmid. The control extracts lacking HA-Swh3 carried the vector pRS426 (A and B) or contained HA-Glc7 expressed from its native promoter on a centromeric plasmid (47) (C to E). (A and C) Proteins (A, 250 μg; C, 500 μg) were immunoprecipitated (IP) with anti-HA (α-HA) antibody as described in Materials and Methods. For the experiment shown in panel C, two steps were added: the lysate was clarified by centrifugation at 13,000 × *g* for 10 min at 4°C before addition of antibody; after incubation, the lysate was centrifuged again at 13,000 × *g* for 10 min. Precipitated proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotted with rabbit polyclonal anti-LexA (α-LexA). The arrow marks the position of LexA-Sth1. The smaller protein is presumably a degradation product. (B and D) Similar immunoblot analysis of the input (10 μg, except 50 μg for the LexA-Snf2 lane). The arrow marks the position of LexA-Sth1. (E) The immunoblot shown in panel C was reprobed with anti-HA antibody to confirm precipitation of HA-tagged proteins. Arrows mark positions of HA-Swh3 (top) and HA-Glc7 (bottom).

the protein. Perhaps a more immediate depletion causes a more uniform arrest phenotype.

Evidence against a role of Swh3 and Sth1 in RNA polymerase I or III transcription. Both Swh3 and Sth1 are essential for viability, but unlike Swi/Snf proteins, neither affects expression of a reporter transcribed by RNA polymerase II. We therefore examined the possibility of a role in polymerase I transcription. The essential function of RNA polymerase I is to transcribe the ribosomal DNA (rDNA), and a multicopy plasmid expressing the rDNA from the *GAL7* promoter suppresses the growth defect caused by defective polymerase I (37). To determine whether Swh3 is essential when the rDNA is transcribed from a polymerase II promoter, we transformed the diploid strain MCY3840 (*SWH3*/*swh3Δ1::HIS3*) with pNOY103, a multicopy *URA3* plasmid carrying the *GAL7*-rDNA fusion (36). Three transformants were subjected to tetrad analysis, and spores were germinated on galactose. Thirty-four tetrads yielded two viable spore clones, and four tetrads yielded only one; all were His^-, and many (32 of 72) were Ura^+. Microscopic examination of the spores that did not yield viable clones revealed that most had germinated and undergone one or two divisions. A parallel experiment with a strain heterozygous for an *sth1* deletion similarly showed failure of the plasmid to rescue the lethality of this mutation. We confirmed that pNOY103 allows growth of spores carrying a disruption of an essential subunit of RNA polymerase I (derived from diploid NOY398), as previously reported (36). Although these results do not exclude a role in RNA polymerase I transcription, Swh3 and Sth1 must have another function essential for viability.

We next considered the possibility that Swh3 and Sth1 are essential for transcription by RNA polymerase III. Previous studies showed that Gal4 DNA-binding domain (GBD) fusions to components of the transcription factor TFIIIB or TFIIIC, including its subunit τ138, can activate a promoter-deficient U6 RNA gene with Gal4 binding sites (UAS_G) located within the B block (B-block-UAS_G-U6) or within the transcribed region (+88-UAS_G-U6) (32). We reasoned that if Swh3 and Sth1 are involved in RNA polymerase III transcription, GBD-Swh3 and GBD-Sth1 fusion proteins might also recruit the polymerase III transcription machinery and activate transcription. However, expression of U6 RNA from these templates was stimulated by GBD-τ138 but not by GBD-Swh3, GBD-Sth1, or GBD, as judged by Northern blotting (data not shown). Thus, these experiments provide no evidence that Swh3 and Sth1 affect RNA polymerase III function.

DISCUSSION

We have here characterized Swh3, a homolog of Swi3, which was identified by its two-hybrid interaction with a small conserved region in the N terminus of Snf2. Evidence that Swh3 is essential for viability, and functionally distinct from Swi3, led us to consider the possibility that the native interacting partner for Swh3 is Sth1, a Snf2 homolog that is also essential. We present genetic and biochemical evidence that in vivo Swh3 associates with Sth1.

The complex containing Swh3 and Sth1 represents a Swi/Snf-related complex by virtue of these two components. Thus, in this respect *S. cerevisiae* resembles higher eukaryotes, which have previously been shown to contain multiple Swi/Snf-related complexes (23, 56). While the work reported here was in progress, Cairns et al. (4) independently identified a large complex containing Swh3, Sth1, and other proteins. It is possible that this complex also contains other Swi/Snf homologs. In addition, the ability of Swh3 to self-associate raises the possibility that a dimer, or higher multimer, of Swh3 resides in the complex. Further work will be required to assess the structural similarity of this complex to the Swi/Snf complex.

From a functional standpoint, the Swh3/Sth1 complex is clearly distinct from Swi/Snf. First, both Swh3 and Sth1 are essential proteins, whereas mutations in most Swi/Snf components cause only defects in activation of a subset of genes. Second, overexpression of Swh3 does not compensate for loss of Swi3 and vice versa; similar results were obtained for Sth1 and Snf2 (29). Third, neither LexA-Swh3 nor LexA-Sth1 (29) significantly activates expression of *lexAop-lacZ* reporters, which by contrast are dramatically activated by LexA fusions to several Swi/Snf proteins (5, 7, 25, 27, 28, 49). Finally, previous work showed that a mutation in *SPT6*, encoding a protein that binds histone H3 and affects chromatin structure (2), suppresses transcriptional defects in *swi*/*snf* mutants but does not suppress *sth1* (29).

The function of the Swh3/Sth1 complex remains unclear.

The finding that mutations in both *SWH3* and *STH1* are lethal strongly suggests that Swh3 and Sth1 together perform an essential function. Previously, it was reported that depletion of the Sth1 protein by shutoff of a *GAL1::NPS1* (*STH1*) promoter fusion arrests cells after one or two divisions at the G_2-to-M transition (51). Here we find that depletion of Swh3 by shutoff of *GAL1::SWH3* leads to aberrant terminal morphologies, but growth did not arrest at a specific stage of the cell cycle. It is possible that this difference results from a real functional distinction between Sth1 and Swh3; alternatively, it could reflect differences in strain background or the kinetics of protein depletion during shutoff. In our experiments, growth did not arrest abruptly but rather continued for six to eight generations. Further analysis using temperature-sensitive mutants will be required to resolve this issue.

The homology to Swi/Snf suggests a role in chromatin remodeling. Moreover, the ATPase activity required for chromatin remodeling by Swi/Snf appears to be conserved; the ATPase domain of Sth1 is required for Sth1 function and is functionally interchangeable with that of Snf2 (26). Nonetheless, a role in chromatin remodeling may or may not be coupled to transcriptional activation. Our data neither support nor rule out a role in transcription. Although Swh3 and Sth1 did not activate transcription of the *GAL1* or *CYC1* promoter, Swh3/Sth1 could contribute to transcriptional activation of a subset of RNA polymerase II promoters, including that of at least one essential gene. Our finding that a *GAL7*-rDNA fusion does not restore viability in *swh3* or *sth1* mutants does not formally exclude that these proteins are required for RNA polymerase I transcription but rather shows that they must have an additional essential function. It also remains possible that Swh3/Sth1 affects RNA polymerase III transcription, although no positive signal was detected in our assays. Alternatively, this complex may have a role in some essential function other than transcription that requires chromatin remodeling.

Are the Swi3 and Swh3 proteins to any extent interchangeable? The genetic evidence, in particular the lack of reciprocal suppression by overexpressed proteins, argues that Swi3 and Swh3 are not interchangeable with respect to the function of Swi3 in the Swi/Snf complex or the essential function of Swh3. However, it remains possible that Swi3 and Swh3 each have yet another function for which the two proteins are interchangeable. The existence of complexes that contain alternately Swi3 or Swh3, or that contain both, is not excluded. Nonetheless, we did not detect two-hybrid interaction between Swh3 and Swi3, Swi3 and Sth1, or Swh3 and the nonactivating ATP-binding site mutant Snf2-K798R. The two-hybrid interaction between Swh3 and the N terminus of Snf2 does not constitute compelling evidence for physiological association between the native Swh3 and Snf2 because this interaction occurs out of context. The overexpressed N terminus of Snf2 does not assemble a complete Swi/Snf complex (and hence does not activate on its own), and domain 2 may therefore be available for interaction with Swh3 that would not occur in the context of an intact complex. This view is supported by the lack of two-hybrid interaction between Swh3 and the full-length Snf2-K798R protein.

The yeast genome contains a number of other Swi/Snf homologs, including homologs of Snf2, Snf5, Tfg3, and Swp73 (5, 7, 8, 24, 52), and other Swi/Snf-related complexes probably remain to be identified. A likely candidate is a NURF-like complex because one of the Snf2 homologs in yeast is most closely related to ISWI (13, 52). Our understanding of the Swi/Snf complex and its constituents should inform efforts to determine the functional roles of related complexes.

ACKNOWLEDGMENTS

We thank M. C. Marsolier and V. Simon for helpful advice; B. Cairns, R. D. Kornberg, and B. Laurent for communication of unpublished results; A. Mitchell for plasmids; and S. Kuchin for criticism of the manuscript.

This work was supported by National Institutes of Health grant GM47259 to M.C.

REFERENCES

1. **Aasland, R.** 1996. The SANT domain: a putative DNA-binding domain in the SWI/SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIIB. Trends Biochem. Sci. **21:**87–88.
2. **Bortvin, A., and F. Winston.** 1996. Evidence that Spt6p controls chromatin structure by a direct interaction with histones. Science **272:**1473–1476.
3. **Brent, R., and M. Ptashne.** 1985. A eukaryotic transcriptional activator bearing the DNA specificity of a prokaryotic repressor. Cell **43:**729–736.
4. **Cairns, B., and R. D. Kornberg.** 1996. Personal communication.
5. **Cairns, B. R., N. L. Henry, and R. D. Kornberg.** 1996. TFG3/TAF30/ANC1, a component of the yeast SWI/SNF complex that is similar to the leukemogenic proteins ENL and AF-9. Mol. Cell. Biol. **16:**3308–3316.
6. **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.
7. **Cairns, B. R., R. S. Levinson, K. R. Yamamoto, and R. D. Kornberg.** 1996. Essential role of Swp73p in the function of yeast Swi/Snf complex. Genes Dev. **10:**2131–2144.
8. **Carlson, M., and B. C. Laurent.** 1994. The SNF/SWI family of global transcriptional activators. Curr. Opin. Cell Biol. **6:**396–402.
9. **Celenza, J. L., and M. Carlson.** 1986. A yeast gene that is essential for release from glucose repression encodes a protein kinase. Science **233:**1175–1180.
10. **Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero, and P. Hieter.** 1992. Multifunctional yeast high-copy-number shuttle vectors. Gene **110:** 119–122.
11. **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.
12. **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.
13. **Elfring, L. K., R. Deuring, C. M. McCallum, C. L. Peterson, and J. W. Tamkun.** 1994. Identification and characterization of *Drosophila* relatives of the yeast transcriptional activator *SNF2/SWI2*. Mol. Cell. Biol. **14:**2225–2234.
14. **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.
15. **Fields, S., and O. Song.** 1989. A novel genetic system to detect protein-protein interactions. Nature **340:**245–246.
16. **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.
17. **Johnson, A. D., and I. Herskowitz.** 1985. A repressor (MATα2 product) and its operator control expression of a set of cell type specific genes in yeast. Cell **42:**237–247.
18. **Johnston, M., and R. W. Davis.** 1984. Sequences that regulate the divergent *GAL1-GAL10* promoter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4:**1440–1448.
19. **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.
20. **Keleher, C. A., M. J. Redd, J. Schultz, M. Carlson, and A. D. Johnson.** 1992. Ssn6-Tup1 is a general repressor of transcription in yeast. Cell **68:**709–719.
21. **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.
22. **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.
23. **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.
24. **Laurent, B. C.** 1996. Personal communication.
25. **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.
26. **Laurent, B. C., I. Treich, and M. Carlson.** 1993. The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. Genes Dev. **7:**583–591.

27. **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.
28. **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.
29. **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.
30. **Lesage, P., X. Yang, and M. Carlson.** 1994. Analysis of the SIP3 protein identified in a two-hybrid screen for interaction with the SNF1 protein kinase. Nucleic Acids Res. **22:**597–603.
31. **Li, L., S. J. Elledge, C. A. Peterson, E. S. Bales, and R. J. Legerski.** 1994. Specific association between the human DNA repair proteins XPA and ERCC1. Proc. Natl. Acad. Sci. USA **91:**5012–5016.
32. **Marsolier, M.-C., N. Chaussivert, O. Lefebvre, C. Conesa, M. Werner, and A. Sentenac.** 1994. Directing transcription of an RNA polymerase III gene via GAL4 sites. Proc. Natl. Acad. Sci. USA **91:**11938–11942.
33. **Miller, M. E., B. R. Cairns, R. S. Levinson, K. R. Yamamoto, D. A. Engel, and M. M. Smith.** 1996. Adenovirus E1A specifically blocks SWI/SNF-dependent transcriptional activation. Mol. Cell. Biol. **16:**5737–5743.
34. **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.
35. **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.
36. **Nogi, Y., L. Vu, and M. Nomura.** 1991. An approach for isolation of mutants defective in 35S ribosomal RNA synthesis in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **88:**7026–7030.
37. **Nogi, Y., R. Yano, and M. Nomura.** 1991. Synthesis of large rRNAs by RNA polymerase II in mutants of *Saccharomyces cerevisiae* defective in RNA polymerase I. Proc. Natl. Acad. Sci. USA **88:**3962–3966.
38. **Owen-Hughes, T., R. T. Utley, J. Côté, C. L. Peterson, and J. L. Workman.** 1996. Persistent site-specific remodeling of a nucleosome array by transient action of the SWI/SNF complex. Science **273:**513–516.
39. **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.
40. **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.
41. **Peterson, C. L., and J. W. Tamkun.** 1995. The SWI-SNF complex: a chromatin remodeling machine? Trends Biochem. Sci. **20:**143–146.
42. **Quinn, J., A. M. Fyrberg, R. W. Ganster, M. C. Schmidt, and C. L. Peterson.** 1996. DNA-binding properties of the yeast SWI/SNF complex. Nature **379:** 844–847.
43. **Rose, M. D., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics, a laboratory course manual. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.
44. **Rothstein, R.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101C:**202–211.
45. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
46. **Smith, J., H. Zou, and R. Rothstein.** 1995. PCR-based gene disruption in *Saccharomyces cerevisiae*. Methods Mol. Cell. Biol. **5:**270–277.
47. **Sutton, A., F. Lin, M. J. F. Sarabia, and K. Arndt.** 1991. The *SIT4* protein phosphatase is required in late G1 for progression into S phase. Cold Spring Harbor Symp. Quant. Biol. **56:**75–81.
48. **Tamkun, J. W., R. Deuring, M. P. Scott, M. Kissinger, A. M. Pattatucci, T. C. Kaufman, and J. A. Kennison.** 1992. brahma: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell **68:**561–572.
49. **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.
50. **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.
51. **Tsuchiya, E., M. Uno, A. Kiguchi, K. Masuoka, Y. Kanemori, S. Okabe, and T. Mikayawa.** 1992. The *Saccharomyces cerevisiae NPS1* gene, a novel *CDC* gene which encodes a 160 kDa nuclear protein involved in G_2 phase control. EMBO J. **11:**4017–4026.
52. **Tsukiyama, T., C. Daniel, J. Tamkun, and C. Wu.** 1995. *ISWI*, a member of the *SWI2*/*SNF2* ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. Cell **83:**1021–1026.
53. **Tsukiyama, T., and C. Wu.** 1995. Purification and properties of an ATP-dependent nucleosome remodeling factor. Cell **83:**1011–1020.
54. **Tyers, M., G. Tokiwa, and B. Futcher.** 1993. Comparison of the Saccharomyces cerevisiae G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2 and other cyclins. EMBO J. **12:**1955–1968.
55. **Vidan, S., and A. P. Mitchell.** Submitted for publication.
56. **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.
57. **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.
58. **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.
59. **Winston, F., and M. Carlson.** 1992. Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet. **8:**387–391.
60. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
61. **Yano, R., and M. Nomura.** 1991. Suppressor analysis of temperature-sensitive mutations of the largest subunit of RNA polymerase I in *Saccharomyces cerevisiae*: a suppressor gene encodes the second-largest subunit of RNA polymerase I. Mol. Cell. Biol. **11:**754–764.