The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation

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The yeast SNF2 (SWI2) protein functions with SNF5, SNF6, SWI1, and SWI3 in the transcriptional activation of many differently regulated genes. These proteins appear to facilitate activation by gene-specific regulatory proteins. SNF2 is highly conserved among eukaryotes and defines a family of proteins with similarity to helicases and nucleic acid-dependent NTPases. Here, we present genetic and biochemical evidence that SNF2 has DNA-stimulated ATPase activity. Mutations in the nucleoside triphosphate (NTP)-binding motif and other conserved motifs impair SNF2 function. Swapping experiments with another member of this family indicate that the helicase-related domains are functionally interchangeable. Finally, bacterially expressed SNF2 protein has ATPase activity that is stimulated by double-stranded DNA, and mutation of the NTP-binding site abolishes this activity. Deletion analysis shows that the helicase-like region of SNF2 is necessary, but not sufficient, for transcriptional activation.

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Activated transcription by eukaryotic RNA polymerase II requires general transcription factors and gene-specific activators. In addition, other classes of coactivators or intermediary proteins are often needed [see Gill and Tjian 1992]. Some of these proteins may facilitate contacts between gene-specific activators and the basal initiation factors or assist activators in overcoming chromatin-mediated repression [see Felsenfeld 1992].

In the yeast *Saccharomyces cerevisiae*, the SNF2/SWI2, SNF5, SNF6, SWI1, and SWI3 proteins function in a related fashion in transcription of many diversely regulated genes [see Laurent and Carlson 1992; Peterson and Herskowitz 1992]. A role in transcriptional activation was indicated by evidence that LexA-SNF2, LexA-SNF5, and LexA-SNF6 hybrid proteins activate expression of a target gene when bound to DNA [Laurent et al. 1990, 1991; Laurent and Carlson 1992]. In vivo, these SNF and SWI proteins function coordinately with different gene-specific activators, including yeast GAL4, *Drosophila* fushi tarazu and bicoid, and rat glucocorticoid receptor expressed in yeast [Laurent and Carlson 1992; Peterson and Herskowitz 1992; Yoshinaga et al. 1992]. The mechanisms by which the SNF and SWI proteins contribute to transcriptional activation are not un-

derstood, but one possibility is that they assist gene-specific activators in relieving repression by chromatin [see Travers 1992; Winston and Carlson 1992].

The SNF2 protein is highly conserved among eukaryotes and defines a new family of proteins with similarity to previously described families that include known helicases and nucleic acid-stimulated NTPases [Davis et al. 1992; Laurent et al. 1992]. The proteins within the SNF2 family are more similar to each other than to other families, although all share seven signature motifs, including nucleoside triphosphate (NTP)-binding motifs [Hodgman 1988a,b; Gorbatenya et al. 1989]. In the SNF2 family, regions of particularly high sequence conservation encompass these motifs, suggesting functional significance. In addition to SNF2, this family includes the yeast proteins STH1 [Laurent et al. 1992], MOT1 [Davis et al. 1992], RAD54 [Emery et al. 1991], RAD5 [Johnson et al. 1992], RAD16 [Bang et al. 1992; Schild et al. 1992], and FUN30 [Clark et al. 1992]; the *Drosophila* proteins brahma [Tamkun et al. 1992] and lodestar [Girdham and Glover 1991]; and the human proteins hSNF2L and ERCC6 [Okabe et al. 1992; Troelstra et al. 1992]. These proteins function in diverse processes that could involve nucleic acid-dependent NTPase or helicase activity: SNF2, MOT1, and brahma are implicated in transcriptional regulation; lodestar is important for chromatid separation during mitosis; and the RAD and ERCC6 proteins are necessary for repair of DNA damage. Thus, it seems plausible that the sequence similarity of SNF2 to known NTPases and helicases reflects function.

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The SNF2 protein also contains at its carboxyl terminus another highly conserved sequence, termed the bromodomain, which serves an unknown function (Haynes et al. 1992; Tamkun et al. 1992). This 77-residue sequence is shared by the yeast proteins STH1 (Laurent et al. 1992), SPT7 (Haynes et al. 1992), and GCN5 (Georgakopoulos and Thireos 1992); the *Drosophila* female sterile homeotic (*fsh*) (Haynes et al. 1989) and *brahma* (Tamkun et al. 1992) proteins; and the human proteins CCG1 (Sekiguchi et al. 1988) and RING3 (Beck et al. 1992).

Here, we take genetic and biochemical approaches to address the functional significance of the helicase-related sequences in the SNF2 family of proteins. We test the effects of mutations in the putative NTP-binding site and other conserved motifs, and we show that the helicase-related region from STH1 can be swapped into the SNF2 protein. Using bacterially expressed protein, we demonstrate that SNF2 has ATPase activity that is stimulated by double-stranded DNA (dsDNA). Finally, we examine the requirement for sequences flanking the helicase-related region in transcriptional activation by SNF2.

Results

Mutation of the nucleotide-binding site compromises SNF2 function

To test the importance of the conserved sequence motifs in SNF2, we first mutated the invariant lysine (Lys-798) in the GXGKT sequence of the "A motif" of the putative NTP-binding site (Walker et al. 1982). Biophysical studies of nucleotide-binding proteins suggest that this lysine can interact with the phosphoryl group of bound NTP (see Story and Steitz 1992), and conservative substitutions of arginine for lysine in various NTP-binding proteins affect function (Sung et al. 1988; Xia and Storm 1990; Parsell et al. 1991). We therefore replaced Lys-798 in SNF2 with arginine, yielding the mutant allele designated K798R.

To assess its functional consequences, we introduced the mutation into the plasmid pLexA-SNF2, which expresses a bifunctional LexA-SNF2 hybrid protein. The mutant derivative pLexA-SNF2-K798R failed to complement growth defects of an *snf2* mutant on raffinose, galactose, or glycerol and showed reduced ability to complement the defect in invertase expression (Table 1), caused by the defect in transcription of the *SUC2* gene (Neigeborn and Carlson 1984; Abrams et al. 1986). We also compared the ability of LexA-SNF2-K798R and LexA-SNF2 to activate the expression of *GAL1-lacZ* target genes when bound to DNA. In a wild-type host, the mutation reduced activation of β -galactosidase expression from target genes containing one or multiple *lexA* operators 35- and 10-fold, respectively (Table 1). In an *snf2* mutant host, activation was reduced similarly (data not shown). The LexA-SNF2 and LexA-SNF2-K798R hybrid proteins were expressed at comparable levels, as determined by immunoblotting (data not

Table 1. Mutations in the helicase-related domain affect SNF2 function

	Invertase activity	β -Gal activity	
		1 op	6 op
pLexA-SNF2	121	70	370
pLexA-SNF2-K798R	18	2	35
pLexA-SNF2 $_{\Delta 1037-1255}$	ND	7	50
pSH2-1	5	<1	<1

Invertase activity was assayed in *snf2* mutant MCY2156 and is expressed as micromoles of glucose released per minute per 100 mg (dry weight) of cells. β -Galactosidase was assayed in MCY829 (wild type), carrying target plasmids 1840 or pSH18-18 with one or six operators (op), respectively. pSH2-1 is the vector. Values for the mutants are averages for four transformants. Values for wild type are averages for two or three transformants and agree with previous data (Laurent et al. 1991). Standard errors were <20%. (ND) Not determined.

shown). These results indicate that the conserved nucleotide-binding motif is important for transcriptional activation by SNF2 and suggest that NTP hydrolysis is an integral function of the SNF2 protein.

To test the importance of other conserved sequences in the helicase-related domain, we constructed pLexA-SNF2 $_{\Delta 1037-1255}$, which encodes a hybrid protein lacking helicase motifs V and VI (see Fig. 4, below). This plasmid failed to complement an *snf2* mutant for growth on raffinose and galactose (data not shown), and β -galactosidase expression from targets with one or multiple operators was substantially reduced relative to pLexA-SNF2 (Table 1). These results suggest that an intact helicase-related region is important for transcriptional activation by SNF2.

Mutations in the nucleotide-binding site of the STH1 protein abolish function

We then mutated the nucleotide-binding site of another member of the SNF2 helicase-related family, the STH1 protein. STH1 is essential for viability, but its function is unknown; the *STH1* gene was isolated based on sequence similarity to *SNF2* (Laurent et al. 1992). We replaced Lys-501 (analogous to SNF2 Lys-798) with arginine or threonine, yielding mutant alleles designated K501R and K501T. Each mutation was introduced into plasmid pSTH1(1291)-*lacZ*, which expresses a fusion protein that restores viability to *sth1* haploid segregants from the diploid MCY2471 (*sth1-2::HIS3/STH1 his3/his3 ura3/ura3*) (Laurent et al. 1992). The mutant derivatives pSTH1-K501R-*lacZ* and pSTH1-K501T-*lacZ* (Fig. 1), were used to transform MCY2471, Ura⁺ transformants were sporulated with selection for the plasmid, and tetrad analysis was carried out. Two transformants carrying pSTH1-K501R-*lacZ* yielded only two viable Ura⁺ His⁻ spores from each of 13 tetrads, and three transformants with pSTH1-K501T-*lacZ* yielded two viable Ura⁺ His⁻ spore clones from each of 12 tetrads. No

Figure 1. STH1- β -galactosidase fusion proteins. The three plasmids contain STH1 codons 1–1291 fused to *lacZ*. pSTH1–K501R–*lacZ* and pSTH1–K501T–*lacZ* carry the K501R and K501T mutations.

viable His⁺ Ura⁺ spore clones were recovered. The mutant proteins are expressed at levels comparable with the wild-type protein, as judged by immunoblot analysis. Thus, STH1 fusion proteins bearing the K501R and K501T mutations fail to provide the STH1 function required for viability. These genetic data suggest that the NTP-binding site is essential for STH1 function.

The STH1 helicase-related domain can be swapped into the SNF2 protein

The sequence conservation among SNF2, STH1, and other members of the family suggests that the helicase-related regions represent functional domains. If so, one would predict that these domains would be exchangeable between family members. The SNF2 and STH1 proteins provide a useful test case for this idea because SNF2 and STH1 are functionally distinct: SNF2 functions as a transcriptional activator, whereas STH1 does not, and increased gene dosage of one does not compensate for loss of the other (Laurent et al. 1992). We therefore swapped the helicase-like domain of STH1 into the LexA–SNF2 protein, replacing SNF2 residues 724–1214 with STH1 residues 426–917. Overall these sequences are 73% identical, with the greatest conservation found in regions encompassing helicase motifs (Laurent et al. 1992). The resulting hybrid protein, expressed from pLexA–SNF2/STH1, was then tested for SNF2 function.

pLexA–SNF2/STH1 complemented partially an *snf2* mutant for expression of invertase, elevating activity fivefold above that conferred by the vector pSH2-1 (Fig. 2), and restored growth partially on raffinose, galactose, or glycerol. The LexA–SNF2/STH1 protein also activated expression of *GAL1-lacZ* target genes containing a single *lexA* operator but did so less effectively than LexA–SNF2 (15-fold lower β -galactosidase activity); however, when the target promoter contained multiple LexA-binding sites, activation by LexA–SNF2/STH1 was comparable with that effected by LexA–SNF2 in wild-type and *snf2* strains (Fig. 2). Thus, the STH1 helicase-related region appears to substitute functionally for the corresponding SNF2 region, although the resulting hybrid protein does not have wild-type SNF2 activity.

Because the hybrid protein was not fully functional, we carried out controls to confirm that the NTP-binding site of the swapped STH1 region is essential for the activity. We constructed pLexA–SNF2/STH1–K501R and pLexA–SNF2/STH1–K501T, carrying mutations in the

NTP-binding site. Neither complemented *snf2* for invertase derepression (Fig. 2) nor for growth on raffinose, galactose, or glycerol. The mutant fusion proteins were also 5- to 10-fold less effective in activating β -galactosidase expression than LexA–SNF2/STH1 (Fig. 2). All hybrid proteins were detected at comparable levels by immunoblotting.

These data indicate that the helicase-related regions of SNF2 and STH1 are interchangeable and argue that they represent functional domains.

SNF2 has ATPase activity that is stimulated by dsDNA

To obtain biochemical evidence that the SNF2 protein has an ATP hydrolysis activity, we expressed SNF2 sequences in bacteria. We constructed several fusions between the *Escherichia coli* maltose-binding protein (MBP) and SNF2 sequences, including fusions to the entire SNF2 protein and to the helicase-related domain alone. Preliminary experiments detected ATPase activity only for the MBP–SNF2_{741–1703} protein containing the helicase-like region and the carboxyl terminus of SNF2. Therefore, an analogous mutant fusion protein containing the K798R substitution, designated MBP–SNF2–K798R_{741–1703}, was expressed to provide a control for further experiments. For simplicity, these proteins will be called MBP–SNF2 and MBP–SNF2–K798R.

The wild-type and mutant fusion proteins were purified from bacteria and tested for ATP hydrolysis activity (see Materials and methods). In the absence of nucleic

	Invertase activity	β -Gal activity			
		WT		<i>snf2</i>	
		1 Op	6 Op	1 Op	6 Op
pLexA-STH1		ND	2	3	ND
pLexA-SNF2		120	120	350	910
pLexA-SNF2/STH1		23	8	150	830
pLexA-SNF2/STH1-K501R		7	1	20	130
pLexA-SNF2/STH1-K501T		7	1	11	160
pSH2-1		5	<1	<1	<1

Figure 2. The STH1 helicase-related sequence substitutes functionally for the SNF2 sequence. For the LexA fusion proteins, STH1 (stippled bar) and SNF2 (open bar) sequences are shown drawn to scale, with the first and last residues indicated and the helicase-related regions overlined. Positions of the K501R and K501T mutations are indicated. (L) LexA DNA-binding domain (not to scale). The host strain for invertase assays was MCY2156 (*snf2*). β -Galactosidase assays were carried out in strains MCY829 [wild type (WT)] or MCY2156 carrying target plasmids 1840 or pSH18-18; (Op) operators. Values are the average activity for at least four transformants. (ND) Not determined; pLexA–STH1 does not complement *snf2* (Laurent et al. 1992).

acid, both preparations exhibited comparable low-level activity. However, ATP hydrolysis by the MBP-SNF2 fusion protein was stimulated more than fivefold by dsDNA, whereas the activity of the mutant protein was not (Fig. 3). Under these conditions, ATP hydrolysis was linear with time for ≥ 60 min (Fig. 3B) and was proportional to the amount of MBP-SNF2 protein (data not shown). The specific activity of the MBP-SNF2 preparation in the presence of dsDNA was ~ 20 pmoles of inorganic phosphate (P_i) released per microgram per minute, which is within the range of activities measured for other nucleic acid-stimulated ATPases (Schwer and Guthrie 1991). Single-stranded DNA (ssDNA) caused a twofold stimulation of hydrolysis by MBP-SNF2, but RNA had no detectable effect (Fig. 3C).

The activity detected for both wild-type and mutant fusion protein preparations in the absence of nucleic acid can probably be attributed to a copurifying contaminant because MBP alone, purified in parallel, showed the same activity (Fig. 3A). For these control experiments, an equal amount of eluate rather than an equal amount of MBP protein was assayed, because MBP was expressed at levels fivefold higher than the fusion proteins.

Thus, MBP-SNF2 has dsDNA-stimulated ATPase activity, which is abolished by a conservative substitution in the NTP-binding motif. These *in vitro* biochemical data are in accord with genetic evidence that the K798R mutation affects SNF2 function *in vivo*.

SNF2 sequences required for transcriptional activation

Two lines of evidence suggest that the helicase-related

region of SNF2 may not be sufficient for transcriptional activation. First, other proteins in the SNF2 family appear to have functions different from transcriptional activation. Second, above we showed that the helicase-like region of STH1 can substitute for the corresponding region of SNF2 and yet, unlike SNF2, the STH1 protein is not a transcriptional activator. This evidence suggests that other parts of SNF2 are essential for the transcriptional activation function. Flanking the helicase-related domain of the SNF2 protein are large amino- and carboxy-terminal regions. The noteworthy feature of the amino terminus of SNF2 is a 29-residue QQA repeat (Fig. 4). The carboxyl terminus includes an 18-residue RG repeat, a nuclear targeting signal (Laurent et al. 1991), and the bromo domain (Tamkun et al. 1992).

To determine the role of these amino- and carboxy-terminal sequences in transcriptional activation, we constructed a series of LexA fusion plasmids (Fig. 4). All of these plasmids express proteins of the expected sizes at levels comparable with LexA-SNF2, as judged by immunoblotting. However, none of these fusion proteins containing portions of the SNF2 sequence stimulated transcription appreciably; in each case, target gene activation was $<1\%$ that effected by LexA-SNF2 (Fig. 4). Although we do not know that any of these proteins folds properly, the LexA-SNF2₇₄₁₋₁₇₀₃ fusion protein contains the same SNF2 sequences that were sufficient for ATP hydrolysis by the MBP-SNF2 protein. We note that LexA-SNF2 _{Δ 1037-1255} and LexA-SNF2-K798R, which carry mutations in the helicase-like domain, each caused greater activation than proteins missing either amino- or carboxy-terminal sequences, suggesting that

Figure 3. The MBP-SNF2 fusion protein has dsDNA-stimulated ATPase activity. (A) ATPase assay. Bacterially expressed MBP (13.5 μ g), MBP-SNF2 (3.7 μ g), and MBP-SNF2-K798R (3.9 μ g) proteins were assayed for ATPase activity in the presence (+) or absence (-) of double-stranded phagemid DNA (1 μ g) (see Materials and methods). Incubation was for 30 min. In the absence of added protein (None), the background hydrolysis was 6 pmoles/ μ l. In separate experiments, an equal amount of plasmid dsDNA containing the *SUC2* promoter and upstream activating sequence (UAS) did not stimulate ATPase activity further. (B) Time course of ATP hydrolysis. MBP-SNF2 and MBP-SNF2-K798R proteins were assayed in the presence or absence of dsDNA as in A. Aliquots were taken after incubation for 10, 20, 40, and 60 min and analyzed for release of P_i . The activity is given as picomoles of P_i released from [γ - 32 P]ATP/ μ l of the reaction mixture. ($\square$) MBP-SNF2 + DNA; ($\blacksquare$) MBP-SNF2-K798R + DNA; ($\circ$) MBP-SNF2 - DNA and MBP-SNF2-K798R - DNA (data points are superimposed). The background was subtracted from all values. (C) Stimulation of ATP hydrolysis by nucleic acid. Hydrolysis of ATP was assayed as in A. Reaction mixtures contained no nucleic acid (open bar), dsDNA (1 μ g) (solid bar), ssDNA (0.3 μ g) (stippled bar), or RNA (1 μ g) (hatched bar) (see Materials and methods). Error bars were calculated from three separate assays.

Figure 4. SNF2 sequences required for transcriptional activation. Expressed proteins are represented as in Fig. 2. (QQA) 29-residue QQA repeat at position 207 (stippled area); (RG) 18-residue RG repeat at position 1505 (thin solid area); (Br) 77-residue bromodomain beginning at residue 1572 (hatched area). The vertical bar at the position of the K768_{am} mutation indicates truncation of the protein. The wild-type host was MCY829 carrying plasmids 1840 or pSH18-18. β-Galactosidase values are the average activity for at least four transformants; (Op) operators. Values for pLexA-SNF2 are from Laurent et al. (1991).

both regions contribute to activation. In summary, the helicase-related region appears necessary but not sufficient for transcriptional activation by SNF2.

Analysis of bromodomain function

The conservation of the bromodomain among diverse eukaryotic proteins suggests an important function. Contrary to this view, previous evidence indicated that the bromodomain is not essential for SNF2 function: the SNF2(1554)-lacZ fusion lacks the bromodomain but complements an *snf2* null mutation (Laurent et al. 1991). However, many helicases are multimeric (Lohman 1992), and it seemed possible that the β-galactosidase moiety could substitute for the bromodomain to promote oligomerization. To test this idea, we expressed SNF2 residues 1–1554 without a β-galactosidase tail. Plasmid pITBL-SNF2₁₋₁₅₅₄ complemented an *snf2* mutant for growth on raffinose, galactose, or glycerol as effectively as pSNF2(1554)-lacZ and restored regulated invertase expression (data not shown). These findings confirm that the SNF2 bromo domain is dispensable.

Discussion

Here, we present genetic and biochemical evidence supporting the functional significance of the helicase-related sequences that are conserved in the SNF2 family. First, we showed that the NTP-binding motif is critical

for the function of SNF2 and the related protein STH1 by mutating the conserved Lys residue. The mutant SNF2-K798R protein was defective, as assayed by complementation of *snf2* mutant defects and by activation of target gene expression, and the mutant STH1-K501R and STH1-K501T products were unable to provide the function essential for viability. Second, deletion of a conserved region encompassing putative helicase motifs V and VI impaired SNF2 function. Third, swapping experiments showed that the helicase-related domain of the STH1 protein can substitute for the corresponding domain of SNF2. Finally, we demonstrated that bacterially expressed MBP-SNF2 fusion protein has dsDNA-stimulated ATPase activity in vitro. This activity is sensitive to the K798R mutation that affects SNF2 function in vivo. These genetic and biochemical data support the conclusion that SNF2 has dsDNA-stimulated ATPase activity that is required for its function in transcriptional activation.

Taken together with the sequence similarity to helicases, these data suggest that SNF2 unwinds duplex DNA. However, we have not been able to demonstrate that the MBP-SNF2 fusion protein has helicase activity. It is possible that helicase activity requires the amino terminus of SNF2, which is missing from the fusion protein, or the presence of other SNF/SWI proteins. Alternatively, DNA-dependent ATP hydrolysis may be the only enzymatic activity associated with SNF2. Helicase activity has not been demonstrated at this time for many proteins containing the signature motifs. NTP-binding proteins undergo conformational changes upon binding and hydrolysis of substrate (see Story and Steitz 1992), and perhaps the mechanism of SNF2 action involves such a conformational change.

Our results suggest that SNF2 has multiple functional domains. The helicase-related region is necessary for transcriptional activation, but it does not appear to be sufficient. First, this region from STH1 can replace the corresponding SNF2 sequence in the SNF2/STH1 hybrid protein but does not confer transcriptional activation function to the native STH1 protein. Second, the MBP-SNF2 protein has ATPase activity in vitro, but a LexA fusion containing the same SNF2 sequence does not activate transcription. Third, transcriptional activation was detected only when the LexA fusion contained the entire SNF2 sequence, and proteins mutant in the helicase-related domain were better activators than proteins missing amino- or carboxy-terminal sequences. These findings suggest strongly that the amino- and carboxy-terminal regions contribute to SNF2 function. These regions may be responsible for interaction with other SNF/SWI proteins, gene-specific DNA-binding activators, general transcription factors, or chromatin components. The possibility that the SNF2 amino- or carboxy-terminal sequences interact with other SNF/SWI proteins is in accord with the model that SNF2 is part of a multiprotein complex comprising also SNF5, SNF6, SWI1, and SWI3 (Laurent et al. 1991; Peterson and Herskowitz 1992).

What is the role of the SNF2 ATPase activity in tran-

scriptional activation? SNF2 and the related SNF/SWI proteins are thought to function coordinately with gene-specific activators in stimulating transcription or alleviating chromatin-mediated repression, or both (Laurent and Carlson 1992; Peterson and Herskowitz 1992). We can imagine a role for a DNA-dependent ATPase in either process. ATP is required for transcriptional initiation by RNA polymerase II (Bunick et al. 1982), and Wang et al. (1992) reported that opening the DNA at the start site requires ATP hydrolysis. In prokaryotic transcription, open complex formation (von Hippel et al. 1984) is an important step for regulation. At bacterial σ^{54} promoters and some T4 promoters, enhancer-binding proteins hydrolyze ATP to catalyze open complex formation, thereby activating transcription (Gralla 1991; Kustu et al. 1991; Weiss et al. 1991). By analogy, we imagine that SNF2 could facilitate transcriptional activation by a mechanism in which its ATPase activity contributes to DNA melting at the start site. If so, it is likely that other proteins can also serve this function because *snf2* null mutants, although unhealthy, are viable.

A second possibility is that the SNF2 ATPase activity helps to remodel chromatin structure, perhaps by displacing nucleosomes or other chromatin-associated proteins (Travers 1992; Winston and Carlson 1992). Genetic evidence suggests that the SNF/SWI proteins function, at least in part, by countering the repressive effects of chromatin: Transcriptional defects of *snf* and *swi* mutants are suppressed by mutations in genes encoding histones (Hirschhorn et al. 1992; Peterson and Herskowitz 1992), *SPT* genes (Neugeborn et al. 1987; Happel et al. 1991), and *SIN1*, encoding an HMG1-like protein (Kruger and Herskowitz 1991). Moreover, the chromatin structure at the *SUC2* promoter is altered in *snf2* and *snf5* mutants (Hirschhorn et al. 1992; Matallana et al. 1992). The demonstration of a DNA-stimulated ATPase activity for SNF2 suggests mechanisms by which SNF2 could contribute to structural changes in chromatin, thereby setting the stage for transcriptional activation.

Materials and methods

Strains and media

S. cerevisiae strains MCY829 (*MAT α his3 Δ 200 lys2–801 ura3–52 SUC2*), MCY2156 (*MAT α snf2–141_{oc} his3 Δ 200 ura3–52 SUC2*), and MCY2471 (*MAT α /MAT α his3/his3 ura3/ura3 ade2/+ +/lys2 sth1–2::HIS3/+ SUC2/SUC2*) were derivatives of S288C. Rich (YPD) and synthetic complete (SC) media (Rose et al. 1990) contained 2% glucose. SC–raffinose and SC–galactose media contained 2% sugar and 1 μ g/ml of antimycin A. *E. coli* strains XL-1 Blue (Stratagene) and CJ236 (*dut, ung*) were used.

Construction of nucleotide binding-site mutations

pIT17 contains the 3.2-kb *KpnI*–*SalI* fragment of pLN138-4 (Abrams et al. 1986) cloned into pBluescript KS– (Stratagene), with a mutation changing the Lys-798 codon to Arg, constructed using the Bio-Rad Muta-Gene kit and oligonucleotide (OL) 29.

pM13–STH1, containing the 2.5-kb *EcoRI*–*KpnI* fragment of pBL51 (Laurent et al. 1992) in M13mp18, was used as template for site-directed mutagenesis with primers OL8 and OL9, replacing the Lys-501 codon with Arg and Thr codons, respectively. pBL51(K501R) and pBL51(K501T) are pBL51 containing these mutations. The *EcoRI*–*NcoI* fragment from each was cloned in YEp353 (Myers et al. 1986), yielding pSTH1–K501R–*lacZ* and pSTH1–K501T–*lacZ*. All mutations were confirmed by sequencing.

LexA–SNF2/STH1 hybrid plasmids

A *NcoI*–*EcoRI* fragment encoding STH1 residues 426–917 was generated by polymerase chain reaction (PCR) amplification of pBL51, using OL3 and OL4. This fragment was swapped into pLexA–SNF2 (Laurent et al. 1991) to replace SNF2 codons 725–1213, yielding pLexA–SNF2/STH1. pLexA–SNF2/STH1–K501R and pLexA–SNF2/STH1–K501T were constructed similarly.

LexA–SNF2 and SNF2 plasmids

LexA fusion plasmids are derivatives of pSH2-1 (Hanes and Brent 1989) and express from the *ADH1* promoter, the amino-terminal 87 residues of LexA fused to the indicated sequences. Expression of fusion proteins was confirmed by immunoblot analysis (Laurent and Carlson 1992). pLexA–SNF2_{14–833} contains the 2.5-kb *EcoRI* fragment of pLexA–SNF2. pLexA–SNF2_{1214–1703} contains the 1.9-kb *EcoRI*–*SalI* fragment of pLN138-4. pLexA–SNF2_{1–1257} contains the *BamHI*–*NruI* and *NruI*–*SalI* fragments generated by PCR amplification of pLN138-4 using primers OL5 and OL6 or OL7 and OL27. pLexA–SNF2_{723–1254} contains the *NcoI*–*BglII* SNF2 fragment modified with linkers. pLexA–SNF2_{741–1703} contains the *BamHI*–*NruI* fragment generated by PCR amplification of pLN138-4 primed by OL7 and OL27 and the 3-kb *NruI*–*SalI* fragment of pMBP–SNF2_{741–1703}. Two independent clones of pLexA–SNF2_{723–1254} and pLexA–SNF2_{741–1703} were tested. To construct pLexA–SNF2 _{Δ 1037–1255}, which contains SNF2 codons 14–1696 with codons 1037–1255 deleted, we first cloned in pRS314 (Sikorski and Hieter 1989) the 6.6-kb partial *BglII*–*SalI* fragment of pLN138-4 with the *BclI*–*BglII* fragment deleted and then cloned the *HincII*–*XhoI* fragment in pSH2-1. pLexA–SNF2–K798R contains the 2.3-kb *EcoRI*–*KpnI* fragment of pLexA–SNF2 and the 3.2-kb *KpnI*–*SalI* fragment of pIT17; the presence of a single mutation was verified by sequencing and fragment swapping. pLexA–SNF2_{14–767} was constructed similarly using a mutated pIT17 from an independent phage with an undirected amber mutation at codon 768. pITBL–SNF2_{1–1554} contains the 5.8-kb pLN138-4 *XbaI* fragment in YEp24.

Target plasmids for activation assay

pLR1 Δ 1 (West et al. 1984) contains a *GAL1*–*lacZ* gene fusion with the UAS_C removed. Plasmids 1840 (Brent and Ptashne 1985) and pSH18-18 (S. Hanes and R. Brent, pers. comm.) were derived from pLR1 Δ 1 by insertion of one or six overlapping *lexA* operators, respectively.

MBP fusion plasmids

MBP fusion plasmids are derivatives of pMAL-c2 (New England Biolabs), which expresses MBP from the inducible *tac* promoter. To construct pMBP–SNF2_{741–1257}, a *BamHI*–*SalI* fragment generated by PCR amplification of pLN138-4 primed by OL27 and OL28 was cloned in pMAL-c2. The *NruI*–*SalI* fragment in this

plasmid was replaced by the *NruI*–*SalI* fragment of pLN138-4, generating pMBP–SNF2_{741–1703}. pMBP–SNF2–K798R_{741–1703} contains a *Bam*HI–*Kpn*I fragment generated by PCR amplification of pLN138-4, using OL28 and OL27, and the *Kpn*I–*Sal*I fragment of pT17.

Preparation of MBP fusion proteins

MBP–SNF2 fusions were expressed in *E. coli* BCM501 (*uvrD::Tn5*; gift of W. Holloman, Memorial Sloan-Kettering, NY). Expression was induced by adding isopropyl β -D-thiogalactopyranoside (0.3 mM) to 1 liter of exponentially growing cells in Luria broth (LB) medium plus 1% dextrose and 100 μ g/ml ampicillin. After 3 hr at 37°C, the cells were harvested by centrifugation and resuspended in 13 ml of ice cold lysis buffer [200 mM Tris-HCl (pH 8.0), 5 mM EDTA, 2 mM dithiothreitol, 2 mM phenylmethylsulfonyl fluoride]. Cells were treated with lysozyme (100 μ g/ml) followed by the slow addition of 13 ml of cold H₂O. The cell suspension was then adjusted to 3 mM MgSO₄ and treated with DNase I (20 μ g/ml) for 10 min on ice, followed by the addition of glycerol to 10%. Cells were sonicated on ice discontinuously for 1 min. Cellular debris was removed by centrifugation at 10,000 rpm in a Sorvall SS34 rotor at 4°C. Amylose resin (0.5 ml, New England Biolabs), equilibrated in buffer A [10 mM sodium phosphate (pH 7.0), 50 mM NaCl, 10% glycerol, 1 mM NaN₃, 1 mM dithiothreitol], was added, and the mixture was incubated 1 hr at 4°C with gentle mixing on a Nutator (Clay Adams). The resin was washed three times with buffer A. The fusion proteins were eluted with 0.5 ml of buffer A containing 10 mM maltose by gentle mixing for 30 min. Protein was diluted 3.4-fold with 25 mM sodium phosphate (pH 7.0) and 10% glycerol and stored at 4°C. The storage buffer was 20 mM sodium phosphate (pH 7.0), 10% glycerol, 15 mM NaCl, 3 mM maltose, 0.3 mM NaN₃, and 0.3 mM DTT. Protein concentrations were determined by Bio-Rad protein assays.

The wild-type and mutant MBP–SNF2 proteins were recovered in equal amounts. In both cases, on Coomassie blue-stained SDS-polyacrylamide gels ~20% of the protein appeared to be >100 kD, and the same pattern of smaller polypeptides, probably the result of protein instability, was detected.

ATPase assay

The hydrolysis of ATP was measured as the release of inorganic phosphate, ³²P_i, from [γ -³²P]ATP using poly(ethyleneimine)-cellulose thin-layer chromatography plates (protocol from W. Holloman). The assay mixture (20 μ l) contained 10 μ l of buffer B [50 mM Tris-HCl (pH 7.0), 20 mM MgCl₂, 2 mM DTT, 2 mM ATP], 1 μ Ci of [γ -³²P]ATP (>5000 Ci/mmol, Amersham Corp.), 9 μ l of MBP fusion protein in storage buffer [20 mM sodium phosphate (pH 7.0), 10% glycerol, 15 mM NaCl, 3 mM maltose, and 0.3 mM DTT], and 1 μ l of H₂O or nucleic acid. After 30 min at 37°C, the reaction was terminated by the addition of 1 μ l of 0.5 M EDTA. A sample (1 μ l) of the reaction mixture was then spotted onto a poly(ethyleneimine)-cellulose plate (J.T. Baker), and chromatography was carried out in 0.8 M LiCl and 0.8 M acetic acid. Plates were autoradiographed at –70°C for 12–16 hr and scanned on a Betascope 603 (Betagen).

dsDNA and ssDNA used in these assays were prepared from the pBluescript KS– phagemid (Stratagene). RNA was prepared by in vitro transcription of pBLU-6, carrying *SNF6* sequences cloned in pBluescript KS–, using T7 RNA polymerase (Pharmacia) [Laurent et al. 1990].

β -Galactosidase and invertase assays

Freshly transformed colonies were grown to mid-log phase in

SC media lacking appropriate supplements to select for plasmids. β -Galactosidase activity was assayed in permeabilized cells (Guarente 1983) and is expressed as described by Miller (1972). At least four transformants of each type were assayed. Secreted invertase activity was assayed in derepressed cells (Neugeborn and Carlson 1984).

Oligonucleotides

Oligonucleotides were synthesized with an Applied Biosystems 394 Synthesizer. Restriction sites are underlined: OL3, 5'-GGC CATGGCAAAAATTCTTCATGGT-3'; OL4, 5'-GGGAATTC GTGGTAATTAATCTTAA-3'; OL5, 5'-CGGGATCCCGATG AACATACCACAG-3'; OL6, 5'-CTCTCATTTGGCGA-3'; OL7, 5'-CCGGATCCCGAACGTCGATTATTAC-3'; OL8, 5'-GGTTTAGGTAGAACCATCC-3'; OL9, 5'-GGTTTAGGTAC AACCATCC-3'; OL27, 5'-CCCCGTCGACTTACAAAGATC TAAGCAACG-3'; OL28, 5'-CCCCGGATCCAACGTCGATT ATTAC-3'; OL29, 5'-GGTCTTGGTAGGACTATC-3'.

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References

- Abrams, E., L. Neugeborn, and M. Carlson. 1986. Molecular analysis of *SNF2* and *SNF5* genes required for expression of glucose-repressible genes in *S. cerevisiae*. *Mol. Cell. Biol.* 6: 3643–3651.
- Bang, D.D., R. Verhage, N. Goosen, J. Brouwer, and P. van de Putte. 1992. Molecular cloning of *RAD16*, a gene involved in differential repair in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 20: 3925–3931.
- Beck, S., I. Hanson, A. Kelly, D.J. Pappin, and J. Trowsdale. 1992. A homologue of the *Drosophila* female sterile homeotic (*fsh*) gene in the class II region of the human MHC. *DNA Sequence* 2: 203–210.
- Brent, R. and M. Ptashne. 1985. A eukaryotic transcriptional activator bearing the DNA specificity of a prokaryotic repressor. *Cell* 43: 729–736.
- Bunick, D., R. Zandomeni, S. Ackerman, and R. Weinmann. 1982. Mechanism of RNA polymerase II-specific initiation of transcription in vitro: ATP requirement and uncapped runoff transcripts. *Cell* 29: 877–886.
- Clark, M.W., W.W. Zhong, T. Keng, R.K. Storms, A. Barton, D.B. Kaback, and H. Bussey. 1992. Identification of a *Saccharomyces cerevisiae* homolog of the *SNF2* transcriptional regulator in the DNA sequence of an 8.6 kb region in the *LTE1-CYS1* interval on the left arm of chromosome I. *Yeast* 8: 133–145.
- Davis, J.L., R. Kunisawa, and J. Thorner. 1992. A presumptive helicase (*MOT1* gene product) affects gene expression and is required for viability in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 12: 1879–1892.
- Emery, H.S., D. Schild, D.E. Kellogg, and R.K. Mortimer. 1991.

- Sequence of *RAD54*: a *Saccharomyces cerevisiae* gene involved in recombination and repair. *Gene* **104**: 103–106.
- Felsenfeld, G. 1992. Chromatin as an essential part of the transcriptional mechanism. *Nature* **355**: 219–224.
- Georgakopoulos, T. and G. Thireos. 1992. Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. *EMBO J.* **11**: 4145–4152.
- Gill, G. and R. Tjian. 1992. Eukaryotic coactivators associated with the TATA box binding protein. *Curr. Opin. Gen. Dev.* **2**: 236–242.
- 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.
- Gorbalenya, A.E., E.V. Koonin, A.P. Donchenko, and V.M. Blinov. 1989. Two related superfamilies of putative helicases involved in replication, recombination, repair and expression of DNA and RNA genomes. *Nucleic Acids Res.* **17**: 4713–4730.
- Gralla, J.D. 1991. Transcriptional control—Lessons from an *E. coli* promoter data base. *Cell* **66**: 415–418.
- Guarente, L. 1983. Yeast promoters and *lacZ* fusions designed to study expression of cloned genes in yeast. *Methods Enzymol.* **101**: 181–191.
- 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.
- Haynes, S.R., B.A. Mozer, N. Bhatia-Dev, and I.B. Dawid. 1989. The *Drosophila fsh* locus, a maternal effect homeotic gene, encodes apparent membrane proteins. *Dev. Biol.* **134**: 246–257.
- Haynes, S.R., C. Dollard, F. Winston, S. Beck, J. Trowsdale, and I.B. Dawid. 1992. The bromodomain: A conserved sequence found in human, *Drosophila* and yeast proteins. *Nucleic Acids Res.* **20**: 2603.
- Hirshhorn, 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.
- Hodgman, T.C. 1988a. A new superfamily of replicative proteins. *Nature* **333**: 22–23.
- Hodgman, T.C. 1988b. A new superfamily of replicative proteins. [Erratum] *Nature* **333**: 578.
- Johnson, R.E., S.T. Henderson, T.D. Petes, S. Prakash, M. Bankmann, and L. Prakash. 1992. *Saccharomyces cerevisiae* *RAD5*-encoded DNA repair protein contains helicase and zinc-binding sequence motifs and affects the stability of simple repetitive sequences in the genome. *Mol. Cell. Biol.* **12**: 3807–3818.
- 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.
- Kustu, S., A.K. North, and D.S. Weiss. 1991. Prokaryotic transcriptional enhancers and enhancer-binding proteins. *Trends Biochem. Sci.* **16**: 397–402.
- 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.
- . 1991. Functional interdependence of the yeast *SNF2*, *SNF5*, and *SNF6* proteins in transcriptional activation. *Proc. Natl. Acad. Sci.* **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.
- Lohman, T.M. 1992. *Escherichia coli* DNA helicases: Mechanisms of DNA unwinding. *Mol. Microbiol.* **6**: 5–14.
- Matallana, 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, New York.
- Myers, A.M., A. Tzagoloff, D.M. Kinney, and C.J. Lusty. 1986. Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions. *Gene* **45**: 299–310.
- Neigeborn, L. and M. Carlson. 1984. Genes affecting the regulation of *SUC2* gene expression by glucose repression in *Saccharomyces cerevisiae*. *Genetics* **108**: 845–858.
- 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.
- Okabe, I., L.C. Bailey, O. Attree, S. Srinivasan, J.M. Perkel, B.C. Laurent, M. Carlson, D.L. Nelson, and R.L. Nussbaum. 1992. Cloning of human and bovine homologs of *SNF2*/*SWI2*: A global activator of transcription in yeast *S. cerevisiae*. *Nucleic Acids Res.* **20**: 4649–4655.
- Parsell, D.A., Y. Sanchez, S.D. Stitzel, and S. Lindquist. 1991. *Hsp104* is a highly conserved protein with two essential nucleotide-binding sites. *Nature* **353**: 270–273.
- 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.
- 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, New York.
- Schild, D., B.J. Glassner, R.K. Mortimer, M. Carlson, and B.C. Laurent. 1992. Identification of *RAD16*, a yeast excision repair gene homologous to the recombinational repair gene *RAD54* and to the *SNF2* gene involved in transcriptional activation. *Yeast* **8**: 385–395.
- Schwer, B. and C. Guthrie. 1991. *PRP16* is an RNA-dependent ATPase that interacts transiently with the spliceosome. *Nature* **349**: 494–499.
- Sekiguchi, T., T. Miyata, and T. Nishimoto. 1988. Molecular cloning of the cDNA of human X chromosomal gene (*CCG1*) which complements the temperature-sensitive *G₁* mutants, *tsBN462* and *ts13*, of the BHK cell line. *EMBO J.* **7**: 1683–1687.
- 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.
- Story, R.M. and T.A. Steitz. 1992. Structure of the *recA* protein-ADP complex. *Nature* **355**: 374–376.
- Sung, P., D. Higgins, L. Prakash, and S. Prakash. 1988. Mutation of lysine-48 to arginine in the yeast *RAD3* protein abolishes its ATPase and DNA helicase activities but not the ability to bind ATP. *EMBO J.* **7**: 3263–3269.
- Tamkun, J.W., R. Deuring, M.P. Scott, M. Kissinger, A.M. Patatucci, 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.
- Travers, A.A. 1992. The reprogramming of transcriptional competence. *Cell* 69: 573–575.
- Troelstra, C., A. van Gool, J. de Wit, W. Vermeulen, D. Bootsma, and J.H.J. Hoeijmakers. 1992. ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's Syndrome and preferential repair of active genes. *Cell* 71: 939–953.
- von Hippel, P.H., D.G. Bear, W.D. Morgan, and J.A. McSwiggen. 1984. Protein-nucleic acid interactions in transcription: A molecular analysis. *Ann. Rev. Biochem.* 53: 389–446.
- Walker, J.E., M. Saraste, M.J. Runswick, and N.J. Gay. 1982. Distantly related sequences in the α - and β -subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO J.* 1: 945–951.
- Wang, W., M. Carey, and J.D. Gralla. 1992. Polymerase II promoter activation: Closed complex formation and ATP-driven start site opening. *Science* 255: 450–453.
- Weiss, D.S., J. Batut, K.E. Klose, J. Keener, and S. Kustu. 1991. The phosphorylated form of the enhancer-binding protein NTRC has an ATPase activity that is essential for activation of transcription. *Cell* 67: 155–167.
- 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.
- Winston, F. and M. Carlson. 1992. Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet.* 8: 387–391.
- Xia, Z. and D.R. Storm. 1990. A-type ATP binding consensus sequences are critical for the catalytic activity of the calmodulin-sensitive adenylyl cyclase from *Bacillus anthracis*. *J. Biol. Chem.* 265: 6517–6520.
- 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.

The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation.

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References

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