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Functional analysis of the DNA-stimulated ATPase domain of yeast SWI2/SNF2

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ABSTRACT

The yeast SWI2/SNF2 polypeptide is a subunit of the SWI/SNF protein complex that is required for many transcriptional activators to function in a chromatin context. SWI2 is believed to be the founding member of a new subfamily of DNA-stimulated ATPases/DNA helicases that includes proteins that function in DNA repair (RAD5, RAD16, ERCC6), recombination (RAD54), transcription (MOT1, ISWI, brm, BRG1, hBRM) and cell cycle control (STH1). We have created a set of 16 mutations within the SWI2 ATPase domain and have analyzed the functional consequences of these mutations *in vivo*. We have identified residues within each of the seven ATPase motifs that are required for SWI2 function. We have also identified crucial residues that are interspersed between the known ATPase motifs. In contrast, we identify other highly conserved residues that appear to be dispensable for SWI2 function. We also find that single amino acid changes in ATPase motifs IV and VI lead to a dominant negative phenotype. None of the 12 SWI2 mutations that disrupt SWI2 activity *in vivo* alter the assembly of the SWI/SNF complex. These studies provide an invaluable framework for biochemical analysis of the SWI2 ATPase and for functional analysis of other SWI2 family members.

INTRODUCTION

The SWI/SNF protein complex, which is composed of 11 different polypeptides (1–3), is required for the induced expression of a large number of diversely regulated yeast genes, including *HO*, *SUC2*, *INO1* and *ADH2* (for review see 4). Furthermore, the efficient functioning of several sequence-specific transcriptional activator proteins, such as yeast GAL4, *Drosphila* ftz, and mammalian steroid receptors, requires an intact SWI/SNF complex (5,6). Mutations that alter chromatin components partially alleviate the defects in transcription due to inactivation of the SWI/SNF complex, and thus it has been proposed that the primary role of this complex is to facilitate the function of gene regulatory proteins in a chromatin environment (7–10). Recent *in vitro* studies are consistent with this role, as the purified SWI/SNF complex can use the energy of ATP hydrolysis to facilitate the binding of a transcriptional activator, GAL4-AH, to a nucleosome (2).

The *SWI2* gene encodes a 200 kDa polypeptide that is the ATPase subunit of the SWI/SNF complex. The sequence of *SWI2* contains seven sequence motifs that are characteristic of members of the DEAD/H superfamily (superfamily 2) of nucleic acid-stimulated ATPases and DNA helicases (11,12). The SWI/SNF complex has DNA-stimulated ATPase activity *in vitro*, but lacks detectable DNA helicase (2,13) or DNA tracking (14) activities. A single amino acid change in the putative nucleotide binding loop (motif I) of SWI2 eliminates SWI2 function *in vivo* (5,13) as well as the ATPase activity of the SWI2 subunit and SWI/SNF complex *in vitro* (2,13). Functionally important amino acids within motifs I and II have also been identified in other members of this superfamily (16–18); however, the importance of residues within motifs Ia, III, IV, V and VI are not known for any DNA-stimulated ATPase.

A large number of proteins have been identified that contain ATPase domains that are more similar to that of SWI2 than other members of the nucleic acid-dependent ATPase and helicase superfamily. This observation led to the proposal that SWI2 may define a new subfamily of DNA-stimulated ATPases (19,20; reviewed in 12,21). This subfamily includes putative functional homologues of SWI2, such as *Drosophila* brm, mouse brg1, human BRG1, and human hbrm, as well as proteins that function in DNA repair, recombination, cell cycle control and transcriptional repression. Viral and bacterial family members have also been described. Although several family members have been shown to exhibit DNA-stimulated ATPase activity, no DNA helicase activity has been described for any member of the SWI2 subfamily. Consistent with these results, Henikoff (22) has suggested that the ATPase motifs of the SWI2 subfamily are most closely related to poxvirus DNA-dependent ATPases.

We have carried out an extensive mutational analysis of the SWI2 DNA-stimulated ATPase domain. Mutations were constructed in each of the seven ATPase/helicase motifs as well as in three additional sequence elements that are highly conserved only in members of the SWI2 subfamily. Residues have been identified in each of these sequence elements that are crucial for SWI2 function *in vivo*. Several additional, highly conserved residues, however, do not appear to play an important role in the activity of SWI2. Furthermore, single amino acid changes in either motif IV or VI result in dominant negative phenotypes that are more potent than previously identified dominant negative *SWI2* alleles.

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Table 1. Strain list

Name	Relevant genotype	Reference
CY26	α *SWI*$^+$*SNF*$^+$	5
CY118	**a** swi2 Δ::*HIS3 HO–lacZ*	isogenic to CY26, 15
CY120	α *swi2* Δ::*HIS3 HO–lacZ*	isogenic to CY26, 15
CY337 (YPH258)	**a** *ura3-52 lys2-801 ade2-101 leu2-Δ1 his3-Δ200*	P. Heiter
CY408	**a** *swi2* Δ::*HIS3* pEG28::*URA3*	isogenic to CY337
CY396	α *swi2* Δ::*HIS3 SWI2*-HA-6HIS::*URA3 HO–lacZ*	27
CY397	α *swi2* Δ::*HIS3 swi2* (K798A)-HA-6HIS::*URA3 HO–lacZ*	27
CY452	α *swi2* Δ::*HIS3 swi2* (P824A)-HA-6HIS::*URA3 HO–lacZ*	isogenic to CY120
CY409	α *swi2* Δ::*HIS3 swi2* (D894A,E895A)-HA-6HIS::*URA3 HO–lacZ*	isogenic to CY120
CY395	α *swi2* Δ::*HIS3 swi2* (D894E,E895Q)-HA-6HIS::*URA3 HO–lacZ*	isogenic to CY120
CY453	α *swi2* Δ::*HIS3 swi2* (P932A)-HA-6HIS::*URA3 HO–lacZ*	isogenic to CY120
CY454	α *swi2* Δ::*HIS3 swi2* (W935A)-HA-6HIS::*URA3 HO–lacZ*	isogenic to CY120
CY455	α *swi2* Δ::*HIS3 swi2* (R994A)-HA-6HIS::*URA3 HO–lacZ*	isogenic to CY120
CY456	α *swi2* Δ::*HIS3 swi2* (H1061A)-HA-6HIS::*URA3 HO–lacZ*	isogenic to CY120
CY457	α *swi2* Δ::*HIS3 swi2* (K1088A)-HA-6HIS::*URA3 HO–lacZ*	isogenic to CY120
CY458	α *swi2* Δ::*HIS3 swi2* (R1164A)-HA-6HIS::*HO–lacZ*	isogenic to CY120
CY519	α *swi2* Δ::*HIS3 swi2* (ΔSTRAGGLG)-HA-6HIS::*HO–lacZ*	isogenic to CY120
CY459	α *swi2* Δ::*HIS3 swi2* (W1185A)-HA-6HIS::*URA3 HO–lacZ*	isogenic to CY120
CY394	α *swi2* Δ::*HIS3 swi2* (R1196A)-HA-6HIS::*URA3 HO–lacZ*	isogenic to CY120

MATERIALS AND METHODS

Strains and media

Strains (Table 1) are isogenic derivatives of the YPH strain set described by Sikorski and Hieter (23). The GAL4 reporter, pEG28 (24), and the yIP5-based *SWI2* plasmids were targeted for integration at the *ura3-52* locus by digestion of the plasmids with *Apa*I or *Stu*I. Strains were grown in either S medium [6.7 g/l yeast nitrogen base without amino acids (Difco Laboratories)], SGS medium (6.7 g/l yeast nitrogen base, 2% galactose, 0.5% sucrose) or YEPD medium (2% yeast extract, 1% bacto-peptone, 2% glucose). S media were supplemented with amino acids as previously described (25). Appropriate amino acids were omitted for selection of plasmids.

Plasmids

Point mutations were generated by oligonucleotide-directed mutagenesis. The *Sac*I–*Kpn*I fragment which contains the ATPase domain of SWI2 was cloned into the RF form of M13mp18 (Pharmacia). An aliquot (100 µl) of phage supernatant was used to infect a dut$^-$ung$^-$ *Escherichia coli* strain (CJ236) and cells were grown in the presence of 20 µg/ml uridine. Single-stranded DNA was purified from the culture supernatant and processed for mutagenesis as described (26). For each mutant template the entire *Sac*I–*Kpn*I insert was sequenced (Sequenase, USB). Mutagenic oligonucleotides (DNA International) were as follows:

P824A: 5′-CAGTGTAGATAAAGCAACAATGAC-3′
W831A: 5′-CACTGCTAGCATTTGACAGTG-3′
D894EE895Q: 5′-GGCCTTGCTCAATAATCATATGG-3′
D894AE895A: 5′-CTATGGCCTGCAGCAATAATCATATGG-3′
P932A: 5′-GGCCCATAATTCTGCCAAGTTG-3′
W935A: 5′-CAATAAGGCAGCTAATTCTGG-3′
R994A: 5′-CCTTTTTCAAAGCACGCAATA-3′
HI061A: 5′-CAAATACAAAAGGAGCATTACA-3′
K1088A: 5′-GTTCAAATGCACCAGCAACACG-3′
D1093A: 5′-GGCAAAATTCTAGCTAATAG-3′-
G1166A: 5′-CCCAAACCAGCCGCTCTAGTTG-3′
W1185A: 5′-GATGAGGATTAGCGTCAGTATC-3′
ΔSTRAGGLG: 5′-GCAGTTTGTAAATTTAATAAGATAAA-GCACAAG-3′
R1196K: 5′-CCTATTCTATGCGCTTTATCCTGAG-3′.

The mutagenized *Sac*I–*Kpn*I fragments were isolated from RF DNA and used to replace the wild-type *SWI2* sequences in plasmid pER13 (*SWI2* in yEP24) to generate the following plasmids: pER14 (P824A), p5-1 (D894EE895Q), pER3 (D894AE895A), pER16 (P932A), pER22 (W935A), pER17 (R994A), pER18 (H1061A), pER19 (K1088A), pER23 (D1093A), pER36 (R1164A), pER21 (W1185A), pER20(G1166A), pER 56 (ΔSTRAGGLG) and p4-1(R1196K). The mutagenized *Sac*I–*Kpn*I fragment was also used to replace the wild-type *SWI2* fragment in plasmid pCP331 [*SWI2-HA-6HIS* in yIP5; (27)] to generate the following plasmids: pER48 (P824A), pER1 (D854AE855A), pER49 (P932A), pER50 (W935A), pER51 (R994A), pER52 (H1061A), pER53 (K1088A), pER54 (R1164A), pER55 (W1185A), pER57 (ΔSTRAGGLG) and pCP437 (R1196K). An *Nco*I–*Sal*I fragment from each of the above yEP24-based vectors was used to replace the wild-type *SWI2* sequences in plasmid pCP337 [*SWI2* in pRS315 (23)] to generate the following plasmids: pER24 (P824A), pER25 (D854AE855A), pER26 (W935A), pER27 (R994A), pER28 (H1061A), pER29 (K1088A), pER37 (D1093A), pER45 (R1164A), pER39 (G1166A), pER38

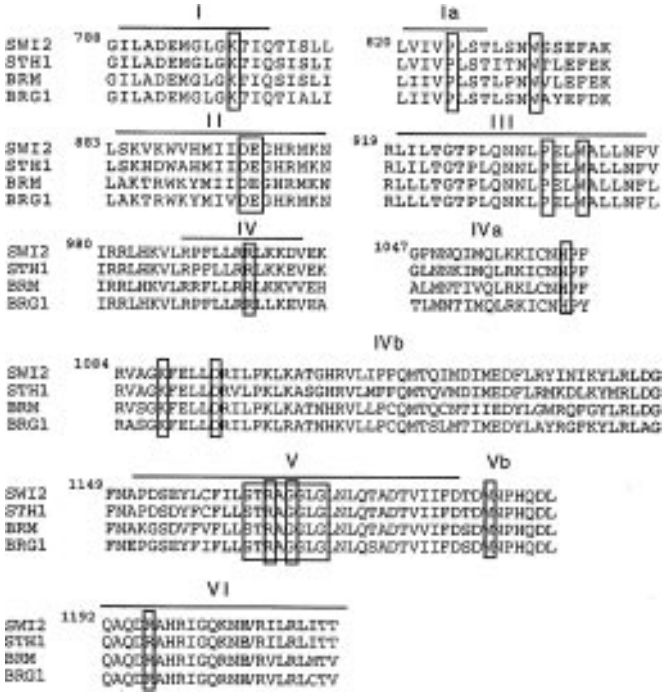

Figure 1. Amino acid sequence alignment of the ATPase domain of SWI2, *Saccharomyces cerevisae* STH1, *Drosophila* brm, and human BRG1 proteins. Sequence motifs characteristic of ATPase/helicase motifs are indicated by a solid line. Sequence motifs indicated by IVa, IVb, and Vb are more highly conserved among proteins most closely related to SWI2, but are not found in more distantly related proteins. Boxed residues were targeted for mutagenesis.

(W1185A), pER58 (ΔSTRAGGLG) and p4-1 (R1196K). The *Nco*I–*Sal*I fragments were also used to replace wild-type sequences in plasmid pCP345 [*SWI2* in pRS424 (23)] to generate the following high copy number plasmids: pER31 (D854AE855A), pER33 (R994A), pER40 (K1088A), pER59 (ΔSTRAGGLG) and pER42 (R1196K). All *SWI2* plasmids contain at least 900 base pairs (bp) of sequence upstream of the translation start site. In every case, the wild-type version fully complemented all growth and transcriptional defects of a *swi2* null allele.

Invertase assays

Invertase assays were performed essentially by the method of Celenza and Carlson (28) as adapted by M. Schmidt (University of Pittsburgh, PA) for smaller culture volumes. Briefly, 4 ml duplicate cultures were grown to mid-log phase in SD media (S with 2% glucose) and one culture was harvested (repressed). The second culture was washed twice with water and suspended in S media plus 0.05% glucose, allowed to grow for 2.5 h at 30°C and harvested (derepressed). The standard colorimetric assay was performed (28). Results are reported as nmol of glucose released/OD_{600}/min.

Preparation of extracts and immunoblotting

Crude whole cell extracts were prepared and analyzed by gel filtration on a Superose 6 gel-filtration column as described previously (27). Superose 6 fractions (0.5 ml) were TCA-precipitated, resuspended in 40 µl of SDS sample buffer, separated on 6% Laemmli gels, and transfered to nitrocellulose. Immunoblots were probed with the 12CA5 monoclonal antibody (Babco, Emeryville, CA) and developed with a chemiluminescent substrate as described (5).

RESULTS

Figure 1 shows the sequence of ATPase motifs I, Ia, II, III, IV, V and VI within yeast SWI2, yeast STH1, *Drosophila* brm and human BRG1. Additional sequence elements that are highly conserved among SWI2 family members are also shown and denoted IVa, IVb, and Vb. Boxed residues indicate conserved residues that were targeted for site-directed mutagenesis. In most instances mutations were designed that resulted in a single amino acid change to an alanine residue; in addition, two sets of double amino acid changes were constructed at positions 894 and 895, D894E,E895Q and D894A,E895A, a conserved lysine within motif VI was changed to an arginine (R1196K), and a small deletion was constructed that results in the removal of eight amino acids from motif V (ΔSTRAGGLG).

The functioning of *swi2* mutants was assessed *in vivo* by complementation of the transcriptional defects that result from the complete deletion of the *SWI2* gene. Three different SWI/SNF-dependent target genes were investigated: (i) an *HO–lacZ* fusion gene, (ii) a GAL4-dependent reporter gene and (iii) the *SUC2* gene.

Table 2. Effect of SWI2 ATPase motif mutations on HO expression

Motif	SWI2 allele	β-galactosidase expression (% wild-type)[a]
	wild-type (CY396)	100
	swi2 Δ	2
I	K798A	2
Ia	P824A	12
II	D894E,E895Q	2
	D894A,E895A	2
III	P932A	97
	W935A	12
IV	R994A	1.5
IVa	H1061A	53
IVb	K1088A	1
	D1093A	ND
V	R1164A	41
	G1166A	ND
	ΔSTRAGGLG	2
Vb	W1185A	37
VI	R1196K	2

[a]β-galactosidase activities of YEPD liquid cultures were performed (25) on at least three independent transformants, and the Miller units (33) were averaged. Results are expressed as % of wild-type (13.2 Miller units). Results varied by less than 20%.
ND, not determined

HO–lacZ

A deletion of the *SWI2* gene results in a 100-fold reduction in expression of a chromosomal *HO–lacZ* fusion gene (25). Mutated

SWI2 genes were initially introduced into a *swi2* deletion strain on high copy number plasmids and a qualitative β-galactosidase filter assay was performed. Eleven mutants showed measurable defects in activity in this assay, whereas four mutants, W831A (Ia), P932A (III), D1093A (IVb), and G1166A (V), showed levels of *HO–lacZ* expression that were indistinguishable from wild-type (data not shown). Wild-type *SWI2* and the remaining 11 mutants were integrated at the *URA3* locus for a quantitative analysis of their defects in *HO–lacZ* expression (Table 2). Seven mutants, K798A (I), D894E,E895Q (II), D895A,E896A (II), R994A (IV), K1088A (IVb), ΔSTRAGGLG (V), and R1196K (VI), had a null phenotype, reducing *HO–lacZ* expression to 1–2% of the wild-type level. Two mutants, P824A (Ia) and W935A (III), reduced *HO–lacZ* expression to 12–15% of the wild-type level, and three mutants, H1061A (IVa), R1164A (V) and W1185A (Vb), had a mild effect, lowering expression to ~40–50% of the wild-type level. These results indicate that at least one residue within each of the 10 targeted domains is important for *SWI2*-dependent expression of an *HO–lacZ* fusion gene.

Table 3. Effect of SWI2 ATPase motif mutations on Gal4 function

Motif	SWI2 allele	β-galactosidase expression (% wild-type)[a]
	wild-type	100
	swi2 Δ	4
I	K798A	7
Ia	P824A	50
II	D894E,E895Q	15
	D894A,E895A	5
III	P932A	ND
	W935A	44
IV	R994A	7
IVa	H1061A	71
IVb	K1088A	6
	D1093A	86
V	R1164A	61
	G1166A	72
	ΔSTRAGGLG	6
Vb	W1185A	71
VI	R1196K	7

[a]Three transformants were grown in SGS medium and analyzed as described in Table 2. Results are expressed as % of wild-type (110.4 Miller units). Values varied by less than 20%.
ND, not determined.

GAL4-dependent transcriptional activation

The ability of GAL4 to activate transcription from templates that contain two low affinity GAL4 binding sites is dependent upon an intact SWI/SNF complex (5). We tested whether activation of transcription by GAL4 was sensitive to a functional SWI2 ATPase domain. *SWI2* mutants were introduced on low copy number plasmids into strain CY408 which contains a deletion of *SWI2* and an integrated GAL4 reporter; expression of β-galactosidase reflects the capacity of GAL4 to activate transcription. The capacity of these *swi2* mutants to support GAL4-dependent activation is similar to our results for expression of the *HO–lacZ* fusion gene (Table 3). The same seven mutants, K798A (I), D894A,E895A (II), D894E,E895Q (II), R994A (IV), K1088A (IVb), ΔSTRAGGLG (V) and R1196K (VI), exhibited a phenotype similar to that of a deletion of *SWI2* (5–15% of the wild-type levels of GAL4-dependent expression). Only three other SWI2 mutants, P824A (Ia), W935A (III), and R1164A (V) had a significant effect on GAL4-dependent expression (50, 44, and 61% of wild-type, respectively). Four mutants had little effect on the ability of GAL4 to activate transcription. These mutants, H1061A (IVa), D1093A (IVb), G1166A (V) and W1185A (Vb), also had small effects on *HO–lacZ* expression (Table 2 and data not shown).

SUC2

The *SUC2* gene encodes the enzyme invertase which is required for yeast to ferment sucrose. *SUC2* expression is repressed on glucose medium and derepressed upon glucose starvation; this transcriptional induction requires a functional SWI/SNF complex (29). We assayed the production of invertase enzyme in strains harboring each of the integrated SWI2 ATPase mutants (Table 4).

Table 4. Effect of SWI2 ATPase motif mutations on invertase expression

Motif	SWI2 allele	Invertase activity[a]		
		Repressed	Derepressed	% Wild-type
	wild-type	7	356	100
	swi2 Δ	11	72	20
I	K798A	12	49	14
Ia	P824A	3	220	62
II	D894E,E895Q	6	77	22
	D894A,E895A	5	42	11
III	P932A	ND	ND	ND
	W935A	5	111	31
IV	R994A	7	40	11
IVa	H1061A	4	161	45
IVb	K1088A	6	26	7
	D1093A	6	419	118
V	R1164A	6	123	35
	G1166A	6	392	110
	ΔSTRAGGLG	4	38	11
Vb	W1185A	4	165	46
VI	R1196K	3	50	14

[a]Two independent transformants were analyzed and the average is shown. Invertase activity is defined as nmol of glucose released/OD_{600}/min. Results varied by less than 20%.
ND, not determined.

The defects in invertase expression due to ATPase motif mutations parallel very closely the results for *HO–lacZ* expression. The seven SWI2 mutants, K798A (I), D894A,E895A (II), D894E,E895Q (II), R994A (IV), K1088A (IVb), ΔSTRAGGLG (V) and R1196K (VI), showed induced levels of invertase expression similar to that of the *swi2* deletion strain. One mutant, P824A (Ia), had only a mild effect on invertase induction, while four mutants, W935A (III), H1061A (IVa), R1164A (V) and W1185A (Vb), had a moderate effect.

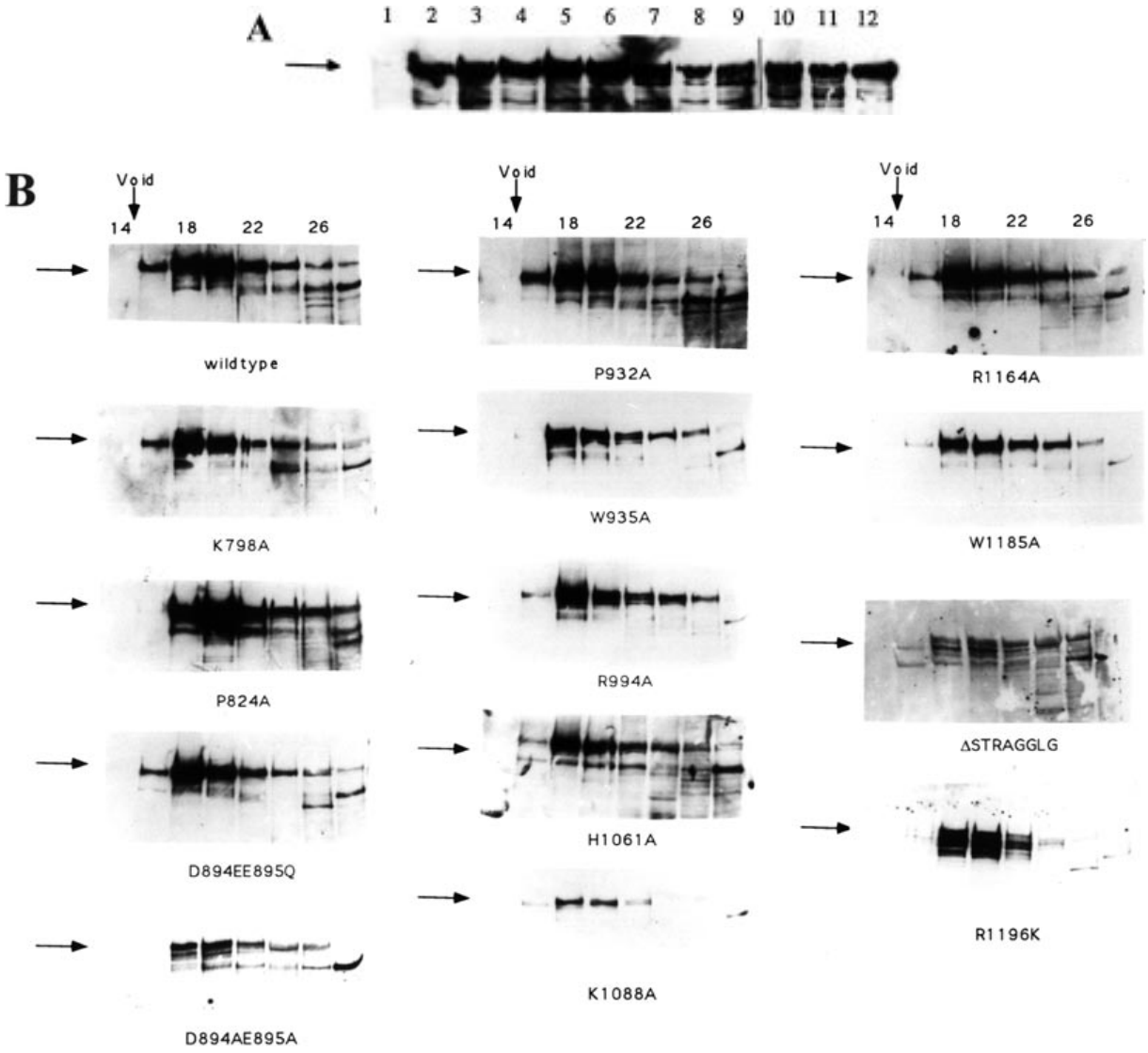

Figure 2. SWI2 ATPase domain mutants are expressed at wild-type steady-state levels and are assembled into SWI/SNF complexes. (**A**) Western blot analysis of whole cell extracts from strains harboring HA epitope-tagged SWI2 alleles. Lane 1, single copy SWI2 in strain CY396. Lanes 2–12 SWI2-HA tagged alleles expressed from high copy vectors in strain CY120; lane 2, SWI2; lane 3, swi2K798A; lane 4, swi2P824A; lane 5, swi2P932A; lane 6, swi2W935A; lane 7 swi2R1164A; lane 8, swi2W1185A; lane 9, swi2ΔSTRAGGLG; lane 10, swi2R994A; lane 11, swi2H1061A; lane 12, swi2K1088A. Equal numbers of cells were used for extract preparation and similar levels of total protein were electrophoresed in each lane as measured by Ponceau S staining. (**B**) Immunoblot analysis of Superose 6 gel-filtration fractions from strains expressing an integrated, single copy of HA epitope-tagged wild-type or the indicated mutant SWI2 proteins. Blots were probed with the 12CA5 monoclonal antibody. Horizontal arrows indicate full length SWI2 protein.

ATPase motif mutations do not affect SWI/SNF complex assembly

The effects of *swi2* mutations on transcription could be due to functional inactivation of the SWI/SNF complex or to a defect in SWI2 stability or SWI/SNF complex assembly. To test the latter two possibilities we analyzed the steady state level of SWI2 protein by western blot and the assembly of the SWI/SNF complex by gel filtration (Fig. 2).

Figure 2A shows a western blot analysis of crude whole cell extracts prepared from strains that harbor wild-type *SWI2* (lanes 1 and 2), or 10 *swi2* mutants on high copy plasmids (lanes 3–12). This analysis required the use of high copy vectors since SWI2 is almost undetectable when expressed from a single copy (lane 1). In almost every case, the steady state level of each SWI2 mutant is similar to the level of wild-type SWI2 expressed from the same high copy vector. Two mutants, K1185A and ΔSTRAGGLG, did show a decrease in their steady state levels compared with wild-type SWI2 on a high copy vector. However, the levels of these two SWI2 mutants were still much higher than wild-type SWI2 expressed at single copy. Since expression of all of these SWI2 mutants from high copy plasmids does not alleviate their transcriptional defects (see above), these results indicate that they are defective for function rather than stability.

Figure 2B shows the results of a gel filtration analysis which monitors the capacity of SWI2 mutants to assemble a SWI/SNF complex. The SWI2 subunit elutes from a Superose 6 column as a peak centered in fraction 19 when the SWI/SNF complex is intact and in fraction 25 when the complex is disassembled (27). Whole cell extracts were prepared from strains harboring each of

the integrated *swi2* mutants and each extract was fractionated on an FPLC Superose 6 gel filtration column (Fig. 2B). In each case the peak of mutant SWI2 subunit eluted between fractions 18 and 20, identical to the elution behavior of wild-type SWI2. Several SWI2 mutants, such as D894AE895A and ΔSTRAGGLG, appeared to be less stable *in vitro*, as evidenced by increased abundance of degradation products and a lower yield of SWI2 during the fractionation. However, in each instance these SWI2 mutants appeared to be competent for assembly *in vivo*, as each of these mutants also eluted as a peak in fractions 18–20. Furthermore, this decrease in stability was not as evident when steady state levels were measured (Fig. 2A). Thus, mutations in the ATPase motifs do not grossly impair SWI/SNF complex assembly. These results are similar to our previous study where we found that a mutation in SWI2 motif I (K798A) had only a minor effect on SWI/SNF complex assembly (27).

ATPase motif mutants exert dominant negative effects on transcriptional activation

Introduction of an *swi2* mutant, *swi2K798A*, on a high copy plasmid into a wild-type strain is able to reduce transcriptional activation by the mammalian glucocorticoid receptor by 50% (15). This result was used to design a similar mutation within a human *SWI2* homologue, BRG1. Overexpression of this BRG1 mutant in a cultured human cell line resulted in a dominant, selective inhibition of transcription driven by the eIFα promoter (15). We tested whether four additional ATPase motif mutants also had a dominant negative effect on GR-dependent transcriptional activation in yeast. Strain CY26 (*SWI2*$^+$) was transformed with three different plasmids: (i) a GR expression vector, (ii) a GR reporter that contains three GR binding sites upstream of a *CYC1–lacZ* fusion gene, and (iii) a high copy number plasmid that contained either wild-type *SWI2*, *swi2K798A* (I), *swi2D894A,E895A* (II), *swi2R994A* (IV), *swi2K1088A* (IVb) or *swi2R1196K* (VI). Expression of *swi2R994K* (IV) and *swi2R1196K* (VI) mutants were more effective than the *swi2K798A* (I) mutant at blocking activation of transcription by GR (Fig. 3), lowering GR-dependent activation to ~20% of the wild-type control. Expression of the other two ATPase mutants also caused a decrease in GR-dependent activation, but the decrease was similar to that found for the *swi2K798A* (I) allele. These results are consistent with the formation of non-functional SWI/SNF complexes that interfere with the ability of the wild-type SWI/SNF complex to facilitate GR function in yeast.

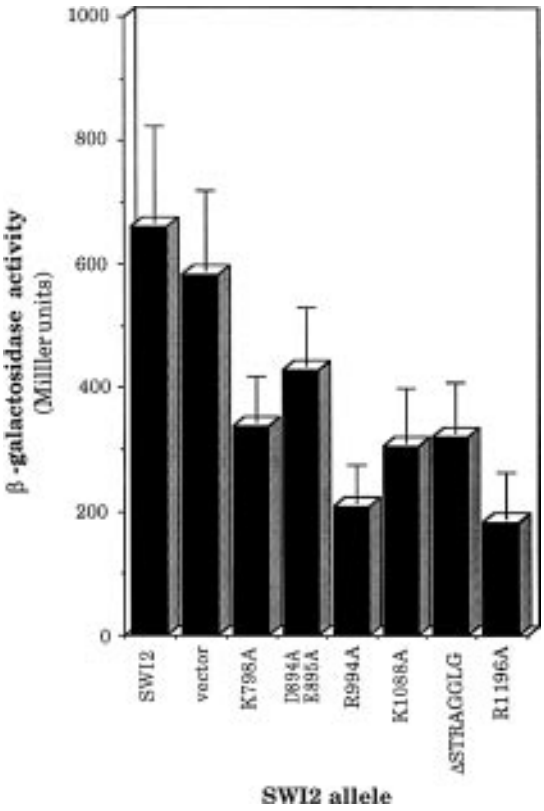

Figure 3. SWI2 ATPase mutants exhibit a dominant negative phenotype. Strain CY26 (*SWI2*$^+$) was transformed with the following three plasmids: pΔ26.x, pN795-LYS2 (GR reporter and expression plasmids, respectively; 15) and a high copy plasmid containing the indicated *SWI2* allele. From six to eight independent colonies were grown to an OD_{600} of 0.4 and deoxycorticosterone (DOC) (Sigma) in ethanol was added to 10 μM. Cultures were grown for an additional 3 h, harvested, and β-galactosidase activities were determined as described in Table 2.

DISCUSSION

SWI2 is a member of a large family of proteins that shares extensive sequence homology within seven sequence motifs characteristic of DNA-dependent ATPases and DNA helicases. We have constructed mutations in each of these motifs and characterized the consequences on SWI/SNF function *in vivo* (summarized in Table 5). We identified residues within each of these motifs that are required for SWI2 to facilitate expression of an *HO–lacZ* fusion gene, activation of transcription by GAL4, and induction of *SUC2* expression. In contrast, some residues were not important for SWI2 function even though they are highly conserved in SWI2 family members (Fig. 1 and Table 5).

Although the SWI2 subfamily of ATPases is comprised of at least 20 proteins, and the entire helicase superfamily 2 contains over 100 members, very little is known about the biochemical functions of the individual ATPase motifs. Motifs I and II are believed to be crucial for ATP binding and ATP hydrolysis, respectively; the function of the other five ATPase/helicase motifs is unknown. Mutations in these motifs may disrupt SWI2 function either by impairing its ability to bind ATP, to hydrolyze ATP, or to interact with its nucleic acid co-factor. Only in the case of the RNA helicase, eIF4A, has an extensive mutational analysis been carried out (17,30,31). In this case a sequence motif related to SWI2 motif VI is believed to be involved in binding the RNA cofactor (31). In addition, mutations in some ATPase/helicase motifs do not alter RNA-stimulated ATPase activity, but do eliminate RNA helicase activity of eIF4A (30). Most of the ATPase/helicase motifs of this RNA helicase, however, are not closely related to those of the SWI2 subfamily. In addition, the SWI2 subfamily also contains additional sequence blocks that are highly conserved between subfamily members, but are not conserved in other members of helicase superfamily 2 (Fig. 1). These additional motifs may be involved in a common biochemical activity that is specific to the SWI2 subfamily of DNA-stimulated ATPases. One possibility is that these motifs are involved in ATP-dependent nucleosome disruption, since many SWI2 subfamily members appear to function in a chromatin context (32). Purification of SWI/SNF complexes that contain mutant SWI2 subunits will allow direct tests of the biochemical function of each sequence element within the ATPase/helicase domain.

Table 5. Effect of SWI2 ATPase motif mutations on SWI/SNF dependent gene expression

Motif	SWI2 allele	HO expression	Gal4 activator function	Invertase expression[a]	Dominant negative[b]
	wild-type	+++[a]	+++[a]	+++	–
	swi2 Δ	–	–	+	–
I	K798A	–	–	+	*
Ia	P824A	+	++	++	ND
	W831A	+++	ND	ND	ND
II	D894E,E895Q	–	+	+	ND
	D894A,E895A	–	–	+	*
III	P932A	+++	ND	ND	ND
	W935A	+	+	+	ND
IV	R994A	–	–	+	**
IVa	H1061A	++	++	+	ND
IVb	K1088A	–	–	–	*
	D1093A	+++	+++	+++	ND
V	R1164A	+	+	+	ND
	G1166A	+++	++	+++	ND
	ΔSTRAGGLG	–	–	+	*
Vb	W1185A	++	++	+	ND
VI	R1196K	–	–	+	**

[a]+++, 80–100% of wild-type activity; ++, 50–79% of wild-type activity; +, 10–49% of wild-type activity; –, 1–9% wild-type activity.
[b]**, 50–79% inhibition of wild-type activity; *, 10–49% inhibition of wild-type activity.
– , no inhibition.
ND, not determined.

Each of the *SWI2* ATPase domain mutants that we have tested exhibit a dominant negative phenotype for GR-dependent transcriptional activation. This phenotype is not surprising since these SWI2 mutants are competent for assembly of functionally inactive SWI/SNF complexes. We were surprised to find that these same *swi2* mutants did not show a dominant negative phenotype for *HO*, *SUC2* or GAL4-dependent expression (unpublished results). One possibility is that transcriptional activation of many SWI/SNF-dependent genes only requires a very low level of active SWI/SNF complex. The inability to observe a dominant negative phenotype may simply reflect the limited overexpression achieved by high copy plasmids. In any event, these results indicate that GR-dependent activation is a sensitive assay system for identification of dominant negative alleles of *SWI2*. The value of this assay system has already been proven, as the *swi2K798A* allele was first identified in this assay, and it has been a useful model to design dominant negative alleles of a mammalian SWI2 homolog (15). In our current study, we have identified two new *swi2* alleles, *swi2R994A* and *swi2R1196K*, that are even more effective than *swi2K798A* as dominant negatives in the GR activation assay. The engineering of similar mutations in other SWI2 family members may result in better dominant negative alleles that will prove invaluable for functional studies.

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