

Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement

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Communicated by Ira Herskowitz, December 16, 1993 (received for review September 14, 1993)

ABSTRACT The *Saccharomyces cerevisiae* SWI1, SWI2 (SNF2), SWI3, SNF5, and SNF6 gene products play a crucial role in the regulation of transcription. We provide here direct biochemical evidence that all five SWI/SNF polypeptides are components of a large multisubunit complex. These five polypeptides coelute from a gel-filtration column with an apparent molecular mass of ≈ 2 MDa. The five SWI/SNF polypeptides do not copurify when extracts are prepared from *swi*⁻ or *snf*⁻ mutants. We show that SWI/SNF polypeptides also remain associated during an affinity-chromatography step followed by gel filtration. Assembly of the SWI/SNF complex is not disrupted by a mutation in the putative ATP-binding site of SWI2, although this mutation eliminates SWI2 function.

The SWI1, SWI2 (SNF2), SWI3, SNF5, and SNF6 gene products are required for the transcriptional induction of a large number of yeast genes (for review, see ref. 1). Furthermore, one or more of these SWI/SNF proteins are required for many transcriptional activator proteins to enhance transcription in yeast, including yeast GAL4 (2), *Drosophila* ftz (2), mammalian steroid receptors (3), and a LexA-Bicoid fusion protein (4). The current hypothesis is that SWI1, SWI2, SWI3, SNF5, and SNF6 function as components of a complex that associates with gene-specific activators and assists in relieving transcriptional repression by chromatin.

Several observations have provided indirect evidence for a SWI/SNF protein complex: (i) the phenotype of multiply defective *swi*⁻ or *snf*⁻ mutants is identical to that of a single *swi*⁻ or *snf*⁻ mutant (2); (ii) all five SWI/SNF proteins are apparently required for transcription of the same large set of genes (2); (iii) the stability of SWI3 protein is reduced in the absence of SWI1 and SWI2 (2); (iv) the SWI3 protein can associate with the mammalian glucocorticoid receptor (GR) *in vitro*, and this interaction requires the SWI1 and SWI2 proteins (3); and (v) analyses of LexA-SWI and LexA-SNF fusion proteins indicate a functional interdependence among SWI and SNF proteins for transcriptional activation (4, 5). These studies, however, have not eliminated the possibility that a subset of the SWI/SNF proteins is required solely for the synthesis, modification, or activity of other SWI/SNF proteins. To test directly the hypothesis that these polypeptides form a protein complex, we have initiated a biochemical analysis of these five polypeptides. We have analyzed the elution of SWI and SNF polypeptides from gel-filtration media and have used an affinity purification scheme to partially purify a SWI/SNF protein complex.

MATERIALS AND METHODS

Preparation of Extracts. Crude whole-cell extracts were prepared by using cells harvested at an OD₆₀₀ 1–2, lysed by glass beads in extraction buffer [40 mM Hepes, pH 7.3/350

mM NaCl/0.1% Tween 20/10% (vol/vol) glycerol/pepstatin A at 2 μ g/ml/leupeptin at 2 μ g/ml/1 mM phenylmethylsulfonyl fluoride/0.097 trypsin inhibitor units per ml of aprotinin] with 5 pulses, each 50 seconds in duration on a BioSpec Products (Bartlesville, OK) beadbeater, and clarified by centrifugation at 100,000 $\times g$ for 1 hr. An aliquot (0.2 ml; 2–3 mg) was loaded onto a fast protein liquid chromatography Superose 6 gel-filtration column (0.2 ml/min, equilibrated in extraction buffer), and 0.5 ml fractions were collected.

Antibodies and Immunoblotting. An epitope-tagged version of SWI2 was created by cloning an oligonucleotide cassette that encodes the hemagglutinin (HA) epitope (6) into the unique *Xho* I site at the C terminus of SWI2. Likewise, an oligonucleotide cassette that encodes six tandem histidines was cloned adjacent and C-terminal to the sequences encoding the HA epitope. The SWI2-HA and SWI2-HA-6HIS fusion proteins fully complemented a *swi2* Δ for defects in growth and transcription of an *HO-lacZ* fusion gene (C.L.P., data not shown). The SWI2-HA-6HIS fusion gene was integrated at the *URA3* locus in strain CY120 (*swi2* Δ ::*HIS3 HO-lacZ*) to generate strain CY396 (*swi2* Δ ::*HIS3 SWI2-HA-6HIS::URA3 HO-lacZ*). Polyclonal antibodies directed against SNF5 and SNF6 proteins were generated by fusing portions of each coding region to glutathione *S*-transferase and injecting these fusion proteins into rats. A 1750-bp *Mse* I-*Kpn* I fragment from *SNF5* (7) and a 733-bp *Nco* I-*Pst* I fragment from *SNF6* (8) were subcloned into the vector pGEX-2TK (International Biotechnologies). For immunoblots, Superose 6 fractions were trichloroacetic acid-precipitated and resuspended in 50 μ l of SDS sample buffer; 10–15 μ l were then separated on either a 6% or 10% Laemmli gel. Immunoblots were probed with either rabbit polyclonal α -SWI3 (2), rabbit polyclonal α -SWI1, rat polyclonal α -SNF5, rat polyclonal α -SNF6, or monoclonal antibody 12CA5 (Babco, Emeryville, CA), and developed with a chemiluminescent substrate as described (2).

Affinity Purification. Extract (5 ml; 75 mg) from a 1-liter culture of strain CY396 was mixed with 0.5 ml of Sepharose CL-4B (Pharmacia), rocked for 5 min, and centrifuged at 1000 $\times g$ for 5 min to remove the resin; the unbound protein was bound batchwise with 0.5 ml of Ni²⁺-nitriloacetic acid-agarose (Qiagen, Chatsworth, CA) for 2 hr at 4°C. Unbound protein was removed by washing four times with 5 ml of extraction buffer, and bound proteins were eluted by batchwise treatment for 15 min at 4°C twice with 1 ml of 0.5 M imidazole, pH 7.9/350 mM NaCl/10% glycerol/0.1% Tween 20/protease inhibitors. Eluates were pooled, concentrated to 0.3 ml by using a Centricon-30 microconcentrator (Amicon), and fractionated on Superose 6.

RESULTS

Five SWI/SNF Polypeptides Coelute on Gel Filtration. A whole-cell extract was prepared from a *SWI*⁺ yeast strain,

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Abbreviations: HA, hemagglutinin; GR, glucocorticoid receptor.
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CY396 (*swi2Δ SWI2-HA-6HIS::URA3*), and applied to a fast protein liquid chromatography Superose 6 gel-filtration column. All chromatography was done in moderate ionic-strength buffer (350 mM NaCl) to reduce weak interactions with DNA or with other proteins. The elution of SWI/SNF polypeptides from the column was followed by immunoanalysis (Fig. 1). The peak of SWI2 (198 kDa; ref. 5) elutes in fraction 19, which indicates an apparent molecular mass of ≈ 2 MDa (this value was obtained by linear extrapolation of the calibration proteins). Likewise, SWI3 (99 kDa; ref. 2), SNF6 (37.6 kDa; ref. 8), and SWI1 (148 kDa; ref. 9) eluted as symmetrical peaks centered on fraction 19, suggesting that they might be associated with the SWI2 polypeptide (Fig. 1). Data are not shown for SWI1, as immunoblots with the polyclonal α -SWI1 antibody were faint and, thus, not well reproduced photographically. SNF5 (102.5 kDa; ref. 7) eluted from Superose 6 in two peaks—one centered at fraction 19 and a second peak centered on fraction 27 (Fig. 1 and data not shown; see also Fig. 2C). The large apparent molecular mass of the putative SWI/SNF complex (2 MDa) suggests that it might contain additional, unidentified components. If the complex contains one copy of each SWI and SNF polypeptide, the predicted molecular mass is ≈ 600 kDa, assuming that the complex is globular (see *Discussion*).

Complex Assembly in *swi*⁻ and *snf*⁻ Mutants. These initial results indicated that all five SWI/SNF polypeptides might be components of a large multisubunit complex. It was also possible, however, that SWI/SNF polypeptides might be eluting together coincidentally or be nonspecifically aggregated. Therefore, we determined whether the removal of a

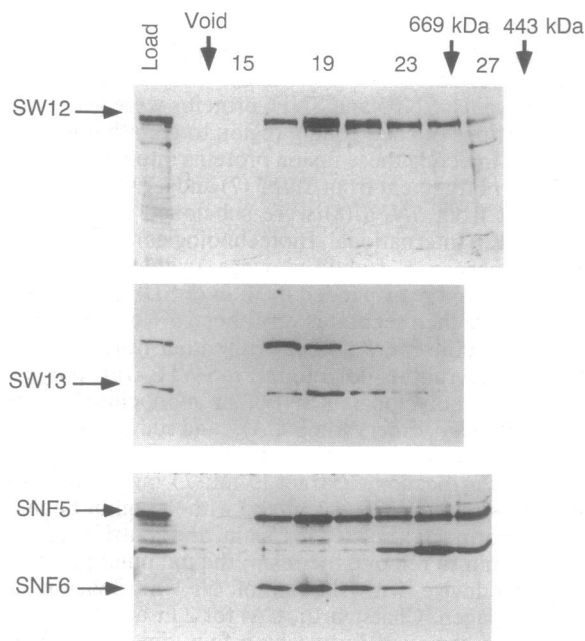


FIG. 1. SWI2, SWI3, SNF5, and SNF6 coelute during chromatography on a gel-filtration column. A whole-cell extract from strain CY396 (*swi2Δ SWI2-HA-6HIS::URA3*; isogenic to strain CY26¹), was fractionated on fast protein liquid chromatography Superose 6, and fractions were assayed for SWI polypeptides by immunoblots. Arrows at left show immunoreactive polypeptides specific for the indicated SWI or SNF gene product; only these polypeptides are absent in extracts prepared from the appropriate deletion strain. An identical elution volume for the SWI/SNF complex was observed in three independent extracts. Small arrows at top depict the peak fractions for elution of a subset of the following calibration proteins—thyroglobulin (669,000; fraction 25), apoferritin (443,000; fraction 28), β -amylase (200,000; fraction 30), and bovine serum albumin (66,000; fraction 33). Void volume was estimated by using high-molecular mass plasmid DNA.

putative subunit from the complex would alter the elution of the other SWI or SNF polypeptides. Extracts were prepared from *swi1*⁻, *swi2*⁻, *swi3*⁻, *snf5*⁻, or *snf6*⁻ strains and fractionated by gel filtration. The results are shown in Fig. 2.

In the absence of SWI1, SWI3, SNF5, or SNF6, the SWI2 polypeptide eluted with an apparent molecular mass of ≈ 700 kDa (peak at fraction 25; Fig. 2A). This elution position represents SWI2 that is apparently not complexed with SWI3, SNF5, or SNF6, as none of these other polypeptides coeluted with SWI2 (Fig. 2B and C). Elution of SWI3, SNF5, and SNF6 was also altered in extracts prepared from *swi*⁻ or *snf*⁻ mutants. In the absence of any one of the other SWI/SNF polypeptides, SWI3 eluted from Superose 6 with an apparent molecular mass of ≈ 1 MDa (peak at fraction 22, Fig. 2B). The SNF6 polypeptide appeared to coelute with SWI3 in the absence of SWI1, SWI2, or SNF5 (Fig. 2C). However, in the absence of SWI3, the apparent molecular mass of SNF6 decreased further to ≈ 66 kDa (peak at fraction 31; Fig. 2C). Interpretation of the SNF5 elution profile is complicated by the second peak of SNF5 protein. It appears, however, that the elution profile of SNF5 resembles that of SNF6—a larger species of SNF5 coelutes with SWI3 in *swi1*⁻, *swi2*⁻, and *snf6*⁻ extracts (peak at fraction 22; Fig. 1C), but in the absence of SWI3 only the second peak of SNF5 protein is observed (peak at fraction 27; Fig. 2C). The apparent molecular mass of this second SNF5 peak is still quite large (≈ 500 kDa); perhaps SNF5 is complexed with other, unidentified polypeptides. In every case removal of one SWI/SNF subunit alters the elution of the other polypeptides; this is consistent with the hypothesis that all five polypeptides are components of the same protein complex.

Affinity Purification of the SWI/SNF Complex. To confirm that SWI/SNF polypeptides are components of a stable complex, we carried out an affinity purification step before gel filtration. A SWI2 polypeptide was constructed that contains six histidines at its C terminus in addition to a HA epitope tag (SWI2-HA-6HIS). Extracts were prepared from a strain that contains SWI2-HA-HIS as the sole source of SWI2. Protein was loaded onto an affinity matrix, Ni²⁺-nitriloacetic acid-agarose (10), and bound proteins were eluted with imidazole buffer. Eluted protein was concentrated and fractionated on Superose 6. The elution of SWI and SNF polypeptides was followed by immunoblotting (Fig. 3).

Affinity purification of SWI2 resulted in the purification of the entire SWI/SNF complex. Each SWI and SNF polypeptide was bound and eluted from the affinity matrix, and furthermore all five SWI/SNF polypeptides coeluted as determined by subsequent gel filtration (Fig. 3; data not shown for SWI1). The peak fraction (fraction 19) was identical to that observed for fractionation of the crude extract. When an extract was prepared from a strain that lacked the tagged SWI2 polypeptide, SWI3, SNF5, and SNF6 were not purified by fractionation on Ni²⁺-nitriloacetic acid-agarose (C.L.P., unpublished work). These results confirm that these five polypeptides are components of a stable protein complex. The overall purification through these two steps is ≈ 1500 -fold. We estimate that complete purification of the SWI/SNF complex will require an additional 70-fold enrichment.

Mutation in the Putative ATP-Binding Loop of SWI2 has only a Minor Effect on SWI/SNF Complex Assembly. SWI2 encodes a DNA-dependent ATPase (11). Single amino acid changes within the putative ATP-binding loop of SWI2 eliminate SWI2 function *in vivo* (11, 12). One possibility is that ATP binding and hydrolysis are required for assembly of SWI2 or other polypeptides into the SWI/SNF complex. To test this possibility, we prepared extracts from a *swi2* mutant, carrying *swi2K798A*, in which the conserved lysine within the putative ATP-binding loop has been changed to an alanine.

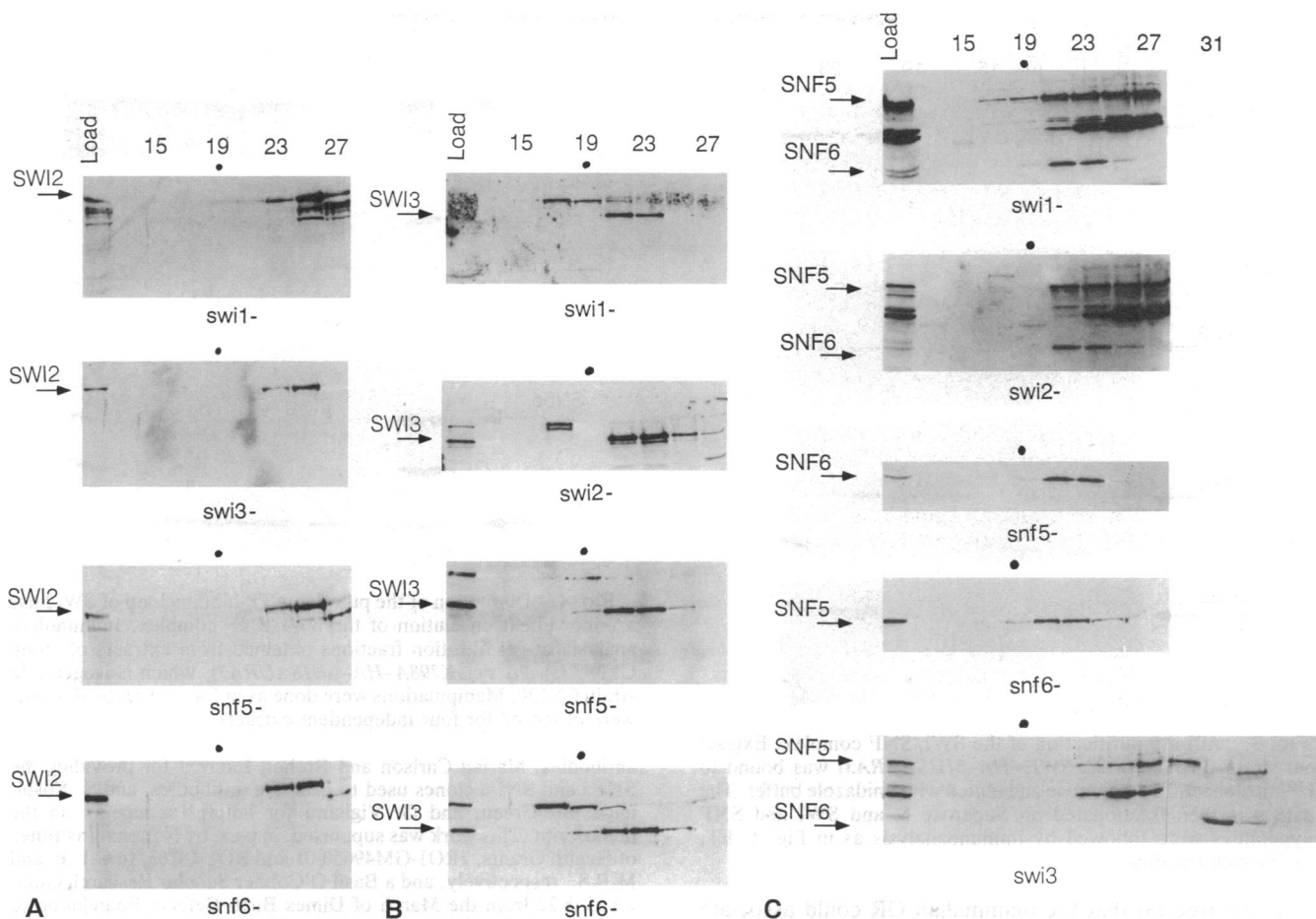


FIG. 2. Fractionation of extracts from *swi*⁻ and *snf*⁻ mutants. Immunoblot analysis of gel-filtration fractions obtained by fractionation of extracts prepared from the *swi*⁻ or *snf*⁻ mutants is indicated below each blot. A dot was placed over fraction 19 of each immunoblot to assist alignment. (A) Elution of SWI3. (B) Elution of SNF5 and SNF6. Strains used were CY405 (*swi1Δ swi2Δ SWI2-HA-6HIS::URA3*), CY406 (*swi2Δ swi3Δ SWI2-HA-6HIS::URA3*), CY402 (*snf5Δ SWI2-HA-6HIS::URA3*), CY403 (*snf6Δ SWI2-HA-6HIS::URA3*), and CY120 (*swi2Δ*). All strains are derivatives of S288C, and strains CY405, CY406, and CY120 are isogenic to strain CY26 (2). Strains CY402 and CY403 are derivatives of strains MCY2099 and MCY2101 (4).

This SWI2 mutant does not complement a *swi2Δ* allele and exerts a dominant negative phenotype in the presence of wild-type SWI2 (12). Laurent *et al.* (11) have shown that changing this conserved lysine to an arginine decreases the ATP hydrolysis activity of a bacterially expressed SWI2 fusion protein. In the case of the *Salmonella typhimurium* MutS ATPase, a Lys → Ala change at this position decreased both ATP binding and hydrolysis (13). Fractionation of *swi2K798A* extracts on Superose 6 indicates that this mutant SWI2 is competent for assembly into a SWI/SNF complex (Fig. 4; peak at fraction 19). We reproducibly observe, however, a higher percentage of SWI2 eluting at smaller apparent molecular masses. Elution of SNF5, SNF6, and SWI3 is also altered by this SWI2 mutation (peaks are shifted from fraction 19 to fraction 21). One possibility is that the putative ATP-binding loop is required for stability of the SWI/SNF complex under these stringent fractionation conditions (350 mM NaCl). It is more likely, however, that this domain is required primarily for the function of the SWI/SNF complex because the K798A mutation does not have a dramatic effect on complex assembly.

DISCUSSION

Numerous studies have suggested that SWI1, SWI2, SWI3, SNF5, and SNF6 might function as components of a protein complex (for review, see ref. 1). We have shown that all five of these polypeptides coelute from a gel-filtration column and

that they copurify through an affinity-chromatography step. We have also found that ATP hydrolysis by SWI2 does not appear to be required for assembly of the SWI/SNF complex, although mutational analyses have suggested that this activity is required for SWI2 function *in vivo* (11, 12).

It is intriguing that the apparent molecular mass of the SWI/SNF complex (2 MDa) is much larger than the sum of the sizes of the known subunits (600 kDa). One possibility is that the SWI/SNF complex is not globular but has an elongated shape that results in an anomalous molecular mass determination. Alternatively, the SWI/SNF complex may contain additional subunits. Fractionation of *swi*⁻ or *snf*⁻ extracts by gel filtration also suggests that the SWI/SNF complex might contain additional subunits. For example, the SWI3 subunit still behaves as a very large protein (1 MDa), even in the absence of other SWI/SNF subunits. We know that the affinity-purified SWI/SNF complex does not contain the TATA box-binding protein (C.L.P., unpublished work); thus the SWI/SNF complex is distinct from the high-molecular mass complexes containing TATA box-binding protein that have been identified in extracts from human and *Drosophila* cells (14) and from the recently described SRB-TATA box-binding protein-RNA polymerase II complex identified in yeast extracts (15). The SWI/SNF complex also does not contain the general transcription factor TFIIB (SUA7; ref. 16), the RNA polymerase II-associated proteins SRB2, SRB4, and SRB6 (15), or the GAL11 transcription factor (ref. 17; C.L.P., unpublished work).

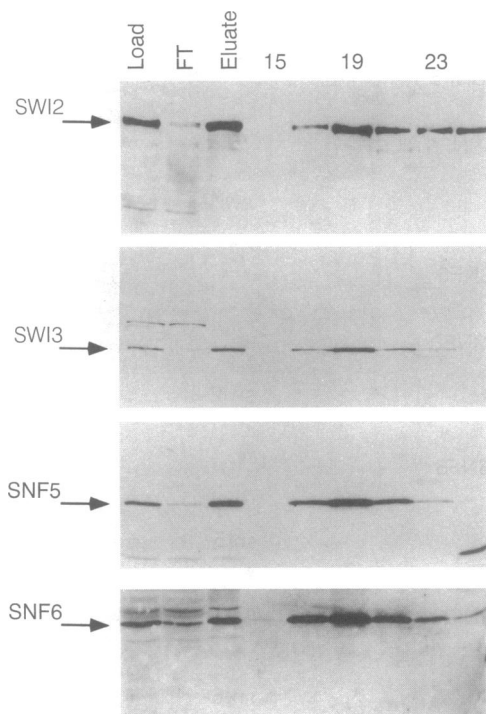


FIG. 3. Affinity purification of the SWI/SNF complex. Extract from strain CY396 (*swi2Δ SWI2-HA-6HIS::URA3*) was bound to Ni²⁺-nitriloacetic acid-agarose and eluted with imidazole buffer. The eluate was then fractionated on Superose 6, and SWI and SNF polypeptides were followed by immunoanalysis as in Fig. 1. FT, flow-through fraction.

We reported (3) that the mammalian GR could associate with SWI3 *in vitro*. This interaction was not seen when extracts were prepared from either a SWI1 or a SWI2 mutant. Based on these results, we proposed that SWI1, SWI2, and SWI3 are components of a protein complex and that the GR can interact only with the intact complex but cannot interact with uncomplexed SWI3. We have not determined whether the GR is capable of coeluting with the SWI/SNF complex on gel filtration. We suspect, however, that the GR-SWI/SNF interaction would not be detected during such fractionation—only 5% of the total SWI/SNF complex in a crude extract can be coimmunoprecipitated with GR even under conditions where GR is in excess (3). Additional binding studies with the purified SWI/SNF complex should facilitate an evaluation of the affinity of the GR for the SWI/SNF complex and should lead to identification of the subunit that interacts with activators such as the GR.

It has been suggested that SWI2 might function as the catalytic subunit of a DNA helicase machine that antagonizes transcriptional repression due to chromatin components (18, 19). Our results have identified this putative protein machine and have suggested that ATP binding or hydrolysis is required for the function of this complex and not for its assembly. Biochemical analyses using the purified SWI/SNF complex will allow direct tests of models for SWI/SNF function in transcriptional enhancement.

We thank E. T. Young for generously providing the α -SWI1 antibodies for this study, A. Koleske and R. A. Young for providing α -SRB2, α -SRB4, α -SRB6 antibodies, J. Perlberg and M. Ptashne for providing α -GAL11 antibodies, D. Reinberg for providing α -SUA7

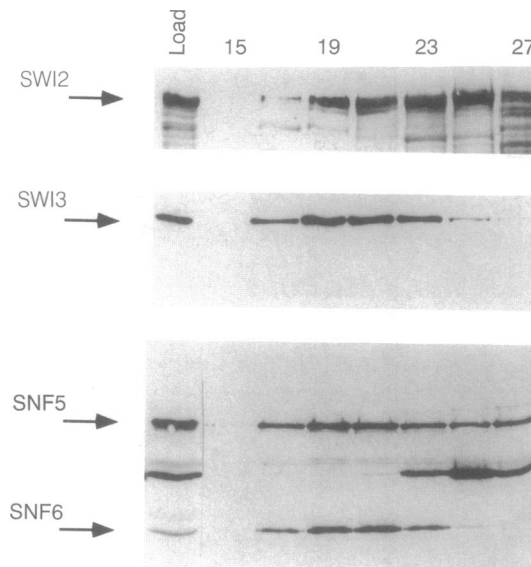


FIG. 4. Disruption of the putative ATP-binding loop of SWI2 has a minor effect on elution of the SWI/SNF complex. Immunoblot analysis of gel filtration fractions obtained from extracts of strain CY397 (*swi2Δ swi2K798A-HA-6HIS::URA3*), which is isogenic to strain CY120. Manipulations were done as in Fig. 1. Identical results were observed for four independent extracts.

antibodies, Marian Carlson and Brehon Laurent for providing the SNF5 and SNF6 clones used to generate antibodies, and S. Yoshinaga, M. Green, and G. Viglianti for helpful comments on the manuscript. This work was supported, in part, by National Institutes of Health Grants, 1R01-GM49650-01 and R01-18163, to C.L.P. and M.P.S., respectively, and a Basil O'Connor Scholar Research Grant FY93-0922 from the March of Dimes Birth Defects Foundation to C.L.P. A.D. is a fellow of the Helen Hay Whitney foundation.

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