Purification of a Human SRCAP Complex That Remodels Chromatin by Incorporating the Histone Variant H2A.Z into Nucleosomes†

Donald D. Ruhl,[‡,§,▽] Jingji Jin,[‡,⊥] Yong Cai,[⊥] Selene Swanson,[⊥] Laurence Florens,[⊥] Michael P. Washburn,[⊥] Ronald C. Conaway,[⊥,#] Joan Weliky Conaway,[⊥,#,@] and John C. Chrivia*[,§]

Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, Saint Louis, Missouri 63104, Stowers Institute for Medical Research, Kansas City, Missouri 64110, Department of Biochemistry and Molecular Biology, Kansas University Medical Center, Kansas City, Kansas 66160, and Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

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ABSTRACT: The Snf-2-related CREB-binding protein activator protein (SRCAP) serves as a coactivator for a number of transcription factors known to interact with CBP. Swr1, the closest *Saccharomyces cerevisiae* ortholog of SRCAP, is a component of the chromatin remodeling complex SWR-C, which catalyzes exchange of the histone variant H2A.Z into nucleosomes. In this report, we use a combination of conventional chromatography and anti-SRCAP immunoaffinity chromatography to purify a native human SRCAP complex with a polypeptide composition similar to that of SWR-C, and we show for the first time that this SRCAP-containing complex supports ATP-dependent exchange of histone dimers containing H2B and H2A.Z into mononucleosomes reconstituted with recombinant H2A, H2B, H3, and H4. Our findings, together with previous evidence implicating H2A.Z in transcriptional regulation, suggest that SRCAP's coactivator function may depend on its ability to promote incorporation of H2A.Z into chromatin.

The Snf-2-related CREB-binding protein activator protein (SRCAP) belongs to the SNF-2 family of ATPases, members of which are involved in a variety of processes including regulation of transcription and remodeling of chromatin (*1*, *2*). Studies to characterize the function of SRCAP indicate that it interacts with the histone acetyltransferase CREB-Binding Protein (CBP) and serves as a coactivator for a number of transcription factors known to interact with CBP including CREB, the androgen receptor, and the glucocorticoid receptor (*3*, *4*).

Information pertaining to the mechanism(s) by which SRCAP might activate transcription has come from recent analyses of its ortholog in *Saccharomyces cerevisiae* termed Swr1. Swr1 copurifies with the histone variant H2A.Z as part of a multiprotein complex called SWR-C (*5*−*7*), which also includes Vps72, Swc1, God1, Aor1,Vps71, Arp4, Arp6, Rvb1, Rvb2, Act1, and Yaf9. The purified SWR-C complex catalyzes ATP-dependent exchange of H2A.Z for nucleosomal H2A (*7*). Consistent with the possibility that SWR-C plays a similar role in vivo, deletion of the Swr1 gene led to a reduction in the amount of H2A.Z incorporated into chromatin (*5*−*7*).

H2A.Z is conserved from yeast to mammals and has been implicated in both positive and negative regulation of transcription (*8*) and, under some conditions, appears to be required for proper recruitment of RNA Pol II and TBP to promoters (*9*). Not surprisingly, deletion of the H2A.Z gene or the Swr1 gene results in decreased expression of an overlapping set of genes (*5*−*7*). Genome-wide mapping studies in *S. cerevisiae* indicate that, in euchromatin, H2A.Z is predominately localized to promoter rather than intergenic regions (*10*−*12*). In mammals, H2A.Z is essential, since deletion of the H2A.Z gene in mice results in an embryonic lethal phenotype (*13*).

In human cells, previous studies have found that SRCAP appears to be a component of a complex that closely resembles the SWR-C complex (*14*) and includes YL1 (Vps72), ZnF-HIT1(Vps71), TIP49a (Rvb1), TIP49b (Rvb 2), BAF53a (Arp4), ARP6, GAS41 (Yaf 9), DMAP1 (Eaf2/ God1), and H2A.Z. However, despite the similarities between the *S. cerevisiae* SWR-C and human SRCAP complexes, previous experiments were not able to show incorporation of H2A.Z into nucleosomes in the presence of SRCAP complexes purified through either FLAG-tagged ZnF-HIT1(Vps71) or through FLAG-H2A.Z (*14*).

In an effort to isolate a catalytically active SRCAP-containing complex, an antibody that recognizes the C-terminal peptide of SRCAP was used to purify a SRCAP-containing complex. In this report, we detail the purification of a SRCAP complex that shares orthologous subunits with

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* Corresponding author. Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, 1402 South Grand Boulevard, Saint Louis, Missouri, 63104. Tel: 314-977-6486. E-mail: Chrivia@slu.edu. Fax: 314-977-6410.

‡ These authors contributed equally.

§ Saint Louis University School of Medicine.

▽ Current address: Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853.

⊥ Stowers Institute for Medical Research.

Kansas University Medical Center.

@ University of Oklahoma Health Sciences Center.

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the yeast SWR-C complex and show for the first time it is capable of remodeling chromatin by catalyzing incorporation of H2A.Z/H2B dimers into nucleosomes.

MATERIALS AND METHODS

SRCAP Antibodies. Anti-SRCAP antibody 90 was generated against SRCAP protein (amino acids 2968−3168). Briefly, a DNA fragment encoding a methionine followed by amino acids 2968−3168 of SRCAP and six C-terminal histidines was generated by PCR and subcloned into pET-15b. This plasmid was introduced into the bacterial strain BL21, and recombinant SRCAP 2968−3168 was purified from extracts using a nickel affinity column. The purified SRCAP 2968−3168 protein was injected into rabbits. The resulting antisera was used in Western blots at a dilution of 1:3000 to recognize a protein of the predicted molecular weight of SRCAP (350 kDa) (Figure S1A, Supporting Information). It was also used at a dilution of 1:100 to immunoprecipate a protein of the same size (Figure S1B, Supporting Information). This immunoprecipitation was blocked by preincubation of the anti-SRCAP antibody 90 with the SRCAP 2968−3168 protein (Figure S1B, Supporting Information).

Antisera against amino acids 3152−3168 of SRCAP was generated by injecting into rabbits the peptide coupled via the N-terminal cysteine to KLH[1] using Sulfo-SMCC (Pierce). Antisera from rabbits 237 and 238 were pooled and purified on a peptide affinity column generated by conjugating the SRCAP 3152−3168 peptide to CNBR Activated Sepharose 4B (Amersham Bioscience). This anti-SRCAP antibody 237/238 recognized the same protein on Western blot as the anti-SRCAP antibody 90 (Figure S2, Supporting Information). This recognition could be blocked by preincubation with the SRCAP 3152−3168 peptide (Figure S2B, Supporting Information).

Preparation of Nuclear Extract. HeLa S3 cells (50 L) were obtained as a pellet from the National Cell Culture Center and nuclei prepared as described by Dignam et al. (*15*) using Buffer A supplemented with 0.2% Triton X-100 and protease inhibitors (0.5 mM PMSF and EDTA free complete mini protease inhibitor, Roche). Nuclei were incubated on ice for 30 min in 100 mL of Buffer B (250 mM NaCl, 20 mM NaH_2PO_4, 30 mM NaP_2O_7, 5 mM EDTA, 10 mM NaF, 0.1% NP-40, and 5 mM DTT, pH 7.2 (and protease inhibitors as previously indicated). The nuclei were centrifuged at 16 000*g* for 10 min at 4 °C (Sorvall RC5B centrifuge, HB4 rotor), the supernatant containing nuclear proteins was removed, and the nuclei were extracted a second time. Supernatants were combined (total of 1125 mg protein) and dialyzed in Spectrapor tubing (MW cutoff 6000−8000) for 14 h at 4 °C against 6 L of modified Buffer B containing 40 mM NaCl. The fold purification for this step is estimated to be at least 5-fold.

DEAE Chromatography. Dialyzed nuclear extract (1125 mg in 200 mL) was applied at a flow rate of 2 mL/min to a 3.0 cm × 23 cm DEAE-Trisacryl Plus M column preequilibrated with 100 mL of Buffer B containing 50 mM NaCl. The flow through was collected as 10 mL fractions, and analysis by Western blot indicated they contained little or no SRCAP protein (data not shown). The column was washed with 100 mL of Buffer B containing 50 mM NaCl, and bound proteins were eluted stepwise by increasing the NaCl concentration from 50 to 100 mM (100 mM fraction), from 100 to 150 mM (150 mM fraction), from 150 to 200 mM (200 mM fraction), and from 200 to 250 mM (250 mM fraction). Two milliliter fractions were collected and analyzed by Western blot. The 150 mM NaCl fraction contained most of the SRCAP protein and 75 mg of total protein giving an estimated 15-fold purification for this step.

Phosphocellulose Chromatography. The 150 mM NaCl eluate from the DEAE column was diluted by the addition of 4 vol of Buffer C (250 mM NaCl, 5 mM EDTA, 10 mM NaF, 0.1% NP-40, and 5 mM DTT, pH 7.2).

The ∼200 mL sample was loaded at a flow rate of 1 mL/min onto a 9 mm × 70 mm phosphocellulose P-11 column preequilibrated with 20 mL of Buffer D (250 mM NaCl, 4 mM NaH_2PO_4, 6 mM NaP_2O_7, 5 mM EDTA, 10 mM NaF, 0.1% NP-40, and 5 mM DTT, pH 7.2). Western blot analysis of flow through fractions indicated that most of the SRCAP was retained on the column (data not shown). The column was washed with 30 mL of Buffer D and the SRCAP complex eluted with Buffer B. As described, Buffer B is identical to Buffer D, except it contains a higher concentration of phosphate (80 versus 16 mM). The fractions containing SRCAP were pooled and found to contain 12.5 mg of total protein. The estimated fold purification of this step was 6-fold.

Affinity Purification of SRCAP-Associated Complex. A total of 250 μL of protein A agarose beads was incubated with either 2.5 mg of affinity-purified anti-SRCAP antibody 237/238 or 2.5 mg of anti-Gal4 antibodies. The anti-Gal4 antibodies have been previously described (*16*). The antibody−beads mixtures were washed with Buffer B and incubated for 18 h at 4 °C with SRCAP-containing P-11 fractions (6 mg of protein). Beads were washed with Buffer B, and SRCAP was eluted by incubation for 30 min at 37 °C with 100 μL of Buffer B containing 2.5 μg/mL of the SRCAP 3152−3168 peptide. The elution was repeated two times, and pooled supernatants (300 μL) contained less than 0.001 mg of protein. Fold purification for this step was ∼6000-fold.

Size Exclusion Chromatography. Nuclear extract (15 mg) was clarified by a second centrifugation at 35 000 rpm (Beckman Optima LE-80K Ultracentrifuge and SW56 rotor) and loaded on a 1.6 cm × 90 cm Sephacryl S300 column preequilibrated in 100 mL of Buffer B. The column was run at a flow rate of 0.2 mL/min, and 1.6 mL fractions were analyzed for absorbance at 280 nm and for the presence of SRCAP by Western blot. To calibrate the column, individual protein standards (Sigma) were run separately. The void volume was determined using linearized plasmid DNA (∼5000 bp).

Western Blot Analysis. Proteins in the size exclusion column fractions were concentrated by boiling followed by centrifugation at 10 000 rpm (Eppendorf microcentifuge), and the pellet was resuspended in 1× SDS−PAGE loading buffer. A portion of each phosphocellulose or DEAE column fraction (25 μL) was mixed with 25 μL of 2× SDS−PAGE loading buffer. Each fraction was subjected to SDS−PAGE

[1] Abbreviations: βME, β-mercaptoethanol; Sulfo-SMCC, sulfo-succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylateper; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; KLH, keyhole limpet hemacyanin.

and Western blotting using a 1:3000 dilution of the anti-SRCAP antibody 90 as described by Swope et al. (*16*). Secondary antibodies conjugated to alkaline phosphatase (Figure S1B, Supporting Information) or conjugated to horseradish peroxidase (Figures1−4, S1A, and S2 of Supporting Information) were used at a dilution of 1:10 000.

Mass Spectrometry. Proteins affinity-purified from the P-11 fractions were size-fractionated by SDS−PAGE, and a Coomassie-stained band identified by Western blot as SRCAP was excised from the gel (Figure S2, Supporting Information). The gel slice was treated as described by Sheffield et al. (*17*) and subjected to MS/MS analysis at the Donald Danforth Plant Science Center.

MudPIT Analysis. Multidimensional protein identification technology (MudPIT) was used to analyze the SRCAP complex affinity-purified from the P-11 fractions. Nonspecifically bound proteins were identified as those that were "purified" in parallel mock affinity purifications using anti-Gal4 antibodies. Identification of proteins was accomplished using a modification of the MudPIT procedure as described by Washburn et al. (*18*). The software algorithm 2-3 (*19*) was used to determine charge state and to delete spectra of poor quality. SEQUEST was used to match MS/MS spectra to peptides in a database containing 27 196 human protein sequences from NCBI. The validity of peptide/spectrum matches was assessed using SEQUEST-defined parameters, cross-correlation score (XCorr), and normalized difference in cross-correlation scores (deltCN). Spectra/peptide matches were only retained if they had a DeltCn of at least 0.08 and minimum XCorr of 1.8 for +1, 2.5 for +2, and 3.5 for +3 spectra. In addition, the peptides had to be at least 7 amino acids long. DTASelect (*20*) was used to select and sort peptide/spectrum matches passing this criteria set. Peptide hits from multiple runs were compared using CONTRAST (*20*).

Preparation of the H2A.Z−H2B Dimer or Histone Octamer. Human histone cDNAs, H2A (accession number BC017379), H2B (NM_003518), H3.3 (BC001124), H4 (BC019846), and H2A.Z (NM_002106), were subcloned into pET-21a for expression in bacteria. C-terminally FLAG-tagged H2A.Z and C-terminally Myc-tagged H2B were used for H2A.Z/H2B dimer. The purification of recombinant histones was performed as described (*21*) with the following modifications. Inclusion bodies containing histones were solubilized in unfolding buffer (7 M Guanidine HCl, 20 mM Tris-HCl, pH 7.5, and 10 mM DTT). After centrifugation for 10 min at 25 000*g* (Beckman-Coulter Avanti J-25I, JA-20 rotor), supernatants were loaded onto a 5 mL Hi-Trap SP-Sepharose column (Amersham). Bound proteins were eluted with a 10 column volume linear gradient of 200−600 mM NaCl in buffer containing 7 M urea, 20 mM $NaC_2H_3O_2$, pH 5.2, 1 mM Na-EDTA, and 5 mM βME. Peak protein fractions were pooled and dialyzed against deionized water containing 5 mM βME and 0.5 mM PMSF. The proteins were lyophilized and dissolved in unfolding buffer. Mixtures containing equal molar amounts of histones (H2A, H2B, H3.3, and H4 for octamer, and H2A.Z-FLAG and H2B-Myc for dimer) were dialyzed against refolding buffer (10 mM Tris-HCl, pH 7.5, 2 M NaCl, 1 mM Na-EDTA, and 5 mM βME) overnight at 4 °C. Dialyzed octamers or dimers were centrifuged for 5 min at 25 000*g* (Beckman-Coulter Avanti J-25I, JA-20 rotor) and applied at a flow rate of 1 mL/min to a 16/60 Superdex 200 column equilibrated in refolding buffer. Fractions of 1 mL were collected and analyzed by SDS−PAGE and Coomassie staining. Fractions containing histone octamers or dimers were pooled, and protein concentration was determined by measuring absorbance at 276 nm.

Preparation of Mononucleosome Substrates for Exchange Assays. A 216 bp DNA fragment was generated by PCR using pGUB-dSH (*25*) as template and the biotinylated oligonucleotides 5′-ACA TTA ACC TAT AAA AAT AGG CGT ATC ACG-3′ and 5′-ATC TAG ACG GCC ACG TGG TTC-3′ as primers. Purified histone octamers (3 μg) and DNA (150 ng) were used to reconstitute mononucleosomes according to the protocol described by Owen-Hughes et al. (*21*).

Exchange Assay. Nucleosomes were incubated for 3 h at room temperature with 200 μL avidin-coupled Dynabeads that had been preequilibrated in 20 mM HEPES, pH 7.5, 200 mM NaCl, 1 mM EDTA, and 10% glycerol. Beads were washed with 0.6 M NaCl, 20 mM HEPES, pH 7.5, 1 mM EDTA, 10% glycerol, 0.5 mM DTT, and 0.5 mM PMSF and resuspended in 200 μL of the same buffer. A total of 7.5 ng DNA equivalents of mononucleosome immobilized on avidin-coupled Dynabeads was preincubated with 2 μL of SRCAP complex, purified as described above, in 50 μL of exchange buffer (25 mM HEPES-KOH, pH 7.6, 0.1 mM EDTA, 5 mM $MgCl_2$, 10% glycerol, 0.02% NP-40, 1 mM DTT, 0.1 mg/mL BSA, and 70 mM KCl) for 30 min at 37 °C. The beads were washed twice with 150 μL of exchange buffer and resuspended in a 100 μL reaction volume with 75 ng of recombinant H2A.Z-FLAG/H2B-Myc dimer, and the reactions were further incubated for 60 min at 37 °C in the presence of ATP or ATPγS. After incubation, the beads were washed with exchange buffer, and the bound proteins were eluted using SDS−PAGE loading buffer and fractionated on a 4−15% gradient SDS−PAGE. The FLAG-tagged H2A.Z was detected by Western blotting using anti-FLAG antibody (Sigma). In the experiment of Figure 4B, reactions were scaled up 5-fold, and the eluted histones were visualized by silver staining.

RESULTS

Purification of a Novel Multiprotein Complex Containing SRCAP. Previous studies have purified SRCAP as part of large multiprotein complexes (*14*, *22*) that did not have detectable histone H2A.Z exchange activity (*14*). To avoid possible disruption of native SRCAP-containing complexes that might account for this observation, a low-salt extraction buffer was used to prepare nuclear extracts from HeLa S3 cells. These extracts were applied to a size exclusion column (Sephacryl S-300HR), and the column fractions were analyzed by Western blot for the presence of SRCAP. This analysis demonstrated that the majority of SRCAP migrated with a molecular weight of 1.5 million Da, indicating large SRCAP complexes were retained following the extraction protocol (Figure 1). Because the SRCAP complex eluted close to the void volume on the S-300HR column, which could be indicative of a protein aggregate, it was run on a second size exclusion column (Sephacryl S-500HR) with a void volume of 20 million Da. On this column, SRCAP also migrated with a molecular weight of approximately 2 million Da (data not shown).

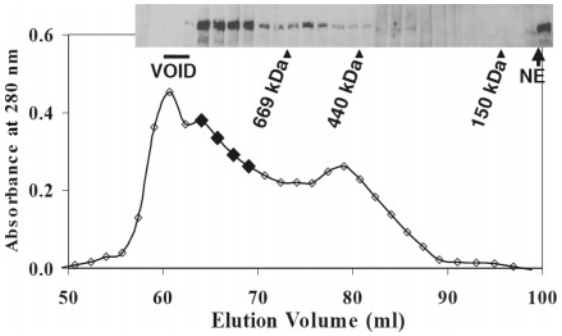

FIGURE 1: SRCAP migrates as a 1.5 million Da complex. Nuclear extract from HeLa S3 cells was loaded on a Sephacryl S300 size exclusion column and the absorbance at 280 nm of fractions plotted against the elution volume. The presence of SRCAP in each fraction was assessed by Western blot using the anti-SRCAP antibody 90 and is shown above the corresponding chromatography fractions. The large, filled diamonds indicate the fractions in which SRCAP was found to be most prevalent. The column was calibrated in separate runs using the indicated size protein standards and linearized DNA to measure the void volume. NE indicates input nuclear extract.

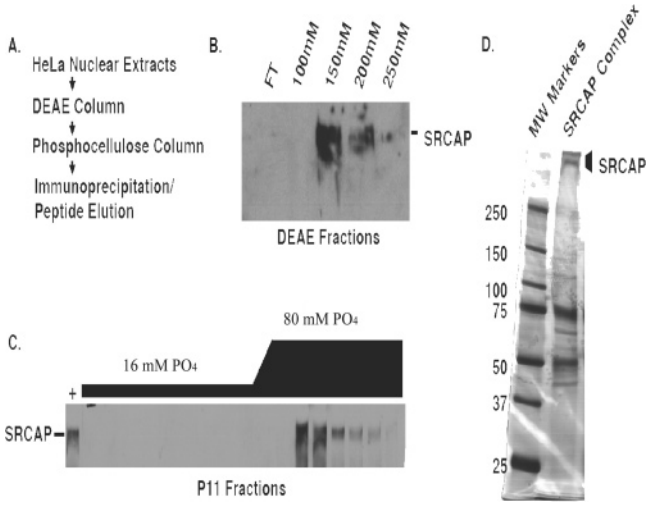

FIGURE 2: Purification of the SRCAP protein complex by ion exchange chromatography. (A) The protocol for purification of the SRCAP complex is shown. (B) Nuclear extracts were applied to a DEAE-Trisacryl Plus M column and bound proteins eluted with 50 mL steps of increased NaCl, e.g., 100, 150, 200, and 250 mM. The presence of SRCAP in these eluted fractions and pooled flow through fractions (FT) was assessed by Western blot using anti-SRCAP antibody 90. (C) The 150 mM NaCl fraction containing the majority of SRCAP was diluted to 10 mM phosphate and applied to a phosphocellulose P-11 column. Bound proteins were eluted by increasing the total phosphate concentration from 16 to 80 mM. The presence of SRCAP in the eluted fractions is shown in the lower panel by Western blot using the anti-SRCAP antibody 90. The phosphate concentration of each fraction is indicated above the corresponding lane of the Western blot. (D) Phosphocellulose fractions containing SRCAP were pooled and incubated with anti-SRCAP antibody 237/238 bound to protein A beads. Bound proteins were eluted by incubation with the SRCAP 3152−3168 peptide, fractionated on a 4−15% SDS−PAGE, and silver stained.

The isolation of nuclear proteins using the low-salt extraction buffer resulted in a 5-fold purification of the SRCAP complex (see Materials and Methods). To further purify SRCAP (see purification schematic, Figure 2A), nuclear extracts were applied to a DEAE-Trisacryl Plus M column and bound proteins eluted by increasing the NaCl concentration in steps from 50 to 250 mM. Western blot analysis of these fractions (Figure 2B) indicated that the majority of the SRCAP eluted in the 150 mM fraction resulting in a 15-fold purification (see Materials and Methods). A smaller amount of SRCAP was routinely observed in the 200 mM fraction, suggesting SRCAP exists in two complexes with different chromatographic properties.

Table 1: SRCAP-Associated Proteins Identified by MudPIT[a]

protein	molecular weight	spectral count[b]	% coverage[c]
SRCAP	315643	91	13.1
Arp6	45810	7	11.4
TIP49a	50228	34	34.4
TIP49b	51157	24	31.1
BAF53a	43236	17	32.8
DMAP1	52993	18	22.9
GAS41	26499	9	19.8
EF1α-like protein	50141	14	15.4
XPG	133329	21	11.1
alpha 1 tubulin	49924	26	25.0
alpha 3 tubulin	50136	26	32.6
beta 5 tubulin	49671	19	32.2
beta actin	41737	16	19.2
cytochrome *c*1	35390	6	13.2

[a] Portions of the anti-SRCAP eluate were subjected to MudPIT as described in Materials and Methods. [b] The *spectral counts* refer to the number of spectra corresponding to peptides derived from each protein. [c] *% coverage* refers to the percent of the total protein sequence detected by mass spectrometry.

To further purify the SRCAP complex, the 150 mM fraction was applied to a phosphocellulose P-11 column. Proteins retained on the column were eluted by increasing the phosphate concentration (Figure 2C), resulting in about a 5-fold further purification of the SRCAP complex (see Materials and Methods). A portion of the eluted SRCAP complex was applied to the Sephacryl S-300HR size exclusion column, and the majority was found to migrate on this column at a molecular weight of 1.5 million Da (data not shown).

As a final purification step, the P-11 column fractions containing the SRCAP complex were incubated with anti-SRCAP antibody 237/238 bound to protein A beads. Elution of the complex with a SRCAP peptide recognized by the antibody resulted in elution of approximately 80% of the bound SRCAP complex from the beads (data not shown), and proteins of the complex could be visualized following SDS−PAGE and silver staining (Figure 2D). As predicted, a protein that migrated with the predicted molecular weight of SRCAP was observed. The identity of this band as SRCAP was confirmed both by Western blot using a second antibody to SRCAP, antibody 90 (Figure S3, Supporting Information), and by MS/MS (see Materials and Methods).

Identification of Proteins Associated with SRCAP. To identify the other proteins visualized in the silver-stained gel, MudPIT analysis was used. Proteins identified in the SRCAP immunoprecipitation were compared to proteins identified in a control sample prepared in parallel using an anti-Gal4 antibody previously described (*16*). This analysis indicated that the immunopurified SRCAP complex is similar in composition to previously identified human complexes (Table 1 and ref *14*) and includes SRCAP, TIP49a, TIP49b, BAF53a, ARP6, GAS41, and DMAP1. On the basis of Western blots, the relative levels of the SRCAP, TIP49a, and TIP49b proteins in this new complex were similar to those in previously isolated SRCAP-containing complexes

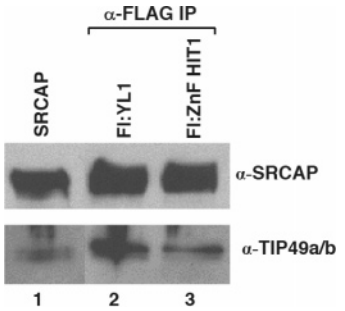

FIGURE 3: Western blot of SRCAP and TIP49a/b. SRCAP and, collectively, TIP49a and TIP49b were detected by Western blotting, affinity SRCAP complexes-purified as described in Materials and Methods (lane 1) or by anti-FLAG immunopurification from Hela cell lines expressing FLAG-tagged YL1 (Fl:YL1, lane 2) or FLAG-tagged ZnF/HIT1 (Fl:ZnF/HIT1, lane 3) (*22*). α-SRCAP, anti-SRCAP antibody; α-TIP49a/b, mixture of anti-TIP49a and anti-TIP49b antibodies.

(Figure 3) purified from cell lines expressing FLAG-tagged YL1 or FLAG-tagged ZnF-HIT1 (*14*). The MudPIT analysis also indicated the presence of additional proteins including EF1α-like protein, XPG-complementing protein, members of the tubulin family (α 1 and 3 and β 5), and β actin. These proteins are frequently found in MudPIT analyses of unrelated protein complexes; thus, it is not clear at this time whether they are contaminants or bona fide subunits of the complex. Some additional proteins such as heat shock 70 kDa protein were also identified by MudPIT as common contaminants of both affinity and mock affinity preparations and may account for the 70 kDa protein band observed in Figure 2D.

Several proteins (H2A.Z, ZnF-HIT1, or YL1) that are found in other SRCAP preparations were not detected in this current SRCAP preparation. H2A.Z has been found to be stripped from other complexes by chromatography on phosphocellulose, suggesting it may have been lost as a result of the purification protocol (J. Jin and Y. Cai, unpublished data).

The SRCAP Complex Incorporates H2A.Z into Mononucleosomes. To test whether the purified SRCAP complex functions to remodel chromatin, an in vitro H2A.Z exchange assay was developed. For this assay, dimers of FLAG-tagged H2A.Z and Myc-tagged H2B were assembled and incubated with the purified SRCAP complex and mononucleosomes that had been assembled on a biotinylated DNA template using core histones H2A, H2B, H3, and H4. Following the exchange reaction, streptavidin magnetic beads were used to separate the mononucleosomes from the free FLAG-tagged H2A.Z and Myc-tagged H2B dimer. Incorporation of FLAG-tagged H2A.Z into the mononucleosome was assessed by anti-FLAG Western blot (Figure 4A). These studies indicated that the purified SRCAP complex was able to catalyze the incorporation of H2A.Z and this activity required ATP and was inhibited by addition of the nonhydrolyzable ATP analogue ATPγS (Figure 4A).

Although it has been assumed that the H2A.Z/H2B dimer is the unit of exchange in the reaction catalyzed by the yeast Swr1 complex, the question of whether the dimer or H2A.Z alone is incorporated into nucleosomes has not been addressed directly in experiments that can distinguish between nucleosomal H2B and H2B from the dimer (*7*). To directly address this issue, the histone subunit composition of the

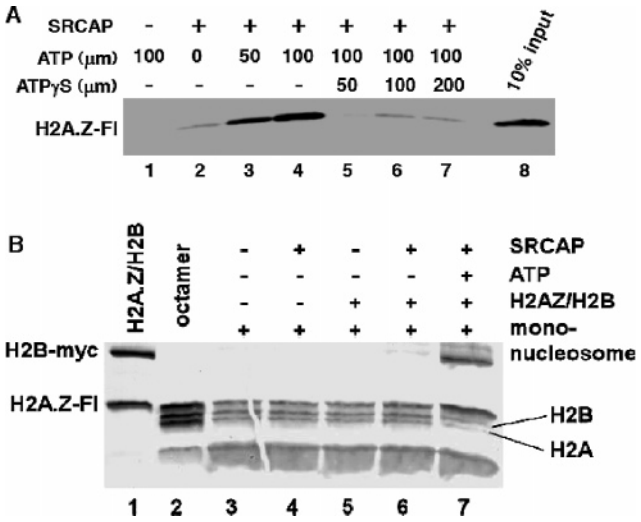

FIGURE 4: Purified SRCAP complex facilitates exchange of H2A.Z/H2B dimers into nucleosomes. (A) ATP-dependent incorporation of H2A.Z-Flag into nucleosomes, detected by Western blotting with anti-FLAG antibody. Preparation of nucleosomal arrays and the exchange assay were performed as described in Materials and Methods in the presence of the indicated concentrations of ATP or ATPγS. (B) ATP-dependent exchange of H2A.Z-Flag/H2B-Myc dimers into nucleosomes visualized by silver staining. The reactions were scaled up 5-fold and were performed without the preincubation/wash step in this experiment. The reactions were performed with or without purified SRCAP, H2A.Z-Flag/H2B-Myc dimer, and ATP in 500 μL exchange buffer for 90 min at 37 °C. The beads were washed three times with exchange buffer, and the bound proteins were analyzed by 18% SDS−PAGE and detected by silver staining. Lane 1 shows the H2A.Z-FLAG/H2B-Myc dimer used in the assays; lane 2 shows the histone octamer used to prepare mononucleosomes.

nucleosome was assessed using silver-stained gels of reaction products from exchange reactions performed with dimers containing FLAG-tagged H2A.Z and Myc-tagged H2B, which is readily distinguished from the untagged nucleosomal H2B. As shown in Figure 4B, the Myc-tagged H2B (upper band in lane 7) and FLAG-tagged H2A.Z (darker band that comigrates with H3) were both incorporated into nucleosomes, with a concomitant loss of untagged H2B and H2A (compare lanes 6 and 7). Notably, this reaction was strongly dependent upon addition of ATP. Taken together, our results indicate that the human SRCAP-containing complex described in this report catalyzes ATP-dependent exchange of H2A.Z/H2B dimers for nucleosomal H2A/H2B.

DISCUSSION

Prior to completion of these studies, the question of whether the SRCAP complex has histone exchange activity was open. Indeed, it had been suggested by Cote and colleagues (*22*) that the Tip60 complex, which contains the SNF2-like helicase p400, might be responsible for H2A.Z exchange in mammalian cells, since (i) in *Drosophila* that activity is carried out by the Tip60 complex, which contains domino (related to both p400 and SRCAP) and (ii) the Tip60 complex includes a number of subunits whose orthologs are present in the SWR-C complex. Further, structural differences between Swr1 and SRCAP suggested the possibility that these proteins had distinct catalytic functions. Notably, in SRCAP, a large spacer of about 1200 amino acids is

inserted between ATPase motif 4 and motif 5, whereas a spacer of only about 400 amino acids is inserted in a similar position within Swr1. Thus, the importance of this study is that in eukaryotic cells a complex distinct from Tip60 can catalyze histone exchange.

The SRCAP complex purified in these studies exhibits the ability to catalyze the exchange of H2A/H2B for H2A.Z/H2B. As noted, similar complexes had been isolated earlier but showed no functional activity in the exchange assay. The most obvious difference in composition between the present complex and those described earlier is the apparent lack of H2A.Z, ZnF/HIT1, and YL1 in the SRCAP-containing complex described in this report. Therefore, it is possible that one or more of these additional proteins might have inhibitory activity; however, this appears to be an unlikely explanation, since orthologous proteins are present in the active yeast SWR-C complex (*7*). A more plausible explanation is that our current preparations of SRCAP complex are more concentrated.

Although it is clear from previous studies that SRCAP plays a role in transcriptional regulation mediated by a number of nuclear receptors (*1*, *4*, *23*), the mechanism by which SRCAP functions in this capacity has not been elucidated. The current studies demonstrate that a purified SRCAP complex has the ability to catalyze exchange of H2A.Z into nucleosomes. A similar activity has been reported for the yeast SWR-C complex, and mutation studies indicate that this catalytic activity is dependent on the ATPase activity of the Swr1 subunit (*7*). By analogy, mutation of the ATP binding site within SRCAP is likely to block the ability of the SRCAP complex to catalyze the exchange reaction. The importance of this site is supported by recent studies with SRCAP transgenic flies that indicate that the ATPase activity of SRCAP is required for in vivo activity (*23*). One model suggested by these studies is that the ability of SRCAP to regulate transcription is linked to the ability to catalyze the exchange of H2A.Z into nucleosomes.

In *S. cerevisiae*, the link between the abilities of Swr1 to regulate transcription and to catalyze incorporation of H2A.Z into nucleosomes has been established (*5*−*7*). H2A.Z has been localized to euchromatic regions near telomeres, adjacent to silenced heterochromatin, and it plays a role in antagonizing the spread of heterochromatin thus retaining the expression of nearby genes (*24*). H2A.Z has also been found within euchromatic regions distal to telomeres, suggesting a further role outside of impeding the spread of heterochromatin (*6*). Results of genome-wide screening studies indicate H2A.Z is associated with about two-thirds of *S. cerevisiae* promoters where it is enriched at the transcription initiation site. Interestingly, H2A.Z is associated with promoters containing highly ordered nucleosomes (*10*−*12*).

An important finding is null mutants of H2A.Z or Swr1 resulted in abnormal regulation of a common set of genes, suggesting a specialized role for Swr1 in H2A.Z deposition (*5*−*7*). SRCAP, the Swr1 mammalian counterpart, may also have this specialized role for H2A.Z. The homology with Swr1, its role in H2A.Z exchange, and the implications in transcriptional regulation begin to bring to light the major function of SRCAP.

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SUPPORTING INFORMATION AVAILABLE

Western blot analysis of the specificity of the anti-SRCAP antibodies and identification of the SRCAP complex in the phosphocellulose column fractions. This material is available free of charge via the Internet at http://pubs.acs.org.

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