The Mammalian YL1 Protein Is a Shared Subunit of the TRRAP/TIP60 Histone Acetyltransferase and SRCAP Complexes* 

Yong Cai‡, Jingji Jin‡, Laurence Floren‡, Selene K. Swanson‡, Thomas Kusch‡, Bing Li‡, Jerry L. Workman‡, Michael P. Washburn‡, Ronald C. Conaway‡,§, and Joan Weliky Conaway‡,∥

From the ‡Stowers Institute for Medical Research, Kansas City, Missouri 64110, the §Department of Biochemistry and Molecular Biology, Kansas University Medical Center, Kansas City, Kansas 66160, and the ¶Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

Received for publication, January 1, 2005, and in revised form, January 10, 2005. Published, JBC Papers in Press, January 11, 2005, DOI 10.1074/jbc.M500001200

The multiprotein mammalian TRRAP/TIP60-containing histone acetyltransferase (HAT) complex performs critical functions in a variety of cellular processes including transcriptional activation, double strand DNA break repair, and apoptosis. We previously isolated the TRRAP/TIP60 complex from HeLa cells (Cai, Y., Jin, J., Tomomori-Sato, C., Sato, S., Sorokina, I., Parmely, T. J., Conaway, R. C., and Conaway, J. W. (2003) J. Biol. Chem. 278, 42733–42736). Analysis of proteins present in preparations of the TRRAP/TIP60 complex led to the identification of several new subunits, as well as several potential subunits including the YL1 protein. Here we present evidence that the YL1 protein is a previously unrecognized subunit of the TRRAP/TIP60 HAT complex. In addition, we present evidence that YL1 is also a component of a novel mammalian multiprotein complex that includes the SNF2-related helicase SRCAP and resembles the recently described Saccharomyces cerevisiae SWR1 chromatin remodeling complex. Taken together, our findings identify the YL1 protein as a new subunit of the TRRAP/TIP60 HAT complex, and they suggest that YL1 plays multiple roles in chromatin modification and remodeling in cells.

The NuA4 complex is a well characterized Saccharomyces cerevisiae histone acetyltransferase (HAT) that acetylates the N-terminal tails of nucleosomal histones H2A and H4. NuA4 plays diverse roles in transcriptional regulation, double strand DNA break repair, and apoptosis (reviewed in Ref. 1). The NuA4 complex is composed of multiple subunits including ataxia telangiectasia mutated/phosphatidylinositol 3-kinase family member Tra1, enhancer of polycomb-like protein Epl1, Eaf2, p33ING3 tumor suppressor-related protein Yng2, actin-related protein ARP4, chromodomain-containing protein Eaf3, Yaf9, Eaf7, and Esa1, a HAT belonging to the MYST family of acetyltransferases.

A NuA4-like HAT complex referred to as the TRRAP/TIP60 HAT complex has been identified in Drosophila melanogaster (2) and mammalian cells (3–5). Studies by our laboratory and others have shown that the multiprotein TRRAP/TIP60 complex includes apparent orthologs of many NuA4 subunits including ataxia telangiectasia mutated/phosphatidylinositol 3-kinase family member TRRAP (transcription/transformation domain-associated protein), enhancer of polycomb proteins EPC1 and EPC-like, Eaf2-like protein DMAP1, Yng2-like protein p33ING3, Arp4-like protein BAF53a, Eaf3-like proteins MRG15 and MRGX, Yaf9-like protein GAS41, Eaf7-like protein MRGBP, and TIP60, an Esa1-like HAT belonging to the MYST family (2–5). Notably, the mammalian TRRAP/TIP60 HAT complex includes an additional set of subunits not present in the S. cerevisiae NuA4 complex. Among these proteins are the SNF2-related helicase p400, the bromodomain-containing TRCp120 coactivator, and the AAA ATPases TIP49a and TIP49b (3–5).

In this report, we identify the YL1 protein (6, 7) as a subunit of the mammalian TRRAP/TIP60 HAT complex. In addition, we demonstrate that the YL1 protein is also a subunit of a novel complex that contains the SNF2-related helicase SRCAP (SW2/SNF2-related CBP activator protein) (8–10) and resembles the recently described S. cerevisiae SWR1 chromatin remodeling complex, which catalyzes incorporation of the histone variant Htz1 (H2AZ) into nucleosomes (11–13).

**EXPERIMENTAL PROCEDURES**

Materials—Anti-FLAG (M2)-agarose, anti-FLAG (M2) monoclonal antibodies, and anti-FLAG peptide were purchased from Sigma. Anti-TRRAP (T-17) antibodies were obtained from Santa Cruz Biotechnology. Anti-TIP60 antibodies were from Upstate-Cell Signaling Solutions. Anti-DMAPI antibodies were purchased from Affinity BioReagents. Anti-MRGBP, anti-TIP49a, anti-TIP49b, anti-YL1, anti-TRCp120, and anti-BAF53a rabbit polyclonal antibodies were raised against recombinant proteins expressed in Escherichia coli (Cocalico Biologicals, Inc.). Anti-p400 antibodies were from Abcam, Inc.

This paper is available on line at http://www.jbc.org

* This work was supported by Grant R37 GM41628 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The amino acid sequence of this protein can be accessed through NCBI Protein Database under NCBI accession number NP_006340. 

* The on-line version of this article (available at http://www.jbc.org) contains supplemental Tables I and II.

** To whom correspondence should be addressed: Stowers Institute for Medical Research, 1000 E. 50th St., Kansas City, MO 64110. Tel.: 816-926-4091; Fax: 816-926-2093; E-mail: jlc@stowers-institute.org.

† The abbreviations used are: HAT, histone acetyltransferase; BAF53, Brg-associated factor; CBP, CREB-binding protein; CREB, cyclic AMP response element-binding protein; DMAP1, DNA methyltransferase 1-associated protein; GAS41, glioma-amplified sequence 1; HPLC, high pressure liquid chromatography; ING, inhibitor of growth; MRG15, mortality factor on chromosome 4-related gene on chromosome 15; MRGX, mortality factor on chromosome 4-related gene on chromosome X; MRGBP, MRG-binding protein; MYST, MZF bZIP2/SAS3-SAS2-TIP90; NuA4, nucleosome acetyltransferase of H4; SRCAP, SW2/SNF2-related CBP activator protein; TRRAP, transcription/transcription domain-associated protein; TIP49a and TIP49b, TATA-binding protein interacting 49-kDa proteins a and b; TIP60, Tat inter-
**Mammalian TRRAP/TIP60 and SRCAP Complexes**

**Tissue Culture**—DNAs encoding N-terminally FLAG-tagged human MRGGBP, YL1, and Zn/PHIT1 were subconed into pcDNA3.1 and stably transfected into HeLa S3 cells. Parental and stably transformed HeLa cells were maintained in Dulbecco’s modified Eagle’s medium with 5% glucose and 10% fetal bovine serum. For large scale cultures, HeLa cells were grown in spinner culture in Joklik medium with 5% calf serum. N-terminally FLAG-tagged human H2AZ was subconed into pcDNA5/FRT and introduced into 293/FRT cells using the Invitrogen Flip-in System. Parental and stably transformed 293/FRT cells were grown in Dulbecco’s modified Eagle’s medium with 5% glucose and 10% fetal bovine serum.

**Anti-FLAG-agarose Chromatography**—Nuclear extracts were prepared according to the method of Dignam et al. (14). Nuclear extracts were adjusted to 0.3 M KCl and 0.5% Triton X-100 and centrifuged at 40,000 rpm for 30 min at 4 °C in a Ti-45 rotor. Following centrifugation, the supernatants were mixed with anti-FLAG (M2)-agarose beads in a ratio of 100 μl of packed beads/60 μl of nuclear extract and gently rocked for 4 h at 4 °C. The beads were washed 3 times with an ~50-fold excess of buffer containing 50 mM Hepes-NaOH (pH 7.9), 0.25 mM KCl, 0.1% volumes/h to a phosphocellulose column (which contained for 30 min at 40,000 rpm in a Ti-45 rotor, the resulting supernatant, and then eluted at 1 ml/min with a 30-ml linear gradient from 0.05 to 0.6 M KCl, flow-through fraction containing 0.1 mM KCl and 0.2 mg/ml FLAG peptide.)

**Fractionation of YL1-containing Species by Phosphocellulose Chromatography**—Approximately 35 ml of nuclear extract prepared according to Dignam et al. (14) from HeLa cells stably expressing FLAG-YL1 were dialyzed against Buffer A (40 mM Hepes-NaOH (pH 7.9), 0.1 mM EDTA, 1 mM dithiothreitol, and 10% (v/v) glycerol) containing 1 mM phenylmethylsulfonyl fluoride to a conductivity equivalent to that of Buffer A containing 0.1 mM KCl. Following centrifugation for 30 min at 40,000 rpm in a Ti-45 rotor, the resulting supernatant, which contained ~200 mg of protein, was applied at 2 packed column volumes of Buffer A containing 0.1 M KCl, flow-through fraction containing 1 mM phenylmethylsulfonyl fluoride to a conductivity equivalent to that of Buffer A containing 0.1 M KCl. The column was washed at the same flow rate with Buffer B containing 0.1 M KCl, and proteins that flowed through the column at 0.1 M KCl were collected. Bound proteins were eluted stepwise at the same flow rate with Buffer A containing 0.3, 0.6, and 1.0 M KCl. One-fifth column volume fractions were collected, and protein-containing fractions from each step were pooled.

**TSK DEAE 5-PW HPLC of the Phosphocellulose 0.1 M KCl Fraction**—The phosphocellulose 0.1 M KCl, flow-through fraction (~25 mg of protein) was centrifuged at 20,000 rpm for 30 min at 4 °C. Using a Beckman System Gold HPLC, the resulting supernatant was applied at 1 ml/min to a TSK DEAE 5-PW HPLC column (7.5 × 75 mm, Tosohaas) pre-equilibrated in Buffer B (20 mM Hepes-NaOH (pH 7.9), 0.5 mM EDTA, 1 mM dithiothreitol, and 10% (v/v) glycerol) containing 0.05 M KCl. A 200–300 nl/ml gradient from 0.05 to 0.6 M KCl in Buffer B, 1.0-ml fractions were collected.

**HAT Assays**—Histone acetyltransferase assays were performed essentially as described (15). Briefly, 40-μl reaction mixtures containing 50 mM Hepes-NaOH, 125 μM ATP, 4 μCi of [3H]acetyl coenzyme A (3.4 Ci/mmol; Amersham Biosciences), 3 μg of long oligonucleosomes prepared as described (16), and anti-FLAG-agarose eluates prepared either from FLAG-MRGGBP or FLAG-YL1 expressing HeLa cells were incubated at 30 °C. After 30 min, aliquots of reaction mixtures were subjected to 18% SDS-polyacrylamide gel electrophoresis (29:1 acrylamide:bis). Proteins were visualized by Coomassie R-250 Blue staining, and radioactively acetylated nucleosomal histones H2A and H4, consistent with the full mass spectrometry spectrum (at 35% collision energy). Mass spectrometer scan functions and HPLC solvent gradients were controlled by the Xcalibur data system (ThermoFinnigan). The software algorithm 2–3 (19) was used to determine charge state and to delete spectra of poor quality. SEQUEST (20) was used to match tandem mass spectra to peptides in a database containing 1,916 human protein sequences downloaded from NCBI on March 24, 2004. The validity of peptide/spectrum matches was assessed using the SEQUEST-defined parameters, cross-correlation score, and normalized difference in cross-correlation scores. Spectra/peptide matches were only retained if they had a normalized difference in cross-correlation scores of at least 0.08 and minimum cross-correlation score of 1.5 for 2 spectra. In addition, it was demanded to be at least seven amino acids long. DTASelect (21) was used to select and sort peptide/spectrum matches passing this criteria set. Peptide hits from multiple runs were compared using CONTRAST (21).

**RESULTS AND DISCUSSION**

**The YL1 Protein Is a Subunit of the TRRAP/TIP60 HAT Complex**—We previously described isolation of the multiprotein TRRAP/TIP60 HAT complex from HeLa cell nuclear extracts (3). Our characterization of the human TRRAP/TIP60 complex led to the identification of the TRCop120, DMAP1, MRGB1, MRGBX, and MRGB proteins as new subunits of the complex. In addition, analysis of proteins present in our most highly purified preparations of the TRRAP/TIP60 complex by mass spectrometry identified the YL1 protein as a potential subunit of the complex.

The YL1 gene was originally isolated by positional cloning as a gene capable of suppressing the anchorage-independent growth of Kirsten sarcoma virus-transformed NIH3T3 cells (6,7). The human YL1 gene encodes a 364-amino-acid protein that appears to be evolutionarily conserved from humans to yeast. The NCBI Conserved Domain Data base (22) identified the human YL1 protein as a potential ortholog of the S. cerevisiae Swr1 chromatin remodeling complex, which catalyzes ATP-dependent incorporation of histone variant Htz1 (H2AZ) into nucleosomes (11–13).

To begin to investigate the possibility that the YL1 protein is a bona fide subunit of the TRRAP/TIP60 HAT complex, we generated a HeLa cell line stably expressing YL1 with an N-terminal FLAG epitope tag and purified YL1 and its associated proteins by anti-FLAG-agarose affinity chromatography. We then compared the nucleosomal HAT activity of anti-FLAG-agarose eluates from HeLa cells stably expressing FLAG-YL1 to that of the TRRAP/TIP60 HAT complex, which had been purified by anti-FLAG-agarose chromatography from nuclear extracts of HeLa cells stably expressing the TRRAP/TIP60 subunit MRGBP with an N-terminal FLAG tag (3). As shown in Fig. 1A, anti-FLAG-agarose eluates from both FLAG-MRGBP- and FLAG-YL1-expressing HeLa cells possessed HAT activity that specifically acetylated nucleosomal histones H2A and H4, consistent with
the possibility that, like MRGBP, YL1 is a subunit of the TRRAP/TIP60 complex. Also consistent with this possibility, analysis of silver-stained SDS-polyacrylamide gels revealed that anti-FLAG-agarose eluates from parental HeLa cells appeared to include a set of proteins similar to those found in preparations of MRGBP-associated proteins; however, some differences in the protein compositions of the two preparations were apparent (Fig. 1B, lanes 2 and 3). Most notably, complexes purified through FLAG-YL1 contained substantially smaller amounts of three polypeptides of ~28–28 kDa, corresponding to MRGBP, MRGX, and MRG15, consistent with our observation that these proteins are found both as components of the TRRAP/TIP60 complex and as free heterodimers consisting of MRGBP and MRGX or MRGBP and MRG15, respectively (3). In addition, complexes purified through FLAG-YL1 contained a reduced level of a doublet of ~120–150 kDa, corresponding to TRCp120 (3). Finally, YL1 also appeared to associate with a number of unique polypeptides.

To compare in more detail the polypeptide compositions of the TRRAP/TIP60 complex purified through FLAG-MRGBP and anti-FLAG-agarose eluates from FLAG-YL1-expressing HeLa cells, we took advantage of MudPIT (17, 23). MudPIT is a sensitive method for detecting proteins present in complex mixtures. In MudPIT, a mixture of proteins is first digested into peptides, which are then separated by two-dimensional cation exchange and reverse phase HPLC and analyzed by in-line tandem mass spectrometry.

Analysis by MudPIT of proteins present in TRRAP/TIP60 complexes purified by anti-FLAG-agarose chromatography from FLAG-MRGBP-expressing HeLa cells identified the YL1 protein (Fig. 2 and supplemental Table I), as well as all of the previously described subunits of the TRRAP/TIP60 complex (3–5). The presence of the YL1 protein in TRRAP/TIP60 complexes purified by FLAG-MRGBP-expressing HeLa cells was confirmed by Western blotting (Fig. 3). Importantly, an analysis of proteins present in anti-FLAG-agarose eluates from FLAG-YL1-expressing HeLa cells by MudPIT (Fig. 2 and supplemental Table I) and by Western blotting (Fig. 3) identified YL1-associated proteins all known subunits of the mammalian TRRAP/TIP60 complex. Together with the results of the HAT assays of Fig. 1A, these results argue that the YL1 protein is a bona fide subunit of the TRRAP/TIP60 complex.

YL1 is also Associated with an SRCAP-containing Complex That Resembles the S. cerevisiae SWR1 Chromatin-remodeling Complex—As expected from the results of the SDS-polyacrylamide gel of Fig. 1B, MudPIT analysis of anti-FLAG-agarose eluates from FLAG-YL1-expressing HeLa cells identified several proteins not found in the TRRAP/TIP60 complex, raising the possibility that the YL1 protein is present in cells not only

![Figure 1](image1.png)

**Fig. 1.** YL1 is associated with HAT activity. A, assay of HAT activities of the TRRAP/TIP60 complex and the purified FLAG-YL1-containing complex. Anti-FLAG-agarose eluates from equivalent numbers of FLAG-MRGBP and FLAG-YL1 expressing HeLa cells were prepared as described under “Experimental Procedures.” As determined by Western blotting (see Fig. 3), the relative concentrations of the TRRAP/TIP60 subunits p400, TRCp120, RAF53a, TIP49a, TIP49b, and TIP60 were similar in anti-FLAG-agarose eluates prepared from the two cell lines and used in HAT assays. HAT assays were performed with long oligonucleosomes and 0.5 μl (lanes 2 and 5), 1.5 μl (lanes 3 and 6), or 4.5 μl (lanes 4 and 7) of the indicated anti-FLAG-agarose eluates as described under “Experimental Procedures.” Proteins were visualized by Coomassie Blue R250 staining (top gel), and acetylated histones were visualized by autoradiography (bottom gel). B, analysis of proteins associated with FLAG-MRGBP, FLAG-YL1, FLAG-ZnF/HIT1, and FLAG-H2AZ. Anti-FLAG-agarose chromatography of nuclear extracts prepared from equivalent numbers of HeLa cells, FLAG-MRGBP, FLAG-YL1, and FLAG-ZnF/HIT1-expressing HeLa cells, and FLAG-H2AZ-expressing human embryonic kidney 293 FRT cells was carried out as described under “Experimental Procedures.” 10 μl of each anti-FLAG-agarose eluate was analyzed by SDS-polyacrylamide gel electrophoresis in a 4–20% gradient gel (Ready Gel, Bio-Rad), and proteins were visualized by silver staining. The positions and relative molecular masses in kDa of protein size standards are indicated at the left of the gel. HeLa, anti-FLAG-agarose eluate from parental HeLa cells; f:MRGBP, FLAG-MRGBP; f:YL1, FLAG-YL1; f:ZnF/HIT1, FLAG-ZnF/HIT1; f:H2AZ, FLAG-H2AZ.

![Figure 2](image2.png)

**Fig. 2.** Identification of proteins associated with FLAG-MRGBP, FLAG-YL1, FLAG-ZnF/HIT1, and FLAG-H2AZ by MudPIT. Aliquots of anti-FLAG-agarose eluates from FLAG-MRGBP-, FLAG-YL1-, FLAG-ZnF/HIT1-, and FLAG-H2AZ-expressing cells were subjected to MudPIT as described under “Experimental Procedures.” Proteins detected by MudPIT are indicated in yellow; proteins not detected are indicated in blue. The numbers represent the number of spectra corresponding to peptides derived from each protein and the percent of the total protein sequence detected by mass spectrometry. *+, spectral counts shown for H2AZ and H2A.F/Z include only those peptides unique to these H2A isoforms. Percent coverage is not shown for H2AZ(H2A.F/Z), because many peptides are shared with other H2A isoforms.
as a subunit of the TRRAP/TIP60 complex but also as a component of one or more additional species. Among the additional proteins associated with YL1 were the SNF2-related helicase SRCAP, the actin-related protein ARP6, and a previously uncharacterized protein encoded by an open reading frame described in the NCBI protein data base as ZnF/HIT1 (accession number NP_006340).

SRCAP is a member of the Swi2/Snf2 family of ATPases. SRCAP was originally identified by its ability to interact with residues 227–460 of the transcriptional coactivator and histone acetyltransferase CBP in a yeast two-hybrid screen (8). Subsequently, SRCAP was found to function as a coactivator for nuclear receptors and to synergize with CBP in this process (9, 10). SRCAP shares significant sequence similarity with the p400 helicase subunit of the SWR1/Tip49 complex—

To explore this possibility further, the phosphocellulose flow-through fraction was subjected to consecutive TSK DEAE 5-PW HPLC and anti-FLAG-agarose chromatography. Notably, YL1 eluted from the TSK DEAE 5-PW column in a single, discrete peak centered around fraction 8 (Fig. 4B), which was then subjected to anti-FLAG-agarose chromatography. MudPIT analysis of proteins present in anti-FLAG-agarose eluates prepared from fraction 8 identified the SRCAP, YL1, DMAP1, BAF53a, ARP6, TIP49a, TIP49b, GAS41, and ZnF/HIT1 proteins (Fig. 4C, column 5 and supplemental Table II), arguing that the YL1 protein is a component not only of the TRRAP/TIP60 HAT complex but also of a multiprotein complex containing the SRCAP protein.

The ZnF/HIT1 Protein Is Associated with the SRCAP Complex—Because of its similarity to the S. cerevisiae SWR1 complex, we sought to determine whether the ZnF/HIT1 protein is a bona fide subunit of the SRCAP complex. To accomplish this, we generated a HeLa cell line stably expressing the ZnF/HIT1 protein with an N-terminal FLAG tag. Nuclear extracts prepared from HeLa cells stably expressing FLAG-ZnF/HIT1 were subjected to anti-FLAG-agarose chromatography. As shown in the silver-stained SDS-polyacrylamide gel of Fig. 1B (lanes 2–4), anti-FLAG-agarose eluates from FLAG-ZnF/HIT1-expressing HeLa cells appeared to include a subset of proteins found in anti-FLAG-agarose eluates from FLAG-MRGBP- and FLAG-YL1-expressing cells. An analysis of proteins present in anti-FLAG-agarose eluates from FLAG-ZnF/HIT1-expressing HeLa cells by MudPIT (Fig. 2 and supplemental Table I) and by Western blotting (Fig. 3) identified, in addition to the ZnF/HIT1 protein, the SRCAP, DMAP1, BAF53a, ARP6, TIP49a, TIP49b, GAS41, and YL1 proteins, arguing that ZnF/HIT1 is a subunit of the SRCAP complex. In addition, we note that no unique subunits of the TRRAP/TIP60 complex were detected by MudPIT in anti-FLAG-agarose eluates from FLAG-ZnF/HIT1-expressing HeLa cells, providing further support for the idea that the YL1 protein is present in cell lysates in multiple, chromatographically distinct species, the TRRAP/TIP60 HAT complex and an SRCAP-containing complex resembling the S. cerevisiae SWR1 chromatin-remodeling complex.

The SRCAP Complex Binds Tightly and Specifically to the Histone H2A Variant H2AZ—As discussed above, our findings argue that human YL1 protein is a subunit of at least two multiprotein complexes, the TRRAP/TIP60 HAT complex and an SRCAP-containing complex similar in subunit composition to the S. cerevisiae SWR1 chromatin-remodeling complex. In yeast, the SWR1 complex is capable of binding tightly and

**Fig. 3.** Identification of proteins associated with FLAG-MRGBP, FLAG-YL1, and FLAG-H2AZ by Western blotting. Anti-FLAG-agarose chromatography of nuclear extracts from FLAG-MRGBP, FLAG-YL1, and FLAG-H2AZ-expressing cells was performed as described under “Experimental Procedures.” Aliquots of anti-FLAG-agarose eluates were analyzed by SDS-polyacrylamide gel electrophoresis in 4–20% gradient gels, and proteins were identified by Western blotting with antibodies that recognize the proteins indicated.
specifically to the histone H2A variant Htz1 and of catalyzing its incorporation into nucleosomes. As summarized in Fig. 2, substantial spectra corresponding to peptides derived from H2AZ (or H2A.F/Z), the mammalian ortholog of yeast Htz1, were detected by MudPIT in fractions containing the SRCAP complex. In contrast, no spectra corresponding to peptides derived from H2AZ were detected in fractions lacking the SRCAP complex, such as those fractions purified from FLAG-MRGBP-expressing HeLa cells. These observations suggest that, like the S. cerevisiae SWR1 complex, the human SRCAP complex might bind H2AZ.

To address this possibility, we generated a human 293 cell line stably expressing human histone variant H2AZ with an N-terminal FLAG tag, and we purified H2AZ-associated proteins by anti-FLAG-affinity chromatography. As shown in the SDS-polyacrylamide gel of Fig. 1B (lanes 4 and 5), anti-FLAG-agarose eluates from FLAG-H2AZ-expressing 293 cells appeared to include a set of proteins similar to those found in anti-FLAG-agarose eluates containing the SRCAP complex from FLAG-ZnF/HIT1-expressing cells. An analysis of proteins present in anti-FLAG-agarose eluates from FLAG-H2AZ-expressing 293 cells by MudPIT (Fig. 2 and supplemental Table I) and by Western blotting (Fig. 3) identified the SRCAP, DMAP1, BAf53a, ARP6, TIP49a, TIP49b, GAS41, ZnF/HIT1, and YL1 proteins, arguing that the SRCAP complex binds stably to histone variant H2AZ. In addition, we note that no unique subunits of the TRRAP/TIP60 complex were detected by MudPIT in anti-FLAG-agarose eluates from FLAG-H2AZ-expressing 293 cells. Thus, like the S. cerevisiae SWR1 complex, the SRCAP complex appears to bind specifically to histone variant H2AZ.

Summary and Perspective—In summary, in this report we present biochemical evidence that the YL1 protein is a subunit of the TRRAP/TIP60 HAT complex. In addition, we present evidence that the YL1 protein is also present in cells as a subunit of the previously uncharacterized mammalian SRCAP complex, which bears a striking similarity to the recently described S. cerevisiae SWR1 chromatin remodeling complex and is composed of the SNF2-related SRCAP helicase, ARP6, ZnF/HIT1, and a subset of proteins including YL1, DMAP1, TIP49a, TIP49b, BAf53a, and GAS41, which are also subunits of the TRRAP/TIP60 HAT complex. Our results are consistent with the findings of Doyon et al. (4), who recently reported that DMAP1 is present in cells as a subunit in one or more additional species that are distinct from the TRRAP/TIP60 complex and include SRCAP and YL1.

Finally, we note that, although the mammalian SRCAP complex has a subunit composition quite similar to that of the S. cerevisiae SWR1 complex and can bind histone variant H2AZ, we have not yet succeeded in assaying SRCAP complex-dependent incorporation of H2AZ into nucleosomes, in either the presence or absence of the TRRAP/TIP60 complex. Although we
observe that crude HeLa cell nuclear extracts catalyze robust incorporation of H2AZ into nucleosomal arrays in vitro (data not shown), we have thus far failed to observe our preparations of the purified SRCAP complex catalyze incorporation of H2AZ into nucleosomes in similar assays. Thus, it is possible that, unlike the S. cerevisiae SWR1 complex, the SRCAP complex is not responsible for H2AZ exchange in mammalian cells or, as seems more likely, that the SRCAP complex participates in H2AZ exchange in mammalian cells but requires assistance from additional, as yet unidentified, factors present in crude nuclear extracts.

REFERENCES
1. Doyon, Y., and Cote, J. (2004) Curr. Opin. Genet. Dev. 14, 147–154
2. Kusch, T., Florens, L., Macdonald, W. H., Swanson, S. K., Glaser, R. L., Yates, J. R., Abmayr, S. M., Washburn, M. P., and Workman, J. L. (2004) Science 306, 2084–2087
3. Cai, Y., Jin, J., Tomomori-Sato, C., Sato, S., Sorokina, I., Parmely, T. J., Conaway, R. C., and Conaway, J. W. (2003) J. Biol. Chem. 278, 42733–42736
4. Doyon, Y., Selleck, W., Lane, W. S., and Cote, J. (2004) Mol. Cell. Biol. 24, 1884–1896
5. Ikura, T., Ogryzko, V., Gigeriev, M., Groisman, R., Wang, J., Horikoshi, M., Scully, R., Qin, J., and Nakatani, Y. (2000) Cell 102, 463–475
6. Horikawa, I., Tanaka, H., Yuasa, Y., Suzuki, M., Shimizu, M., and Oshimura, M. (1995) Exp. Cell Res. 220, 11–17
7. Horikawa, I., Tanaka, H., Yuasa, Y., Suzuki, M., and Oshimura, M. (1995) Biochem. Biophys. Res. Commun. 208, 999–1007
8. Johnston, H., Kneer, J., Chackalaparampil, I., Yaciuk, P., and Chirivia, J. (1999) J. Biol. Chem. 274, 16370–16376
9. Monroy, M. A., Ruhl, D. D., Xu, X., Graner, D. K., Yaciuk, P., and Chirivia, J. C. (2001) J. Biol. Chem. 276, 40721–40726
10. Monroy, M. A., Schott, N. M., Cox, L., Chen, J. D., Ruh, M., and Chirivia, J. C. (2003) Mol. Endocrinol. 17, 2519–2528
11. Kabor, M. S., Venkatasubramanyam, S., Meneghini, M. D., Gin, J. W., Jennings, J. L., Link, A. J., Madhani, H. D., and Rine, J. (2004) PLoS Biol. 2, E131
12. Krogan, N. J., Baetz, K., Keogh, M. C., Datta, N., Sawa, C., Kwik, T. C., Thompson, N. J., Davey, M. G., Pootoolal, J., Hughes, T. R., Emil, A., Buratowski, S., Hieter, P., and Greenblatt, J. F. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 13513–13518
13. Mizuguchi, G., Shen, X., Landry, J., Wu, W. H., Sen, S., and Wu, C. (2004) Science 303, 343–348
14. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1489
15. Eberharter, A., John, S., Grant, P. A., and Workman, J. L. (2004) Methods 15, 315–321
16. Owen-Hughes, T., Utle, R. T., Steger, D. J., West, J. M., John, S., Cot, J., Havas, R. M., and Workman, J. L. (1999) Methods Mol. Biol. 119, 319–331
17. Washburn, M. P., Wolters, D., and Yates, J. R., III (2001) Nat. Biotechnol. 19, 242–247
18. McDonald, W. H., Ohi, R., Miyamoto, D. T., Mitchison, T. J., and Yates, J. R. (2002) Int. J. Mass. Spectrom. 219, 245–251
19. Sadygov, R. G., Eng, J., Durr, E., Saraf, A., McDonald, H., MacCoss, M. J., and Yates, J. R., III (2002) J. Proteome Res. 1, 211–215
20. Eng, J. K., McCormick, A. L., and Yates, J. R., III (1994) J. Am. Soc. Mass. Spec. 5, 976–989
21. Tabb, D. L., McDonald, W. H., and Yates, J. R., III (2002) J. Proteome Res. 1, 21–26
22. Marchler-Bauer, A., Anderson, J. B., DeWeese-Scott, C., Federova, N. D., Geer, L. Y., He, S., Hurwitz, D. I., Jackson, J. D., Jacobs, A. R., Lanczycki, C. J., Liebert, C. A., Liu, C., Madej, T., Marchler, G. H., Mzumder, B., Nikol-skaya, A. N., Panchenko, A. R., Rao, B. S., Shoemaker, B. A., Simonov, V., Song, J. S., Thiesen, P. A., Vasudevan, S., Wang, Y., Yamashita, R. A., Yiu, J. J., and Bryant, S. H. (2003) Nucleic Acids Res. 31, 383–387
23. Wolters, D., Washburn, M. P., and Yates, J. R. (2001) Anal. Chem. 73, 5683–5690