A Mammalian Chromatin Remodeling Complex with Similarities to the Yeast INO80 Complex*

Received for publication, August 18, 2005, and in revised form, October 11, 2005 Published, JBC Papers in Press, October 17, 2005, DOI 10.1074/jbc.M509128200

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The mammalian Tip49a and Tip49b proteins belong to an evolutionarily conserved family of AAA+ ATPases. In Saccharomyces cerevisiae, orthologs of Tip49a and Tip49b, called Rvb1 and Rvb2, respectively, are subunits of two distinct ATP-dependent chromatin remodeling complexes, SWR1 and INO80. We recently demonstrated that the mammalian Tip49a and Tip49b proteins are integral subunits of a chromatin remodeling complex bearing striking similarities to the S. cerevisiae SWR1 complex (Cai, Y., Jin, J., Florens, L., Swanson, S. K., Kusch, T., Li, B., Workman, J. L., Washburn, M. P., Conaway, R. C., and Conaway, J. W. (2005) J. Biol. Chem. 280, 13665–13670). In this report, we identify a new mammalian Tip49a- and Tip49b-containing ATP-dependent chromatin remodeling complex, which includes orthologs of 8 of the 15 subunits of the S. cerevisiae INO80 chromatin remodeling complex as well as at least five additional subunits unique to the human INO80 (hINO80) complex. Finally, we demonstrate that, similar to the yeast INO80 complex, the hINO80 complex exhibits DNA- and nucleosome-activated ATPase activity and catalyzes ATP-dependent nucleosome sliding.

The related mammalian Tip49a and Tip49b proteins are members of a family of AAA+ (associated with various cellular activities) ATPases with roles in DNA repair, recombination, and transcriptional regulation (1, 2). In Saccharomyces cerevisiae, the Tip49a and Tip49b proteins (also known as Rvb1 and Rvb2) participate in chromatin remodeling as subunits of the multiprotein SWR1 and INO80 ATP-dependent chromatin remodeling complexes (3–5).

The SWR1 complex remodels chromatin by catalyzing ATP-dependent replacement of H2A-H2B histone dimers in nucleosomes by dimers containing histone variant Htz1 (referred to as H2AZ in mammalian cells) (3). In addition to Tip49a and Tip49b, the SWR1 complex includes the SNF2 family helicase Swr1, actin-related proteins Arp4 and Arp6, YEATS domain family member Yaf9, bromodomain protein Bdf1, and additional proteins Swc3–Swc7, which are of unknown function (3–5).

The INO80 complex catalyzes ATP-dependent sliding of nucleosomes along DNA and, based on genetic and other evidence, may be involved in the repair of DNA double strand breaks and in transcriptional regulation (6–12). The INO80 complex includes Tip49a and Tip49b, the SNF2 family helicase Ino80, actin-related proteins Arp4, Arp5, and Arp8, YEATS domain family member Taf14, HMG (high mobility group) domain protein Nhp10, and six additional proteins designated les1–les6 (6, 13). Thus, the SWR1 and INO80 complexes share three proteins (Tip49a, Tip49b, and Arp4) and contain additional homologous components. In addition, each of the two complexes has a number of unique subunits.

The orthologs of the Tip49a and Tip49b AAA+ ATPases also play roles in chromatin remodeling in higher eukaryotes. Tip49a and Tip49b are subunits of the mammalian and Drosophila melanogaster TRRAP-TIP60 histone acetyltransferase (HAT) complexes (14–17). In addition to Tip49a and Tip49b, the TRRAP-TIP60 complex includes ATM/ phosphatidylinositol 3-kinase 3-kine family member TRRAP, the SNF2 family p400 or Domino helicase, actin-related protein Arp4, bromodomomain-containing protein BRD8, the enhancer of polycomb (EPC) and/or enhancer of polycomb-like (EPC-like) protein, inhibitor of growth 3 (ING3), DNA methyltransferase 1-associated protein (DMAP1), MRG15 and/or the related MRGXF protein, the MRGXP protein, and TIP60, a HAT belonging to the MYST family. Characterization of the activities associated with the higher eukaryotic TRRAP-TIP60 complex revealed that it possesses HAT activity similar to that of the S. cerevisiae NuA4 HAT complex, which acetylates histones H2A and H4 (reviewed in Ref. 18). The human and D. melanogaster TRRAP-TIP60 complexes were found to play critical roles in double-stranded DNA break repair (16, 17). Notably, the D. melanogaster TRRAP-TIP60 complex is capable of acetylating nucleosomal phospho-H2Av and replacing it with unmodified H2Av, indicating that in flies this single complex performs functions closely related to those of the yeast NuA4 HAT and SWR1 histone exchange complexes (17).

We recently identified a new mammalian Tip49a- and Tip49b-containing ATP-dependent chromatin remodeling complex that bears striking similarity to the S. cerevisiae SWR1 complex (19). Purification of this complex revealed that it includes the SNF2 family and SWR1-related SRCAP helicase, as well as orthologs of most of the known subunits of the S. cerevisiae SWR1 complex. In the course of our characterization of the structure and function of the SRCAP complex, we identified an additional mammalian Tip49a- and Tip49b-containing chromatin remodeling complex. Here we describe the properties of this

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§ This work was supported by National Institutes of Health Grants R01GM416178 (to R. C. C.) and R01GM47867 (to J. L. W.) and by Grant S437-05 from the Leukemia and Lymphoma Society (to T. Y.). 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.
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1 The abbreviations used are: HAT, histone acetyltransferase; ATPγS, adenosine 5’-O-(thiotriphosphate); HEK, human embryonic kidney; hINO80, human INO80-like protein; HPLC, high pressure liquid chromatography; MudPIT, multidimensional protein identification technology; NFKB, nuclear factor related to κB-binding protein; ORF, open reading frame; Taf, TATA-binding protein-associated factor; Tip49a and Tip49b, TATA-binding protein interacting 49-kDa proteins a and b.
new chromatin remodeling complex, which includes orthologs of 8 of the 15 subunits of the *S. cerevisiae* INO80 chromatin remodeling complex as well as at least 5 additional subunits unique to the human INO80 (hINO80) complex.

**EXPERIMENTAL PROCEDURES**

*Generation and Growth of Mammalian Cell Lines*—Full-length cDNAs encoding the human Tip49a, Tip49b, Arp8, PAPA-1 (hles2), C18orf37 (hles6), Amida, and FLJ90652 proteins or a fragment of FLJ20309 (residues 106–544) were obtained from the American Type Culture Collection, subcloned with FLAG tags into pcDNA5/FRT, and introduced into HEK293/FRT cells using the Invitrogen Flip-in system. Full-length cDNAs encoding the human PAPA-1 and C18orf37 proteins were subcloned with FLAG tags into pcDNA3.1 and introduced into HeLa S3 cells. Parental and stably transformed HEK293/FRT and HeLa S3 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.

**Anti-FLAG Agarose Chromatography**—Whole cell extracts were prepared from HEK293/FRT cells as follows. Cells were grown to 70–80% confluence in four to five 15-cm dishes. Cells were washed in dishes with phosphate-buffered saline and then lysed by resuspension in buffer (1 ml/dish) containing 40 mM Hepes-NaOH (pH 7.9), 0.45 mM NaCl, 1.5 mM MgCl₂, 10% glycerol, 1 mM dithiothreitol, and 0.2% Triton X-100. The resulting suspension was transferred to centrifuge tubes and incubated with rotation at 4 °C for 30 min. The cell lysate was centrifuged at 40,000 rpm for 60 min at 4 °C in a 70.1 Ti rotor (Beckman-Coulter). The resulting supernatant was subjected to anti-FLAG agarose chromatography. Nuclear extracts were prepared from HeLa S3 cells according to the method of Dignam et al. (20). Whole cell or nuclear extracts were adjusted to 0.3 mM NaCl and 0.2% Triton X-100 and centrifuged at 40,000 rpm for 30 min at 4 °C in a Ti-45 rotor. Supernatants were then mixed with anti-FLAG (M2) agarose beads (SIGMA) in a ratio of 100 packed beads/6 ml of supernatant and gently rocked for 4 hr at 4 °C. The resulting supernatant was subjected to anti-FLAG agarose chromatography. Nuclear extracts were prepared from HeLa S3 cells according to the method of Dignam et al. (20).

**ATPase Assays**—Reactions contained 20 μl of 50 mM Hepes-NaOH (pH 7.6), 70 mM NaCl, 5 mM MgCl₂, 0.5 mM EGTA, 0.1 mM MgATP, 10% glycerol, 0.02% Nonidet P-40, 0.2 mM dithiothreitol, 100 μg/ml bovine serum albumin, 40 μM ATP, 0.2 μM of [α-32P]ATP (400 Ci/mmol, Amersham Biosciences). Where indicated, reaction mixtures contained the hINO80 complex purified from HeLa cells expressing FLAG-hles2 (PAPA-1) and 150 ng of mononucleosomes or long oligonucleosomes prepared from HeLa cells as described (21). After incubation at 37 °C for 30 min, reactions were stopped by the addition of 2 μl of 20 mM EDTA (pH 8.0). A 2-μl aliquot of each reaction mixture was spotted onto a cellulose polyethyleneimine TLC plate (JT Baker). The plate was then developed with 0.375 M potassium phosphate (pH 3.5). Reaction products were detected and quantitated using a Typhoon phosphorimaging device (GE Healthcare).

**Nucleosome Remodeling Assays**—A 216-bp DNA fragment (dSH-A) was generated by PCR from pGUB-dSH in the presence of [α-32P]dCTP, pGUB-dSH was generated by deleting the Sall to HindIII fragment of pGUB (22). Mononucleosomes were reconstituted on this labeled DNA fragment by dilution transfer from HeLa long oligonucleosomes. ~3 μg of nucleosomes was mixed with ~1 pmol of [32P]-labeled DNA fragment in 25 μl of buffer containing 1.0 mM NaCl, 10 mM Tris-HCl (pH 8), 1 mM EDTA (pH 8.0), 0.5 mM phenylmethylsulfonyl fluoride, and 5 mM dithiothreitol. After 30 min at 30 °C, the mixture was sequentially adjusted to 0.8, 0.6, and 0.4 mM NaCl by dilution with 10 mM Tris-HCl (pH 8), 1 mM EDTA (pH 8.0), 0.5 mM phenylmethylsulfonyl fluoride, and 5 mM dithiothreitol, with a 30-min incubation at 30 °C between each dilution. Final dilutions to 0.2 and 0.1 mM NaCl were made using the same buffer plus 0.1% Nonidet P-40, 20% glycerol, and 200 μg/ml bovine serum albumin. After reconstitution, the mononucleosomes were stored in 30-μl aliquots at ~20 °C. hINO80 complex purified from HeLa cells expressing FLAG-PAPA-1 (hles2) was incubated at 37 °C with 2.5 μl of reconstituted mononucleosomes (~0.01 pmol of labeled mononucleosome, ~0.25 pmol of unlabeled oligonucleosomes) in buffer containing 20 mM Hepes-NaOH (pH 7.9), 50 mM NaCl, 4.5 mM MgCl₂, 2 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 45 μg/ml bovine serum albumin, 10% glycerol, 0.02% Triton X-100, 0.02% Nonidet P-40, and 1 mM ATP. After a 30-min incubation, 0.5 μg of HeLa cell long oligonucleosomes (21) and 0.75 μg of salmon sperm DNA (which had been sonicated, boiled, and quick-chilled) were added, and reactions were incubated for an additional 30 min at 37 °C to remove DNA- or nucleosome-binding proteins that would alter mononucleosome electrophoretic mobility. The reaction products were then applied to 5% polyacrylamide gels (37.5:1 acrylamide:bis-acrylamide) gels in 0.5× Tris borate-EDTA (23) and subjected to electrophoresis at 4 °C for 4.5 h at 200 V. Gels were dried and exposed to a storage phosphor screen overnight.

**Mass Spectrometry**—Identification of proteins was accomplished as described (19) using a modification of the multidimensional protein identification technology (MudPIT) procedure of Washburn et al. (24). Briefly, trichloroacetic acid-precipitated proteins were reduced, alkylated, and digested with modified trypsin (Roche Applied Science). Peptide mixtures were applied to a three-phase microcapillary HPLC column (25) packed with 5 μm C18 reverse phase resin (Aqua, Phenomenex), followed by strong cation exchange resin (Partispher SCX, Whatman) and then by 5 μm C18 reverse phase resin (Aqua, Phenomenex), and equilibrated in 5% acetonitrile, 0.1% formic acid (Buffer A). Peptides were sequentially eluted from the SCX resin to the reverse phase resin with six steps of increasing salt concentration. After each step, peptides were eluted from the reverse phase resin with a gradient of acetonitrile into a Deca-XP ion trap mass spectrometer equipped with a nano-liquid chromatography electrospray ionization source (ThermoFinnigan). The program 2-3 (26) was used to determine the charge state and to delete poor quality spectra. The SEQUEST algorithm (27) was used to match tandem mass spectrometry spectra to human peptides extracted from the NCBI NR data base (27,196 human protein sequences as of March 24, 2004). Spectra/peptide matches were only retained if they had a normalized difference in cross-correlation scores of at least 0.08 and a minimum cross-correlation score of 1.8 for +1, 2.5 for +2, and 3.5 for +3 spectra and if the peptides were at least 7 amino acids long.

**RESULTS AND DISCUSSION**

*Human Tip49a and Tip49b Are Associated with a Human INO80-like Protein (hINO80)*—As part of our characterization of the structures and functions of mammalian TRRAP-TIP60 and SRCAP chromatin remodeling complexes, we generated cell lines stably expressing either Tip49a or Tip49b with N-terminal FLAG epitope tags and then purified...
Tip49a- and Tip49b-associating proteins by anti-FLAG agarose immunopurification. As a control for the specificity of immunopurification, extracts prepared from untransformed, parental cells were subjected to the same procedure. As shown in Fig. 1, anti-FLAG agarose eluates from FLAG-Tip49a- and FLAG-Tip49b-expressing cells appeared to include similar sets of proteins (compare lanes 1 and 2).

To identify and compare FLAG-Tip49a- and FLAG-Tip49b-associating proteins, we took advantage of MudPIT (24, 28), a sensitive method for identifying proteins present in complex mixtures. In a MudPIT experiment, a mixture of proteins is first digested into peptides, which are then fractionated by two-dimensional strong cation exchange and reverse phase HPLC and analyzed by in-line tandem mass spectrometry.

As summarized in Fig. 2, MudPIT analyses of anti-FLAG agarose eluates from FLAG-Tip49a- and FLAG-Tip49b-expressing cells identified, in addition to the known subunits of the TRRAP-TIP60 and SRCAP complexes, a collection of proteins not previously found in either TRRAP-TIP60 or SRCAP preparations or MudPIT control samples. Among these proteins was a previously uncharacterized SNF2 family helicase encoded by the KIAA1259 ORF. As suggested by analysis of symmetrical best matches in database searches and by phylogenetic

![FIGURE 1. SDS polyacrylamide gel of the mammalian INO80-related chromatin remodeling complex.](image1)

**FIGURE 2. Consensus subunits of the mammalian INO80-related chromatin remodeling complex.** Aliquots of the anti-FLAG agarose eluates, indicated at the top of each column, were subjected to MudPIT as described under “Experimental Procedures.” Boxed numbers represent the normalized spectral count, which is equivalent to the number of individual spectra identifying a particular protein divided by the molecular weight of the protein \( \times 10^5 \). The MudPIT data sets for the FLAG-MRGBP and FLAG-ZnF/HIT1 preparations were described previously (19) and are included here for comparison purposes. Although both MRG15 and MRGX share regions of sequence similarity with S. cerevisiae Eaf3, MRGX lacks the chromodomain found in Eaf3.
inference, the 1561-amino acid KIAA1259 protein is an apparent human ortholog of the *S. cerevisiae* Ino80 helicase (29). Also among these proteins were the actin-related proteins Arp5 and Arp8, each of which has a yeast ortholog found in the INO80 complex but not in the SWR1 complex (Fig. 2, lanes 1 and 2).

**Subunit Composition of the hINO80 Complex**—To investigate the possibility that some or all of these proteins were subunits of a mammalian INO80 complex, we generated an HEK293/FRT cell line stably expressing Arp8 with an N-terminal FLAG tag. Extracts prepared from these cells were subjected to anti-FLAG agarose chromatography, and proteins present in anti-FLAG agarose eluates were identified by MudPIT. As shown in Fig. 2, Arp8 copurified with hIno80, Tip49a and Tip49b, Baf53a (Arp4), Arp5, and an additional seven proteins that were not present in either the TRRAP-TIP60 or SRCAP complexes. These proteins included the “Pim-1 kinase-associated protein-associated protein 1” (PAPA-1, GI 13775202) (30), Amida (also known as TCF3(E2A) fusion partner in childhood leukemia, GI 7019371) (31, 32), nuclear protein 1” (PAPA-1, GI 13775202) (30), Amida (also known as TCF3(E2A) fusion partner in childhood leukemia, GI 7019371) (31, 32), nuclear factor related to k5-binding protein (NFRKB, GI 23346420) (33), microspherule protein 1 (MCRS1 or MSP58, GI 28983564) (34), and previously uncharacterized proteins encoded by the FLJ90652 (GI 27734727), C18orf37 (GI 34916002), and FLJ20309 (GI 3848718) genes. To determine whether these proteins were present in the same complex, we generated additional HeLa and HEK293/FRT cell lines stably expressing full-length PAPA-1, C18orf37, Amida, or FLJ90652, all with N-terminal FLAG tags, or a C-terminally FLAG-tagged fragment of FLJ20309 (residues 106–544). As shown in Fig. 1 (compare lanes 4 and 5), anti-FLAG agarose eluates prepared from FLAG-PAPA-1 expressing HeLa cells and from FLAG-FLJ90652 expressing HEK293/FRT cells appeared to include similar sets of proteins. Furthermore, MudPIT analyses revealed that FLAG-tagged PAPA-1, C18orf37, Amida, FLJ20309, and FLJ90652 each copurified with the hINO80 helicase and the Tip49a, Tip49b, PAPA-1, C18orf37, Arp4, Arp5, Arp8, Amida, NFRKB, MCRS1, FLJ90652, and FLJ20309 proteins, which argues that they are all components of a multienzyme hINO80-containing complex (Fig. 2). Notably, unique subunits of the TRRAP-TIP60 or SRCAP complexes were not detected by MudPIT in any of these purified samples (Fig. 2).

Sequence analysis suggests that several of these hINO80-associated proteins are previously unrecognized orthologs of subunits of the yeast INO80 complex. PAPA-1 is orthologous to the les6 subunit of the yeast INO80 complex, and we henceforth designate it hles2. When hles2 is used as a query in a PSI-BLAST search, the PAPA-1 ortholog from *Arabidopsis* passes the threshold of 0.001 at the fourth iteration followed by vertebrate co-orthologs at iterations 7–10. We note that although hles2/PAPA-1 is annotated in the NCBI data base as high mobility group AT-hook 1-like 4, it does not appear to contain recognizable AT-hook or high mobility group DNA-binding domains. Instead, the N-terminal half of the protein consists of a predicted long helical region, whereas the C-terminal half is globular and contains several conserved cysteine residues (data not shown).

The human C18orf37 protein is orthologous to the les6 subunit of the yeast INO80 complex. Comparison of protein family alignments using the pairwise Hidden Markov Model-based algorithm, HHsearch (35), indicates that both proteins contain a modified zinc ribbon, most closely related to the YL1 family of putative transcriptional activators (36, 37). We previously identified the YL1 protein as a component of the mammalian TRRAP-TIP60 and SRCAP complexes (19).

The five remaining proteins appear to be unique to the human INO80 complex. NFRKB is a large (more than 1300 amino acids) protein. The C-terminal half of NFRKB contains low complexity, mucin-like repeats. The N-terminal half of this protein is broadly conserved in metazoans, in plants, and in *Giardia*, but it appears to have no homologs in fungi. MCRS1/MSP58 contains a forkhead-associated (FHA) domain in its N terminus. Although there are FHA-like domains in fungi, the N-terminal 350 amino acids of MCRS1 represent a distinct domain, well conserved in metazoans, plants, and
some protists, but not found in the available fungal genomes. The FLJ20309 protein has a characteristic pattern of conserved histidine and cysteine residues. Like the MCRS1 protein, FLJ20309 orthologs appear to be present in multicellular eukaryotes but not in fungi. Amida, or FLJ90652, contains a putative DNA-binding domain of the b-ZIP type. The FLJ90652 protein has an N-terminal domain that is distantly related to b-ZIP domains.

**Table One**

| Yeast INO80 | Human INO80 | Molecular function and domain structure |
|------------|-------------|----------------------------------------|
| Complex    | GI no."a"   | Complex                               | GI no."a"                      |
| Ino80      | 6321289     | KIAA1259                               | 33469139                       | SNF2-like helicase                        |
| Arp8       | 2492678     | Arp8                                   | 39812115                       | Actin-related protein                     |
| Arp5       | 1730738     | Arp5                                   | 31542680                       | Actin-related protein                     |
| Arp4       | 728794      | BAF53a/Arp4                            | 30089997                       | Actin-related protein                     |
| Rvb1       | 6320396     | TIP49a                                 | 4506753                        | AAA + ATPase                              |
| Rvb2       | 6325021     | TIP49b                                 | 5730023                        | AAA + ATPase                              |
| ies2       | 6324114     | PAPA-1                                 | 13775202                       | Helical, perhaps nonglobular, N-terminal half and mostly β-strand C-terminal half |
| ies4       | 6324763     | C18orf37                               | 34916002                       | Modified zinc ribbon, distantly related to YL-1 family of putative transcription factors |
| ies6       | 6320791     | Amida / TCF3 (E2A) fusion partner      | 7019371                        | b-ZIP-type, possibly DNA-binding, protein |
|            |             | FLJ90652                               | 2773427                        | Conserved in metazoa (not C. elegans)     |
|            |             | NFRKB                                  | 23346420                       | Conserved in metazoa (not C. elegans), plants, Giardia; nonglobular mucin-like C-half |
|            |             | MCRS1                                  | 2384717                        | FHA" domain in the C terminus             |
|            |             | FLJ20309                               | 38488718                       | Two copies of CxxHxxHhxHxxC signature; orthologs in plants and metazoan |
| Taf14      | 461510      |                                       |                                 | YEATS domain                              |
| Nhp10      | 6320202     |                                       |                                 | HMG" type II domain; binds kinked, looped, distorted, or four-way DNA |
| ies1       | 14318508    |                                       |                                 |                                             |
| ies3       | 6323081     |                                       |                                 |                                             |
| ies4       | 6324763     |                                       |                                 |                                             |
| ies5       | 6320938     |                                       |                                 |                                             |

*a* GI, GenInfo Identifier, a unique identifier for protein or nucleic acid sequences in the NCBI Entrez protein and nucleotide data bases.

*b* FHA, forkhead-associated.

*c* HMG, high mobility group.

The hINO80 Complex Catalyzes ATP-dependent Nucleosome Sliding—

Previous studies revealed that the S. cerevisiae INO80 complex possesses both DNA- and nucleosome-activated ATPase and ATP-dependent nucleosome sliding activities. To investigate the possibility that the mammalian INO80-related complex possesses similar activities, we assayed anti-FLAG agarose eluates from FLAG-hIes2-expressing HeLa cells for ATPase and nucleosome remodeling activities.

As shown in Fig. 3, the hINO80 complex, immunopurified from FLAG-hIes2-expressing cells, catalyzed ATP hydrolysis in a reaction that was strongly dependent on the addition of DNA or nucleosomes. Although both mononucleosomes and oligonucleosomes stimulated ATPase more strongly than did DNA, free histone octamers had no effect on the reaction.

To determine whether the hINO80 complex could catalyze ATP-dependent nucleosome mobilization, anti-FLAG agarose eluates from FLAG-hIes2 expressing HeLa cells were mixed with mononucleosomes reconstituted on a [32P]labeled, 216-base pair DNA fragment in the presence of ATP or a mixture of ATP and ATPyS, a potent inhibitor of many ATPases. At the conclusion of the reaction, HeLa oligonucleosomes and free DNA were added as competitor to remove DNA- or nucleosome-binding proteins that would alter mononucleosome electrophoretic mobility. Reaction products were then fractionated on native polyacrylamide gels. The electrophoretic mobility of mononucleosomes depends on the position of the nucleosome on the DNA. The mobility of a nucleosome positioned in the middle of the DNA fragment is slow, whereas nucleosomes positioned more laterally migrate more rapidly (38, 39). The reconstituted nucleosomes used in our experiments include a mixture of centrally located and lateral nucleosomes, with the majority of nucleosomes located at the DNA ends (Fig. 4A, lane 4, and B, lane 1). Upon the addition of increasing amounts of the purified hINO80 complex, nucleosomes were shifted to a more central position (Fig. 4A, lanes 5 and 6). Similar results were obtained when hINO80 complexes were immunopurified from cells expressing FLAG-Amida or FLAG-FLJ90652 (Fig. 4A, compare lane 11 with lanes 12 and 13). The change in nucleosome position catalyzed by the hINO80 complex depends upon ATP (Fig. 4B) and is inhibited by ATPγS. Thus, like the yeast INO80 complex, the hINO80 complex can support ATP-dependent nucleosome sliding. Notably, both the yeast and human INO80 complexes move nucleosomes from a lateral to a central position.

Summary and Perspective—In this report we have exploited a MudPIT-based subtractive proteomics approach to identify and characterize a novel mammalian ATP-dependent chromatin remodeling complex that shares structural and functional properties with the S. cerevisiae INO80 complex (TABLE ONE). This new mammalian INO80-related chromatin remodeling complex contains the INO80-like, SNF2 family helicase encoded by the previously uncharacterized KIAA1259 ORF, as well as several additional proteins that appear to be orthologs of subunits of the yeast INO80 complex; these include the Tip49a and Tip49b AAA" ATPases, the actin-related proteins Arp4, Arp5, and Arp8, hIes2 (PAPA-1), and hIes6 (C18orf37). Notably, the hINO80 complex contains five additional proteins that appear to lack yeast orthologs; these include the b-ZIP domain-containing Amida protein, the forkhead-associated domain-containing MCRS1 protein, the NFRKB protein, and...
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proteins encoded by the FLJ90652 and FLJ20309 ORFs. Finally, we show that, despite these apparent differences in the subunit compositions of the yeast and human INO80 complexes, these complexes possess similar chromatin remodeling activities. Future studies investigating the contributions of the individual subunits of the hINO80 complex should provide more in-depth insights into the functional similarities and differences between the yeast and mammalian chromatin remodeling complexes.

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