Largest subunits of the human SWI/SNF chromatin remodeling complex promote transcriptional activation by steroid hormone receptors

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Running title: Molecular analysis of the largest human SWI/SNF subunits
SUMMARY

The mammalian SWI/SNF-related complexes facilitate gene transcription by remodeling chromatin using the energy of ATP hydrolysis. The recruitment of these complexes to promoters remains poorly understood and may involve histone modifications or direct interactions with site-specific transcription factors or other cofactors. Here we report the isolation of two related but distinct cDNA clones, hOsa1 and hOsa2, that encode the largest subunits of human SWI/SNF. hOsa1 is identical to previously reported BAF250, and hOsa2 shares a high degree of sequence similarity with hOsa1. Mass spectrometric analysis and immunoblotting with antibodies specific to hOsa1 or hOsa2 demonstrate the presence of both proteins in SWI/SNF-A but not in the related PBAF complex purified from HeLa cells. Co-precipitation studies indicate that hOsa1 and hOsa2 associate with BRG1 and hBRM through the C-terminal domain of hOsa. We define multiple domains within hBRM and BRG1 that interact with the hOsa C-terminus. In cultured mammalian cells hOsa1 and hOsa2 stimulate transcription by the glucocorticoid, estrogen and androgen receptors. The GR-mediated activation is not observed with the C-terminal domain or with the hOsa2 polypeptide lacking the ARID DNA binding domain. These results suggest that hOsa1 and hOsa2 participate in promoting transcriptional activation by the steroid hormone receptors.
INTRODUCTION

Modulation of chromatin structure plays a fundamental role in gene expression because transcription factors must contend with nucleosomes, which are generally thought to inhibit transcription. Genetic and biochemical approaches have led to the discovery of multiple protein complexes that are thought to activate or repress transcription by targeting histones or nucleosomes (reviewed in (1-5)). A recent explosion of research in yeast, fruit flies, and mammals has uncovered at least two classes of complexes, those that enzymatically modify histones and those that remodel nucleosomes in an ATP-dependent manner. Cells contain multiple distinct chromatin remodeling complexes, each of which includes a central ATPase subunit. The yeast SWI/SNF complex was the first such complex characterized (reviewed in (6,7)). Homologous complexes have since been identified in Drosophila and mammals (8). At least two distinct ATPase subunits, termed hBRM and BRG1, have been identified in mammalian SWI/SNF-related complexes and are homologous to the yeast SWI2/SNF2 and Drosophila Brahma (Brm) proteins (9-12). Mammalian cells appear to contain multiple SWI/SNF-related complexes (13) and immunoprecipitation experiments using antibodies specific for hBRM or BRG1 suggest that these subunits are present in distinct complexes (14,15). Purification and characterization of these separate complexes demonstrate differences in activity and subunit composition, implying distinct regulatory roles (15,16). Indeed, recent in vitro transcription studies have shown that PBAF (SWI/SNF-B) is selectively required for activated transcription from target genes in the context of chromatin, whereas human SWI/SNF-A and ACF failed to activate transcription from the same templates (16). Consistent with the notion that each complex confers unique gene regulatory activity, targeted disruption of the Brg1 gene in the mouse resulted in homozygotes that died during the preimplantation stage, whereas
Brm mutant homozygotes were viable and fertile (17,18). Taken together, these results suggest that different chromatin remodeling complexes have distinct functions during mammalian development.

Many studies have focused on the mechanisms by which the SWI/SNF-related complexes alter nucleosomes, as well as how the complexes are targeted to specific genes for remodeling. Biochemical changes in the nucleosomes that occur during remodeling have provided mechanistic insights into the functions of SWI/SNF-related complexes (reviewed in (5,7)). Studies suggest that these complexes reposition nucleosomes by sliding of the DNA or triggering conformational changes in the nucleosomes to expose DNA. In certain promoter contexts, SWI/SNF may be recruited directly by gene-specific transcriptional regulators. This is supported by the findings that some activators interact functionally and physically with SWI/SNF complexes (19-23). In addition, recruitment of SWI/SNF to the HO endonuclease promoter in vivo requires binding of the site-specific SWI5 activator (24,25). At least two SWI/SNF subunits possess a DNA binding domain: the ARID domain (AT-rich interacting domain) present in yeast SWI1 and Drosophila Osa (26), and the HMG domain in BAF57, a subunit unique to metazoans (27). Whether these DNA binding domains play a direct role in chromatin remodeling or in the recruitment of SWI/SNF to specific genes remains to be established.

We previously reported that the protein product of the Drosophila gene osa (28) is a homologue of yeast SWI1 and component of the Drosophila Brahma (SWI/SNF) complex (29). Osa is required for proper photoreceptor differentiation and embryonic segmentation in Drosophila and it is thought to function as a transcription factor that antagonizes the Wingless signaling pathway (28). osa was previously identified in a screen for the trithorax (trx) group of genes required for the expression of Drosophila homeotic genes (30). Osa has been shown to
regulate the expression of the Antennapedia gene and to interact genetically with brahma (brm). Osa possesses an ARID DNA binding domain found in yeast SWI1 and shared by many other transcription factors (26,31,32). It is possible that Osa binds to AT-rich regions of DNA to help target the Brahma complex; however the ARID domain of Osa appears to bind DNA without sequence specificity (29). To identify the human homologues of Osa, we used defined probes from two related but distinct human EST sequences homologous to osa (28), and isolated cDNA clones encoding two human proteins we named human hOsa1 and hOsa2. We found that both hOsa proteins stimulate transcriptional activation by the steroid hormone receptors GR, ER and AR.
EXPERIMENTAL PROCEDURES

Cell lines and plasmids

HeLa cells were maintained in Dulbecco’s Modified Eagle Medium (Invitrogen) supplemented with 10% fetal bovine serum (HyClone), L-glutamine and penicillin/streptomycin and cultured in a humidified incubator with 5% CO₂. HeLa tet-off cells (Clontech) were grown in media containing 30ng/ml doxycycline. To induce the expression of tet-regulated constructs, doxycycline was removed from the media. T47D and C33A cells were maintained using the same conditions as described above.

The cytomegalovirus (CMV) enhancer/promoter constructs expressing rat GR, human ER and AR, as well as the luciferase reporter plasmids were gifts of M. Garabedian (NYU School of Medicine). Human BRG1 and hBRM constructs in a CMV expression vector were gifts of S. Goff (Columbia University). The plasmid encoding KIAA1235 (GenBank Accession # AB033061) was a gift of T. Nagase (Kazusa DNA Research Institute). hOsa derivatives were subcloned into a CMV-based mammalian expression construct pCGT that added a T7 epitope tag at the N-terminus ((33); a gift of A. Wilson, NYU School of Medicine), or subcloned into pcDNA (Invitrogen) that added an HA epitope tag at the N-terminus.

Isolation of cDNA clones encoding hOsa1 and hOsa2

The NTera2D1 line of human teratocarcinoma cDNA library (34) was screened with EST clones M85358 and AA243104 encoding a portion of hOsa1 and hOsa2, respectively. Fragments derived from the 5’ end of the initial cDNA isolate were used to extend the 5’ end of hOsa2. To isolate hOsa2 cDNA fragments contiguous with clone 18A and 16e, two sets of primers were used in PCR reactions using a cDNA library from human liver (Clontech). The
first reaction used primer 18A F1 (5’-GGCATGGGCTTGGGCAA-3’) and 16e R1 (5’-ATGCTGGAGATGAGG-3’) and the second reaction used primer 18A F2 (5’-GGCAAGGACAGATGAGG-3’) and 16e R2 (5’-GTTGCTCTGATCCCCTT-3’). The 5’ end primers were derived from 18A-specific sequence and the 3’ end primers were derived from regions specific to 16e. Fragments of expected size and sequence were obtained from these reactions, confirming the presence of hOsa2 cDNA containing the sequence of both 18A and 16e.

Northern blot analysis

Human multiple tissue and cancer cell line northern blots (Clontech) were probed with a 400 bp fragment from the 5’ end of hOsa1, and three separate fragments corresponding to 18A-specific region, a region overlapping between 18A and 16e, and 16e/4AT-specific region of hOsa2. 18A-specific P1 probe was created by PCR using primers 18A-5 (5’-CCGACATGGAGCAGCCG-3’) and 18A-3 (5’-TGTAGCCCGGATAATGG-3’). An Eco RI-Bgl II fragment of 16e was used as the P3 probe for the overlapping region, and an Eco RI-Bam HI fragment of 4AT was used as the P5 probe for 16e/4AT-specific region of hOsa2. Probes used for BRG1 and hBRM were 540 bp and 550 bp fragment, respectively, derived from the 5’ end of the coding sequence. The blot was stripped and exposed to film between hybridization with different probes.

Polyclonal antibody production

DNA fragments generated by PCR using primers that introduced a Bam HI site at the 5’ end and a stop codon followed by an Eco RI site at the 3’ end were subcloned into pGEX2T
(Amersham Pharmacia Biotech) and pRSET-A (Invitrogen), digested with Bam HI and Eco RI to create GST and polyhistidine-tagged fusions, respectively. hOsa1 DNA was amplified with 5’ primer (5’-GGATCCGTGAGGACGGAGCCTGGAA-3’) and 3’ primer (5’-GAATTCAATGTTTTGCTGGGCATTGGT-3’) where introduced restriction sites are underlined and stop codon indicated in bold type. To amplify the corresponding region of hOsa2 DNA, 5’ primer (5’-GGATCCAGGCATGAACCTTATGGGCA-3’) and 3’ primer (5’-GAATTCAAGATCCGTGTGTCTGGATCT-3’) were used. The GST fusion proteins expressed in E. coli were purified using glutathione sepharose 4B resin (Amersham Pharmacia Biotech) and injected into rabbits for polyclonal antibody production (Cocalico Biologicals, Inc.). The same DNA fragment of hOsa1 and hOsa2 subcloned into pRSET-A vector was used to produce polyhistidine- and T7-tagged fusion proteins in E. coli. The crude and purified antisera were tested for reactivity using the polyhistidine-tagged fusion protein. To eliminate the antibodies against GST and those that potentially cross-reacted with hOsa2, the antiserum raised against GST-hOsa1 was first incubated with GST immobilized on a resin followed by a resin cross-linked with GST-hOsa2. Similar treatments were carried out with the antiserum raised against hOsa2. In some cases, antibodies were affinity-purified using resins with the cross-linked antigen.

**Protein purification and mass spectrometry**

Purification of SWI/SNF and PBAF from HeLa nuclear extract was described (16) and mass spectrometry was performed as described (16).
Co-precipitation assay

C33A cells grown to 70-80% confluency in 100 mm plates were transfected with 10µg each of plasmid DNA by the calcium phosphate precipitation method. Mini-nuclear extracts were prepared as described (35) and immunoprecipitations were carried out with α-HA (BAbCO), α-T7 (Novagen), α-hBRM (Santa Cruz Biotechnology), or α-BRG1 (Santa Cruz Biotechnology) antibody. Immunoprecipitated products were separated by SDS-PAGE, subjected to immunoblotting with indicated antibody and detected by Enhanced Chemiluminescence (Amersham Pharmacia Biotech).

In vitro protein binding assay

GST fusion proteins were expressed in E. coli strain BL21LysS and purified from cell lysates using glutathione sepharose 4B (Amersham Pharmacia Biotech) in HEMG buffer (25mM HEPES-KOH (pH 7.9), 0.1mM EDTA, 12.5mM MgCl2, 20% glycerol) containing 0.1M KCl and 0.1% NP-40. Approximately 1 µg of purified recombinant GST protein was used in each binding reaction. In vitro translated 35S-methionine labeled proteins were synthesized using the TNT-coupled reticulocyte lysate system (Promega) as per manufacturer’s instructions. DNA fragments for in vitro translation were generated by PCR with a 5’ primer that included a T7 RNA polymerase promoter and rabbit β-globin translation initiation sequence as described (33). 7.5 µl of radiolabeled reticulocyte lysate was added to each binding reaction in 75 µl of HEMG buffer containing 0.1M KCl and 0.1% NP-40. Binding reactions were performed at 4°C for 2-3 hours with nutation. After five 1ml washes with the same buffer, 2X SDS-PAGE sample buffer was added and bound proteins were separated by SDS-PAGE and analyzed by autoradiography.
Transient transfection and luciferase assay

T47D cells seeded at a density of $7 \times 10^5$ cells per 35 mm plate were transfected the next day using Lipofectamine Plus reagent (Invitrogen). Typically, 0.1 µg of RSV-lacZ plasmid, 0.05 µg of luciferase reporter plasmid (XG₆TL (36), XETL (37), or MMTV-luciferase) were transfected with or without 0.05 µg each of plasmid expressing a steroid hormone receptor (rat GR, human ER, or human AR) and 0.05-1.5 µg of plasmid expressing hOsa1, hOsa1C, hOsa2, hOsa2C, hOsa2∆ARID, BRG1 or hBRM. Appropriate ligand at the indicated final concentration was added 24 hrs following transfection (100nM dexamethasone, 100nM 17β-estradiol, or 10nM R1881) and the cells were lysed after 20 hrs by the addition of lysis buffer (Promega). Luciferase assays were carried out as described using a Lumat LB 9509 luminometer (EG&G Berthold) (38). Data shown are from a representative experiment carried out a minimum of three times.
RESULTS

Isolation of cDNA clones encoding hOsa1 and hOsa2.

We isolated cDNA clones for hOsa1 and hOsa2 from the NTera2D1 human teratocarcinoma cDNA library (34) utilizing human EST clones with sequence similarity to Drosophila osa (28). Comparison of the published Drosophila Osa (28) and the predicted human hOsa1 and hOsa2 amino acid sequence revealed three highly conserved regions: the ARID DNA binding domain and Homology Regions I and II (Fig. 1A, B). There is 48% amino acid identity between Drosophila and human Osa in these regions, whereas sequences flanking the conserved regions are quite dissimilar. hOsa1 and hOsa2 proteins are approximately 60% identical. hOsa1 is nearly identical but 286 residues shorter than the recently reported BAF250 (39). This difference is reflected by immunoblot experiments that compare recombinant hOsa1 with the endogenous hOsa1 protein (Fig. 3B). Previously reported p270 is a partial clone of BAF250/hOsa1 (40,41) and a partial human cDNA sequence representing a spliced form of BAF250/hOsa1 has also been reported (42).

Our longest hOsa2 cDNA clone is a composite of clone 18A and 16e (Fig. 1B, C). It is 7.8kb in length, containing 6.5kb of coding and 1.3kb of 3’ untranslated sequence, and is consistent with mRNA size of ~8kb in the northern blot (Fig. 2). Importantly, recombinant hOsa2 protein expressed in HeLa cells is indistinguishable in size from the endogenous protein on an SDS-polyacrylamide gel, suggesting that it is nearly full-length (Fig. 3C).

Expression of hOsa1 and hOsa2 mRNA in human tissues and cell lines.

Northern blot analysis of hOsa1 and hOsa2 mRNA was carried out under stringent hybridization conditions using blots containing RNA isolated from multiple human tissues (Fig.
High levels of mRNA expression were observed in heart, skeletal muscle and kidney, especially for hOsa2. In cancer cell lines, high levels of expression of hOsa1 and hOsa2 mRNA were detected in lymphoblastic leukemia MOLT-4 and Burkitt’s lymphoma Raji cells. Immunoblotting of the Raji cell nuclear extract with hOsa-specific antibodies confirmed higher levels of hOsa proteins compared to the nuclear extracts from other cell lines (data not shown). We also compared the expression patterns of hOsa mRNA to those of hBRM and BRG1. In the tissue blot, the relative patterns of hBRM and BRG1 mRNA were similar to those of hOsa1 and hOsa2 (Fig. 2A). The expression patterns were also similar in the cancer cell line blot, although BRG1 mRNA was additionally detected in promyelocytic leukemia HL-60 and chronic myelogenous leukemia K-562 cells at levels similar to MOLT-4 and Raji cells (Fig. 2B)

Detection of endogenous and recombinant hOsa1 and hOsa2 proteins in HeLa cells.

Rabbit polyclonal antibodies were generated against hOsa1 or hOsa2 polypeptide (Fig. 3A) and used to detect endogenous and recombinant hOsa1 and hOsa2 proteins expressed in HeLa cells (Fig. 3B, C). The endogenous hOsa1 protein migrated slightly slower than the recombinant hOsa1 consistent with the notion that the recombinant hOsa1 lacks the N-terminal residues (Fig. 3B). The size of the endogenous hOsa2 was similar to that of the recombinant hOsa2 protein.

Immunoprecipitations carried out in HeLa nuclear extracts with hOsa-specific antisera demonstrated co-precipitation of BRG1 (Fig. 3D), hBRM (Fig. 3E) and other known subunits of human SWI/SNF such as BAF155 and BAF170 (data not shown). The results indicate that hOsa-specific antibodies are capable of immunoprecipitating the components of the endogenous
SWI/SNF. In addition, reciprocal immunoprecipitations performed with antibodies against BRG1 or BAF155 demonstrated co-precipitation of hOsa1 and hOsa2, confirming the association of hOsa proteins with the endogenous SWI/SNF components (data not shown).

**Identification of hOsa1 and hOsa2 as the largest subunits of purified SWI/SNF-A.**

To determine whether the gene products of hOsa1 and hOsa2 are associated with human SWI/SNF, we carried out mass spectrometric analysis of the two largest bands present specifically in SWI/SNF-A and not the related PBAF complex purified from HeLa nuclear extracts (16) (Fig. 4A). Analysis of the tryptic peptides obtained from the two bands identified peptides that matched with both hOsa1 and hOsa2. Although the upper band yielded more hOsa1 peptides and the lower band contained more peptides matching with hOsa2, it was evident that polypeptides corresponding to both hOsa1 and hOsa2 were represented in both bands. Immunoblotting of purified SWI/SNF-A with antibodies specific to hOsa1 and hOsa2 confirmed that both proteins are present in these two bands (Fig. 4B). As expected, purified PBAF contained BAF180, but not hOsa proteins. These findings indicate that hOsa1 and hOsa2 represent the largest subunits of the human SWI/SNF-A complex.

**hOsa proteins associate with hBRM and BRG1.**

Because hOsa1 and hOsa2 represent the largest subunits of human SWI/SNF-A, we investigated their ability to associate with hBRM or BRG1, essential ATPase subunits of SWI/SNF-related complexes. To test this, we transfected T7-tagged hOsa1 or hOsa2 with HA-tagged BRG1 or untagged hBRM into C33A cells, a cervical tumor cell line shown previously to lack hBRM and BRG1 (10,14). We transfected plasmids expressing T7-tagged hOsa1 and HA-
tagged BRG1 singly and together into C33A cells. Immunoprecipitation of transfected cell lysates with α-HA (Fig. 5A) or α-T7 (Fig. 5B) antibodies demonstrated co-precipitation of T7-hOsa1 and HA-BRG1. Similar experiments performed with T7-hOsa1 and hBRM showed that hOsa1 associated with hBRM (Fig. 5C, D). Co-transfection of plasmids expressing T7-hOsa2 (clone 4AT containing residues 916-2165) and HA-BRG1 or hBRM showed that hOsa2 associated with HA-BRG1 and hBRM (Fig. 6). Because the C-terminus of hOsa1 and hOsa2 shares sequence homology with Drosophila Osa (Osa Homology Domain, Fig. 1B), we transfected hOsa1C or hOsa2C singly or together with hBRM (Fig. 7A) or HA-BRG1 (Fig. 7B). Immunoprecipitations with antibodies against hBRM or BRG1 demonstrated that the C-terminal domain of hOsa1 and hOsa2 was sufficient to co-precipitate hBRM and BRG1.

We also examined in vitro translated 35S-methionine labeled polypeptide fragments of hBRM and BRG1 for binding to recombinant GST-hOsa1 and GST-hOsa2 C-terminal fusions purified from E. coli (Fig. 8). Similar amounts of labeled in vitro translated products were used in each binding reaction, examples of which are shown in Fig. 8A. We analyzed a collection of polypeptide fragments of hBRM and BRG1, and identified multiple regions involved in binding to the GST-hOsa C-terminus. hBRM polypeptides most efficiently retained by hOsaC contained residues 350-470 (+++ and residues 710-830 (++) (Fig. 8B). The region spanning residues 830-1306 (+, dashed line) was also found to contain at least three separate polypeptides that were weakly retained by GST-hOsaC. Remarkably, the pattern of binding was quite similar for the BRG1 polypeptides with residues 380-558 (+++ and residues 746-864 (++) demonstrating strong interactions, and the region spanning residues 864-1230 (+) showed weak binding mediated by at least three separate polypeptide fragments. The similar physical interactions observed between hOsa proteins and BRG1 or BRM correlate with the high degree of sequence
conservation between hBRM and BRG1. These data indicate that hBRM and BRG1 interact with the C-terminus of hOsa1 and hOsa2 through multiple domains that include two strong interaction domains within the N-terminal half of hBRM and BRG1.

**hOsa1 and hOsa2 stimulate GR-dependent transcriptional activation.**

Components of the yeast SWI/SNF complex are required for transcriptional activation by GR in yeast (43). Overexpression of hBRM, a human homologue of yeast SWI2/SNF2, increased activation of transcription by GR when cotransfected into C33A cells lacking endogenous hBRM (10). The N-terminal transactivation domain of GR was also reported to recruit SWI/SNF to activate transcription in yeast and mammalian cells (44). To determine whether hOsa1 and hOsa2 affect transcription by GR in a similar manner, we transfected hOsa1 and hOsa2 expression constructs (shown schematically in Fig. 9A), a GR-expressing plasmid, and a GR-responsive XG46TL reporter plasmid in the presence of dexamethasone (a GR ligand) into the breast cancer cell line T47D, previously found to lack hOsa1 ((39), data not shown). We found that both hOsa1 and hOsa2, but not their C-terminal domain enhanced GR-mediated activation by at least 4 fold (Fig. 9B, C and Fig. 10B). Immunoblotting of the transfected cell lysates indicated that hOsaC proteins were expressed at levels at least comparable to or greater than hOsa1 or hOsa2 (Fig. 9B and Fig. 10C). The hOsa proteins and their derivatives had little effect on basal transcription in the absence of the ligand, or on the expression of the cotransfected RSV-lacZ reporter gene (data not shown). As expected, control experiments with hBRM or BRG1 augmented transactivation by GR 4-6 fold (data not shown). Thus, the overexpression of hOsa1 and hOsa2 can increase transcriptional activation by GR.
The C-terminal domain of hOsa shares two highly conserved regions (HRI and HRII) with its *Drosophila* homologue (Fig. 1B, 9A). Genetic studies in *Drosophila* indicate that this domain functions in a dominant negative manner (45). The expression of hOsa2 C-terminal domain at high levels repressed GR-mediated transcription as compared to the stimulatory effect observed with the longer form of hOsa2 (Fig. 9D). Moreover, co-transfection of BRG1 and hOsa2 demonstrated an additive effect on the activation by GR; however, the C-terminus of hOsa2 repressed transactivation by GR with or without BRG1 (Fig. 9D). Similar results were obtained with the C-terminal domain of hOsa1 (data not shown). Protein binding assays indicated an association between hOsa1C, hOsa2C with BRG1 and hBRM (Fig. 7 and 8) and the overexpressed hOsaC proteins can associate with the endogenous SWI/SNF components (data not shown). Thus, it is possible that the conserved C-terminal domain binds to BRG1 or hBRM to form complexes incapable of increasing GR-mediated transcription. Such a model points to an important functional role for the N-terminus of the hOsa proteins. We also examined transcriptional effects of hOsa1 and hOsa2 on the thymidine kinase (*tk*)-luciferase reporter in T47D cells (Fig. 9E). The *tk*-luciferase reporter did not respond to the transfected hOsa, indicating that the stimulatory effects of hOsa are specific to activation by GR.

The transcriptional effects of hOsa1 and hOsa2 were further demonstrated by examining the activity of a GR-dependent XG46TL reporter plasmid in HeLa cells that stably expressed T7-epitope tagged hOsa1 or hOsa2(4AT) (data not shown). Consistent with the transient transfection results, we observed 3-4 fold enhancement of GR-mediated reporter activity in the cells stably expressing hOsa1 or hOsa2 compared to control HeLa cells (data not shown).

To determine whether the ARID domain is required to stimulate GR-mediated transcription in T47D cells, we examined a construct deleted for the ARID sequence
(hOsa2ΔARID, Fig. 10A). The ARID sequence in Osa and BAF250 proteins has been shown to interact with DNA albeit with no apparent sequence specificity (29,41). Surprisingly, hOsa2ΔARID failed to stimulate transcription by GR (Fig. 10B), although hOsa2ΔARID was expressed at least comparable to or greater than hOsa2 (Fig. 10C). Like hOsa2, hOsa2ΔARID was found to associate with the endogenous SWI/SNF subunits (data not shown). These results suggest that the ARID sequence may play an important role in the activity of hOsa2.

**hOsa1 and hOsa2 stimulate ER- and AR-mediated transcriptional activation.**

Mammalian SWI/SNF complexes were also implicated in mediating transcriptional activation by the estrogen receptor (ER), although its effect on androgen receptor (AR) has not been reported (12,46,47). We examined the effects of hOsa1 and hOsa2 on transcription by ER and AR in T47D cells and found that hOsa1 and hOsa2 stimulated ER- and AR-mediated transactivation (Fig. 11). hOsa2 increased the activation by ER by 6-fold compared to 2.5-fold increase by hOsa1 (Fig. 11A). Both hOsa proteins promoted activation by AR by 5-fold (Fig. 11B). Surprisingly, hBRM, but not BRG1, increased transcriptional activation by ER and AR, in contrast to the reported co-activator activity of BRG1 on ER transcription in SW13 cells (46). Differences between the previous study and what we observe may be attributed to different cell lines used because SW13 cells lack the BRG1 protein present in T47D cells. Indeed, similar analysis performed in C33A cells lacking BRG1 demonstrated co-activator activity by overexpressed BRG1 for transactivation by ER and AR (data not shown). Analysis of hOsa functions in T47D cells suggest that hOsa proteins or protein complexes may serve as transcriptional co-regulators that promote the activity of GR, ER and AR.
DISCUSSION

We have isolated cDNA clones encoding two related proteins, hOsa1 and hOsa2, corresponding to the largest subunits of human SWI/SNF. The SWI/SNF-A complex purified from HeLa cells contained proteins migrating at ~250kD and ~210kD, both of which reacted with antibodies generated against hOsa1 and hOsa2. Mass spectrometric analysis confirmed both proteins were present with a greater number of peptides unique to hOsa1 identified in the 250kD band, and a greater number of peptides unique to hOsa2 identified in the 210kD band. Studies have demonstrated that two related SNF-2 ATPase subunits BRG1 and hBRM of SWI/SNF are associated with distinct SWI/SNF complexes (14,15). Because no BRM-specific peptides were identified by mass spectrometry in highly purified SWI/SNF-A or PBAF complexes (16), it is possible that either hOsa1 or hOsa2, or both, are also present in other distinct SWI/SNF-related complexes. hOsa1 is likely identical to previously reported BAF250/p270/OSA1 (39,41,42) and studies have shown that BAF250 stimulates GR-mediated transcription in T47D cells potentially via an interaction of GR with the C-terminus of BAF250 (39). We found both hOsa proteins to stimulate transactivation by GR, and in vitro binding experiments indicate that GR can associate with the C-terminus of hOsa1 and hOsa2 (T.F. and N.T., unpublished data).

While this work was in progress, Hurlstone et al (48) and Kato et al (49) reported protein sequences of 1740 residues long (which was named hELD/OSA1) and 1486 residues long (named p250R), respectively, that are nearly identical to the sequence encoded by our hOsa2 clone 16e. Based on the mass spectrometry and immunoblotting experiments (Fig. 4), we think our longest hOsa2 cDNA(18A+16e) encoding 2165 residues represents the near full-length form corresponding to the ~250kD SWI/SNF-A subunit. In agreement with our studies, the C-
terminal domain of the clone reported by Hurlstone et al was shown to interact with BRG1 (48). Unfortunately, in their paper Hurlstone et al called their clone hELD/OSA1, when in fact the name OSA1 had already been given a year earlier by Kozmik et al to a partial human (and mouse) cDNA sequence representing a spliced form of BAF250/p270 (42). We decided to be consistent with the earlier work of Kozmik et al and chose the name hOsa1 to represent our clone that is most similar to BAF250/p270. Thus, hOsa2 was chosen as the name for our second related clone (which is similar to hELD/OSA1 reported by Hurlstone et al). Interestingly, the paper by Kato et al contained data suggesting that the two hOsa proteins may be present in separate SWI/SNF-related complexes (49).

The *Drosophila* Osa protein has been shown to be an integral component of the *Drosophila* SWI/SNF-related Brahma complex (29). Genetic analysis of the *osa* mutants has indicated that Osa can promote both the activation and repression of certain target genes (29). Recent studies suggest that Osa acts as a direct repressor of Wingless (Wg) target genes (45). In the wing imaginal disc, *nubbin*, which encodes a POU-domain protein normally activated in the wing pouch by Wg, is misexpressed in the notum in *osa* mutants and its expression can be blocked by overexpression of full-length Osa. *Distalless*, another gene activated by Wg, can also be repressed by Osa, and in this case it is shown that the repression occurs downstream of upregulation of the level of the coactivator Armadillo (fly homologue of mammalian β-catenin). Furthermore, loss of two other components of the Brahma complex, *brahma* or *moira*, also results in the ectopic expression of Wg target genes. Thus, the repressive function of *Drosophila* Osa seems to result from its association with the Brahma complex.

The C-terminal domain of hOsa shares significant sequence similarities with the C-terminus of the *Drosophila* Osa. Genetic studies suggest that this domain functions as a
dominant negative: ectopic expression of Osa C-terminus causes activation of a Wg target gene nubbin and destabilization of endogenous Osa. We have noticed that high levels of expression of hOsa C-terminus repress GR-mediated transcription in transfected cells, consistent with its potential role as a dominant negative. Surprisingly, the deletion of ARID in hOsa2 failed to support activation by GR. In contrast to ARID present in transcription factors such as Bright and Dri (31,32), the ARID of Drosophila Osa and human BAF250/p270 has been reported to bind to DNA in a sequence-independent manner. The function of ARID in the context of SWI/SNF is not known, but it has been speculated that it might play a role in the recruitment of SWI/SNF to the target gene promoter. It is possible that ARID might gain target specificity in the presence of other subunits of SWI/SNF or through interactions with other cofactors. Its interaction with DNA is also likely required for the biochemical process of chromatin remodeling. Our data points to a potentially important role for ARID in mediating transcriptional activation by hOsa2.

Recent biochemical studies found that the human PBAF (SWI/SNF-B) chromatin remodeling complex is specifically required for transcriptional activation of chromatin templates by nuclear hormone receptors, as well as transcription from chromatin templates directed by Sp1 and SREBP-1a in vitro (16). Surprisingly, although capable of remodeling these same chromatin templates, human SWI/SNF-A and Drosophila ACF failed to support activated transcription reconstituted with purified factors from the same templates. Immunoblotting experiments indicate that hOsa1 and hOsa2 are absent in purified PBAF. We speculate that SWI/SNF-A may activate transcription from a subset of gene promoters different from those dependent on PBAF, or that SWI/SNF-A may be involved in the transcriptional repression of specific genes. The further characterization of the biochemical properties of subunits unique to specific SWI/SNF-
related complexes should facilitate our understanding of the role of distinct chromatin remodeling complexes in the regulation of gene expression.
ACKNOWLEDGMENTS

We thank Bryan Lemon, Catharine Boothroyd and Dana Peck for expert technical assistance, Michael Garabedian, Gerald Crabtree, Stephen Goff, Takahiro Nagase and Angus Wilson for generously providing the reagents used in this study. We thank Jessica Treisman and Russell Collins for the initial collaborative studies, Bryan Lemon, Michael Garabedian and Angus Wilson for their valuable suggestions throughout the project and critical reading of the manuscript.

This work was supported in part by a grant from the American Cancer Society (RSG-01-248-01-CCE). The National Science Foundation is thanked for its support of the Computing Resources through grant BIR-9318128. N.T. was supported in part by The Irma T. Hirsch Trust.

The nucleotide sequences of hOsa1 and hOsa2 have been deposited in the GenBank database (accession nos. AF521670 and AF521671).
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FIGURE LEGENDS

Figure 1 Comparison of hOsa1 and hOsa2 cDNAs.

(A) An alignment of the amino acid sequences predicted from the cDNA clones isolated for hOsa1 (1999 residues) and hOsa2 (Clone 16e+18A=2165 residues). The BestFit program of the SeqWeb sequence analysis software and the Clustal W sequence analysis program of MacVector were used. Identical residues (vertical bars) and similar residues (represented by one or two dots) were determined by the criteria of Henikoff and Henikoff (50). For hOsa2, the additional 98 amino acids at the N-terminus predicted from the contiguous sequences deposited in GenBank database are indicated in italic (schematically shown in C). Within this N-terminal extension, three methionines are underlined: the first methionine is in-frame with an upstream stop codon; however, the DNA sequences surrounding the second and third methionine show better fit with the consensus translational start sequence (51). hOsa2 also contains a portion of the previously reported KIAA1235 sequence (AB033061) with the exception of 53 amino acids present in KIAA1235 but missing from hOsa2 (indicated by a triangle after lysine 961 of hOsa2). hOsa1 isolated in this study is nearly identical to previously reported p270 (AF265208) and BAF250 (AF231056). Although hOsa1 extends the predicted amino acid sequence reported for p270, it lacks the N-terminal 286 residues reported for BAF250. Within these N-terminal residues (thin underline in the figure) lie two blocks of sequence similarity with the predicted N-terminus of hOsa2. hOsa1 and hOsa2 sequences are divergent in the N-terminus; however, six linear blocks of sequence similarity are evident (see boxed residues and schematic in B). The entire cDNA sequence of hOsa1 differs in several places with that reported for BAF250, but agrees with overlapping p270 sequences. Nearly three-quarters of hOsa1 and hOsa2 show sequence similarity (boxed); present within these sequences are those that are homologous with
the *Drosophila* Osa protein (indicated by a thick underline). They correspond to ARID, Homology Region I and Homology Region II (shown schematically in B). Seven LXXLL motifs, many of which are conserved between the two hOsa isoforms are indicated by asterisk. Of these, motif 1, 2, 5 and 6 are also conserved between human and fly proteins. Ten proline-tyrosine dipeptide sequences were found within less than 200 residues of the glutamine-rich N-terminal region of hOsa1 (two were found in hOsa2, indicated in bold).

**(B)** A schematic comparison of hOsa1, hOsa2 and *Drosophila* Osa(Eld) (AF053091). ARID, Homology Regions I and II that are conserved among the three proteins are indicated. Additional sequences conserved between hOsa1 and hOsa2 corresponding to the boxed regions in A are shaded. Numerical ratios represent percent identity / percent similarity within defined DNA segments according to the sequence analysis software. The EST sequence used for the initial screening of the cDNA library is indicated by a thick bar (GenBank #M85358 for hOsa1 and #AA243104 for hOsa2). The N-terminal 98 residues predicted from contiguous sequences in the database are represented as a dotted box in hOsa2. The N-terminal 286 residues of BAF250 lacking in hOsa1 are shown as the dotted N-terminal extension of hOsa1. The sequence of hOsa1 and hOsa2 used as polypeptide antigens to raise hOsa-specific antibodies are indicated by a thick bar labeled Ab. Clone 16e and 4AT of hOsa2 encode the C-terminal 1747 residues (419-2165) and 1250 residues (916-2165), respectively.

**(C)** A schematic representation of the cDNA clones isolated for hOsa2. A probe derived from the most 5’ end of the longest hOsa2 cDNA clone 16e resulted in the isolation of clone 18A, which contained 534 identical nucleotides to the 5’ end of clone 16e, but differed in the 3’ end, suggesting that the two products could be produced by differential splicing. The C-terminus of clone 18A terminates after ten residues and thus represents a much smaller protein. Although
we were unable to isolate cDNA clones containing a sequence contiguous with clone 18A and clone 16e, we have successfully PCR amplified cDNA fragments that contained both sequences using the indicated primers P2 and P4. Because clone 18A shares sequence similarities with the hOsa1 N-terminus, we think clone 18A represents the N-terminus of hOsa2. Our α-hOsa2 antibody detects endogenous proteins of 250kD and 210kD (Fig. 4); the smaller protein may be encoded by clone 16e. Peptides corresponding to the N-terminal sequences in clone 18A were also identified by mass spectrometry of the 250kD band. The 5’ end of clone 18A is identical to the previously reported DAN15 cDNA (52), part of the genomic clone E7 associated with the expansion of CAG repeats (53). The positions of these sequences with respect to clone 18A are shown. The N-terminal 98 amino acid residues of hOsa2 in A were predicted from the translation of DAN15 cDNA and the open reading frame of Clone E7 (assuming the sequence lies within an exon contiguous with DAN15 cDNA).

**Figure 2** Northern blot analysis of hOsa1 and hOsa2 mRNA.

(A) Human multiple tissue RNA blot was probed with hOsa1, hOsa2 (P5 in Fig. 1C), hBRM, BRG1 and β-actin under stringent hybridization conditions. The blot was stripped between hybridizations with different probes. PBL, peripheral blood leukocyte.

(B) Human cancer cell line blot was probed with hOsa1, hOsa2 (P1 in Fig. 1C), hBRM, BRG1 and β-actin as above. hOsa2 probes P3 and P5 (see Fig. 1C) also demonstrated similar patterns of hybridization (data not shown). A potential spliced variant of hOsa2 mRNA (indicated by *) was most visible with probe P1.

**Figure 3** Endogenous and recombinant hOsa1 and hOsa2 proteins.
(A) The specificity of antibodies directed against hOsa1 and hOsa2. Polypeptide sequences chosen as antigens to raise antibodies to hOsa1 or hOsa2 were tagged with polyhistidine (His), expressed and purified from E. coli. Immunoblotting of His:hOsa1 and His:hOsa2 with α-His antibody (left panel), α-hOsa1 antibody (middle panel), and α-hOsa2 antibody (right panel) indicates that the antibody against each form of the hOsa protein is highly specific. The immunoblots shown here were prepared from separate gels; thus, the position of His:hOsa2 differs slightly between lanes 1 and 5. A similar analysis to demonstrate antibody specificity was carried out with recombinant hOsa proteins expressed in mammalian cells (data not shown).

(B) Nuclear extracts were prepared from HeLa tet-off cells (Clontech) mock-transfected (lane 1) or transfected with a plasmid expressing the longest cDNA clone encoding hOsa1 tagged with a FLAG tag (lane 2). The immunoblot was probed with α-hOsa1 antibody that had been pre-incubated with hOsa2 antigen polypeptide to deplete any antibody that reacted with hOsa2.

(C) Nuclear extracts were prepared from HeLa tet-off cells mock-transfected (lane 1) or transfected with a plasmid expressing FLAG-tagged hOsa2 cDNA clone 18A+16e (lane 2). The immunoblot was probed with hOsa2-specific antiserum pre-incubated with hOsa1 antigen polypeptide to deplete any antibody that reacted with hOsa1. The recombinant hOsa2(18A+16e) protein is similar in size to the endogenous hOsa2 present in HeLa cells in lane 1.

(D) Immunoprecipitation of endogenous BRG1 by α-hOsa1 and α-hOsa2 antibody. HeLa nuclear extract (input, lanes 1 and 4) was incubated with hOsa1-specific antiserum (lane 2), hOsa2-specific antiserum (lane 5), or corresponding preimmune serum (lanes 3 and 6) and immunoprecipitated products were separated by SDS-PAGE and detected by immunoblotting with α-BRG1 antibody.

(E) Immunoprecipitation of endogenous hBRM by α-hOsa1 and α-hOsa2 antibody.
Figure 4 Identification of hOsa1 and hOsa2 in SWI/SNF-A purified from HeLa nuclear extract.

(A) Mass spectrometric identification of polypeptides from silver-stained SDS 7% polyacrylamide gel of purified PBAF and SWI/SNF-A (16). Tryptic peptides obtained from the two largest protein bands present in SWI/SNF-A, but not in PBAF, were analyzed by mass spectrometry. Number of tryptic peptides representing unique or shared masses that matched hOsa1 or hOsa2 protein sequence is indicated.

(B) Immunoblotting of purified SWI/SNF-A confirms the presence of hOsa1 and hOsa2. 3 μg of phosphocellulose 0.5M fraction (P0.5M, lane 1), 0.3 μg of purified SWI/SNF-A (lane 2), 0.3 μg of purified PBAF (lane 3), and 3 μg of phosphocellulose 1M fraction (P1M, lane 4) were separated by SDS 7% polyacrylamide gel and probed with indicated antibodies. Consistent with the data in A, the top two bands in SWI/SNF-A reacted with both α-Osa1 and α-Osa2 antibody.

Figure 5 T7-tagged hOsa1 co-precipitates with BRG1 and hBRM.

(A) T7-hOsa1 and HA-BRG1 were expressed singly or together in C33A cells. Proteins were immunoprecipitated (IP) from the nuclear extract with α-HA and probed with α-T7 (upper panel) or α-BRG1 (lower panel) antibody.

(B) The reciprocal experiment of A. Proteins were immunoprecipitated (IP) with α-T7 and probed with α-BRG1 (upper panel) or α-T7 (lower panel) antibody.

(C) T7-hOsa1 and hBRM were expressed singly or together in C33A cells. Proteins were immunoprecipitated (IP) from the nuclear extract with α-hBRM and probed with α-T7 (upper panel) or α-hBRM (lower panel) antibody.
(D) The reciprocal experiment of C. Proteins were immunoprecipitated (IP) with α-T7 and probed with α-hBRM (upper panel) or α-T7 (lower panel) antibody.

**Figure 6** T7-tagged hOsa2 co-precipitates with BRG1 and hBRM.

(A) T7-hOsa2 (clone 4AT containing residues 916-2165) and HA-BRG1 were expressed singly or together in C33A cells. Proteins were immunoprecipitated (IP) from the nuclear extract with α-HA and probed with α-T7 (upper panel) or α-BRG1 (lower panel) antibody.

(B) The reciprocal experiment of A. Proteins were immunoprecipitated (IP) with α-T7 and probed with α-BRG1 (upper panel) or α-T7 (lower panel) antibody.

(C) T7-hOsa2(4AT) and hBRM were expressed singly or together in C33A cells. Proteins were immunoprecipitated (IP) from the nuclear extract with α-hBRM and probed with α-T7 (upper panel) or α-hBRM (lower panel) antibody.

(D) The reciprocal experiment of C. Proteins were immunoprecipitated (IP) with α-T7 and probed with α-hBRM (upper panel) or α-T7 (lower panel) antibody.

**Figure 7** The C-terminal domain of hOsa1 and hOsa2 is sufficient for co-precipitation of hBRM and BRG1.

(A) hBRM and HA-hOsa1C (residues 1349-1999) or HA-hOsa2C (residues 1518-2165) were expressed singly or together in C33A cells. Proteins were immunoprecipitated (IP) from the nuclear extract with α-hBRM and probed with α-HA antibody. The same immunoblot probed with α-hBRM confirms the presence of hBRM (data not shown).

(B) Similar to A except HA-BRG1 was cotransfected instead of hBRM and proteins were immunoprecipitated using α-BRG1 antibody.
**Figure 8** hBRM and BRG1 bind to the C-terminal domain of hOsa1 and hOsa2 *in vitro*.

(A) The C-terminus of hOsa1 (residues 1373-1999) and hOsa2 (residues 1542-2165) were expressed in *E. coli* as fusions to the glutathione-S-transferase (GST) protein and purified using glutathione sepharose beads. *In vitro* binding reactions were carried out with purified GST protein and *in vitro* translated, $^{35}$S-methionine labeled fragments of hBRM (residues indicated in parentheses; the numbering is according to the sequence in the database under GI:414117).

(B) Summary of hBRM and BRG1 truncations examined for *in vitro* binding to the C-terminal domain of hOsa1 (1373-1999) and hOsa2 (residues 1542-2165). Comparable amounts of *in vitro* translated products were used in each binding reaction. hBRM or BRG1 polypeptides that demonstrated significant binding (≥20%, +++), moderate binding (~10%, ++) weak binding (<5%, +) are shown. Polypeptide fragments that showed no binding are indicated in gray (-). Positions of minimal binding regions are summarized above the full-length representation of hBRM and BRG1. In many cases, binding of hBRM or BRG1 was additionally examined with GST fusions to the C-terminal half-domains of hOsa1 (residues 1373-1652 and 1653-1999) and hOsa2 (residues 1542-1818 and 1819-2165) with similar results to the full-length C-terminus. By contrast, hOsa1 subdomain containing residues 1443-1663 and hOsa2 subdomains containing residues 1314-1627 or 1612-1829 bound poorly to hBRM and BRG1, or did not bind at all (data not shown). Conserved motifs in hBRM and BRG1 identified through Conserved Domain Database Search (NCBI) are as follows: TCH, domain in transcription and CHROMO domain helicases; DEXDc, DEAD-like helicases superfamily; HELICc, helicase superfamily C-terminal domain; BROMO, bromo domain. The residue numbers of hBRM and BRG1 are according to the sequence in the GenBank database under GI:414117 and GI:902046, respectively.
**Figure 9** Expression of hOsa1 and hOsa2 in breast cancer cell line T47D augments GR-mediated activation of the reporter gene.

(A) A schematic representation of the hOsa constructs used in the study. The C-terminal constructs contained the conserved Osa Homology Domain (Fig. 1B).

(B) Construct expressing T7-hOsa1 (1.5, 1, 0.5 µg) or T7-hOsa1C (0.5, 0.05 µg) was transiently transfected with Lipofectamine Plus into T47D cells together with a GR-responsive XG₄₆ TL luciferase reporter plasmid and a rat GR expression plasmid in the presence of 100nM dexamethasone. Immunoblotting of transfected cell lysates with α-T7 antibody demonstrated the expression of T7-hOsa1 (lane 2, 1.5 µg) and T7-hOsa1C (lane 3, 0.5 µg and lane 4, 0.05 µg).

(C) Similar to B except hOsa2(16e)- or hOsa2C-expressing construct was transfected. hOsa1 and hOsa2, but not their C-terminal derivatives, enhanced GR activation by at least 4 fold in several independent experiments. Expression of the hOsa proteins did not affect basal transcription in the absence of the ligand or the cotransfected RSV-lacZ reporter activity (data not shown).

(D) Expression of hOsa2 and hOsa2C in T47D cells show opposite effects on GR-, BRG1-mediated activation of the reporter. Indicated plasmids expressing BRG1 (0.5 µg), hOsa2(16e) or hOsa2C (1 µg) were transiently transfected using Lipofectamine Plus into T47D cells together with a GR-responsive XG₄₆ TL luciferase reporter plasmid and a rat GR expression plasmid in the presence of 100nM dexamethasone.

(E) hOsa1 and hOsa2 do not affect transcription from the thymidine kinase (tk)-luciferase reporter. T47D cells were transiently transfected as in B with tk-luciferase reporter (0.5 µg) and indicated expression plasmids (1.5 µg). Reporter constructs such as tk-luciferase and others
(data not shown) did not respond to the transfected hOsa protein, indicating that the stimulatory effects of hOsa are specific to transcription by GR.

**Figure 10** The ARID DNA binding domain of hOsa2 is required to promote GR-mediated activation of transcription in T47D cells.

(A) A schematic representation of the hOsa2 derivatives used in the experiment.

(B) T47D cells were transiently transfected with a GR-responsive XG₄₆TL luciferase reporter plasmid, a rat GR expression plasmid, and indicated T7-tagged hOsa2 derivative (hOsa2(16e): 1.5, 1, 0.5 µg; hOsa2C: 0.1, 0.05, 0.03 µg; hOsa2ΔARID: 1.5, 1, 0.5 µg). hOsa2(16e) enhanced activation by GR, but not hOsa2C or hOsa2 lacking ARID (hOsa2ΔARID).

(C) The expression of T7-hOsa2 derivatives was confirmed by immunoblotting of the cell lysates examined in B with α-T7 antibody.

**Figure 11** hOsa1 and hOsa2 stimulate ER- and AR-mediated transcription in T47D cells.

(A) Transfection assays similar to those described in Fig. 9 were carried out with an ER-expressing plasmid and an ER-responsive XETL luciferase reporter plasmid in the presence of 17β-estradiol.

(B) Transfection assays were carried out with an AR-expressing plasmid and an AR-responsive MMTV-luciferase reporter plasmid in the presence of R1881.
B.

hOsa2

hOsa1

Osa (Drosophila)

ARID
Homology Region (HR) I
Homology Region (HR) II
Osa Homology Domain

C. hOsa2 Clones

Clone 16e

Clone 18A

DAN15
Clone E7 ORF

FIGURE 1
**FIGURE 2**

A. DNA blot analyses of normal and tumor tissues. (A) shows the expression of hOsa1, hOsa2, hBRM, and BRG1 in various tissues. The DNA sizes are indicated in kilobases (kb). The tissues analyzed include brain, heart, skeletal muscle, colon, thymus, spleen, kidney, small intestine, placenta, lung, and PBL.

B. Expression of hOsa1, hOsa2, hBRM, and BRG1 in different cancer cell lines. The cell lines include promyelocytic leukemia, HeLa S3, chronic myelogenous leukemia, Burkitt's lymphoma, colorectal adenocarcinoma, lung carcinoma, and melanoma. The expression levels are indicated in the same order as in panel A.
FIGURE 3

A. 

B. 

C. 

D. 

E.
FIGURE 4
FIGURE 5

A. (α-T7 Immunoblot)  
B. (α-BRG1 Immunoblot)  
C. (α-T7 Immunoblot)  
D. (α-hBRM Immunoblot)
FIGURE 6

A. 

B. 

C. 

D.
FIGURE 7

A. α-HA Immunoblot

B. α-HA Immunoblot
### FIGURE 8

**A.**

[Image of a gel electrophoresis result showing bands at kDa values of 50, 37, and 25. Arrows indicate bands for hBRM(530-830) and hBRM(830-1070).]

| Lane | Description |
|------|-------------|
| 1    | Input (20%) |
| 2    | GST-hOsa2C  |
| 3    | GST-hOsa1C  |
| 4    | GST         |
| 5    | Input (20%) |
| 6    | GST-hOsa2C  |
| 7    | GST-hOsa1C  |
| 8    | GST         |

**B.**

[Diagram showing the structure of hBRM and BRG1 with annotations for various domains and regions.]

| Region            | hBRM        | BRG1        |
|-------------------|-------------|-------------|
| 1-540             | +++         | +           |
| 1-350             | -           | ++          |
| 350-540           | +++         | +           |
| 350-470           | +++         | +           |
| 470-710           | -           | +           |
| 530-1070          | ++          | +           |
| 530-830           | ++          | +           |
| 530-710           |            | +           |
| 710-830           | ++          | -           |
| 830-1070          | +           | ++          |
| 830-950           | +           | ++          |
| 950-1070          | +           | ++          |
| 1060-1586         | +           | ++          |
| 1060-1306         | +           | ++          |
| 1306-1586         | -           | ++          |
| 1232-1306         | -           | ++          |

**Input (20%)**

- GST-hOsa2C
- GST-hOsa1C
- GST

---

**hBRM(530-830)**

**hBRM(830-1070)**

---

**BRG1**

**Input (20%)**

- GST-hOsa2C
- GST-hOsa1C
- GST

---

**TCH**

**DXDC**

**HELIC**

**Bromo**
**FIGURE 9**

A. 

hOsa1  
(1)  
(1999)  

hOsa1C  
(1349)  
(1999)  

hOsa2(16e)  
(419)  
(2165)  

hOsa2C  
(1518)  
(2165)  

B. 

Luciferase units (x10^4)  

GR: -+++++  
hOsa1:  
hOsa1C:  

C. 

Luciferase units (x10^4)  

GR: -+++++  
hOsa2(16e):  
hOsa2C:  

D. 

Luciferase units (x10^4)  

GR: -+++++  
BRG1:  
hOsa2(16e):  
hOsa2C:  

E. 

Luciferase units (x10^3)  

GR: -+++++  
+  
+  
+  
+  
+  

**FIGURE 9**
FIGURE 10

A. 

hOsa2(16e)  

hOsa2C  

hOsa2 ΔARID

B. 

Luciferase units (x10^5)

hOsa2(16e)  
hOsa2C  
hOsa2ΔARID

C. 

α-T7 Immunoblot

GR: - + + + + + + + + + + 

kD 200 124 80

hOsa2(16e)  hOsa2ΔARID  hOsa2C
Figure 11: Luciferase units (x10^4) for A. ER and B. AR treatments with various combinations of proteins.
Largest subunits of the human SWI/SNF chromatin remodeling complex promote transcriptional activation by steroid hormone receptors
Hiroko Inoue, Takako Furukawa, Stavros Giannakopoulos, Sharleen Zhou, David S. King and Naoko Tanese

J. Biol. Chem. published online August 27, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M205961200

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