Cloning and characterization of hELD/OSA1, a novel BRG1 interacting protein

Adam F. L. HURLSTONE*, Ivan A. OLAVE†, Nick BARKER*‡, Mascha VAN NOORT* and Hans CLEVERS*¹

*University Medical Centre Utrecht, Department of Immunology, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands, †Departments of Developmental Biology and Pathology, Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305, U.S.A., and ‡Semaia Pharmaceuticals BV, Buntlaan 44, 3971 JD Driebergen, The Netherlands

A highly conserved multisubunit enzymic complex, SWI/SNF, participates in the regulation of eukaryote gene expression through its ability to remodel chromatin. While a single component of SWI/SNF, Swi2 or a related protein, can perform this function *in vitro*, the other components appear to modulate the activity and specificity of the complex *in vivo*. Here we describe the cloning of hELD/OSA1, a 189 KDa human homologue of *Drosophila* Eld/Osa protein, a constituent of *Drosophila* SWI/SNF. By comparing conserved peptide sequences in Eld/Osa homologues we define three domains common to all family members. A putative DNA binding domain, or ARID (AT-rich

DNA-interacting domain), may function in targetting SWI/SNF to chromatin. Two other domains unique to Eld/Osa proteins, EHD1 and EHD2, map to the C-terminus. We show that EHD2 mediates binding to Brahma-related gene 1 (BRG1), a human homologue of yeast Swi2. EHD1 and EHD2 also appear capable of interacting with each other. Using an antibody raised against EHD2 of hELD/OSA1, we detected Eld/Osa1 in endogenous SWI/SNF complexes derived from mouse brain.

Key words: ELD/OSA, SWI1, chromatin, SWI/SNF, BRG1.

INTRODUCTION

In eukaryote cells, DNA is associated with histones and non-histone proteins to form chromatin, the basic building block of which is the nucleosome. Besides facilitating DNA compaction, chromatin also exerts an overall repressive effect on transcription by excluding the basal transcription machinery [1–3] and transcription factors [4,5] from their binding sites in promoter and enhancer regions. Chromatin encompassing the regulatory regions of inactive genes often comprises an ordered array of nucleosomes. Transcriptional activation is accompanied by localized disruption of nucleosomes concomitant with increased accessibility to the unmasked DNA [6–9]. The binding of transcription factors to target gene promoters, when it can occur unaided, is generally insufficient to induce such nucleosome disruption [10–13]. Additional factors are required to achieve effective remodelling of chromatin as a prerequisite to gene activation.

Two broad classes of multisubunit enzymic complexes exist in eukaryote cells that function synergistically to remodel chromatin. One class of complex comprises the nuclear histone acetyltransferases (HATs) (for reviews see [14,15]). The second class of complex is distinguished by inclusion of a member of the Swi2/Snf2 subfamily of DNA-stimulated ATPases, of which yeast Swi2/Snf2 is the prototype. Indeed, the yeast SWI/SNF complex, a 2 MDa complex consisting of at least ten distinct peptides besides Swi2/Snf2 [16,17], is a paradigm for this second class of chromatin remodelling complex. Yeast strains deficient in essential SWI/SNF subunits demonstrate altered chromatin structure at target gene promoters [18–20]. Purified SWI/SNF facilitates the binding of transcription factors to chromatin [21–23]. The activation of various reporter gene constructs by yeast, *Drosophila*, and mammalian transcription factors is compromised in SWI/SNF-deficient yeast strains [24,25], an in-

dication that SWI/SNF-like complexes are entailed in transcription in other eukaryote species. SWI/SNF proteins are indeed evolutionally conserved [26–31], although complexes derived from higher eukaryotes contain subunits additional to those found in yeast [28,30,32–34]. Moreover, SWI/SNF complexes purified from mammalian cells display markedly heterogeneous subunit composition [35,36]. In humans, two Swi2/Snf2 homologues have so far been isolated, BRAHMA (BRM) and Brahma-related gene 1 (BRG1), that exist within mutually exclusive SWI/SNF complexes [36].

SWI/SNF binds DNA directly but does not exhibit significant sequence specificity *in vitro* [37]. Mutation of *swi2* however affects less than 6% of all yeast genes [38,39]. Thus SWI/SNF is probably targetted to a subset of genes *in vivo* via interaction with sequence-specific transcription factors. Indeed, a number of transcription factors have now been identified that specifically bind SWI/SNF, and the interaction appears critical for optimal target gene activation. These factors include yeast Gal4 [21,40,41], Swi5 [40,42] and Gcn4 [40,43]; *Drosophila* Zeste [44] and Trithorax (Trx) [45]; mammalian glucocorticoid receptor [25,46], C/EBP β [47], c-Myc [48], erythroid Krüppel-like factor (EKLf) [49], ALL-1 [45], and p53 [34]. In general, this recruitment appears to be mediated by acidic activation domains within the transcription factors [40,41]. The Snf5 subunit of SWI/SNF mediates binding of the SWI/SNF complex to Trx/ALL-1 and c-Myc transcription factors, while the Zeste transcription factor contacts Moira and Eld/Osa within *Drosophila* SWI/SNF [44,45,48], implying that multiple surfaces are likely to exist within the SWI/SNF complex for interaction with transcription factors.

While earlier studies delineated a role for SWI/SNF in transcriptional activation, affected genes were mainly derepressed in *swi2* mutant yeast [38,39], implying a more substantial

Abbreviations used: ARID, AT-rich DNA-interacting domain; BRM, BRAHMA; BRG1, Brahma-related gene 1; BAF, BRG1/BRM-associated factor; EHD, Eld/Osa homology domains; EST, expressed sequence tag; HA, haemagglutinin; hELD/OSA1, human homologue of *Drosophila* Eld/Osa protein; HATs, nuclear histone acetyltransferases; ORF, open reading frame; PQRs, proline and glutamine rich regions; 5'-RACE, 5'-rapid amplification of cDNA ends.

¹ To whom correspondence should be addressed (e-mail H.Clevers@lab.azu.nl).

requirement for SWI/SNF in transcriptional repression. In human cells, SWI/SNF has been demonstrated to repress endogenous *c-fos* expression dependent on co-expression of retinoblastoma protein [50], a molecule known to interact with BRG1 and BRM [51–53]. Intriguingly, this complex can also contain histone deacetylase, a protein also believed to regulate chromatin structure [54], but whose effects are opposite to those of HATs with which SWI/SNF co-operates to achieve optimal transcriptional activation [55–57].

Several SWI/SNF subunits possess DNA-binding domains. Swi2/Snf2 homologues themselves can bind chromatin through HMG (high-mobility-group) I/Y-like AT hooks [58]. In higher eukaryotes, BRG1/BRM-associated factor (BAF)57 homologues (HMG-box-like factors) bind DNA adopting a four-way junction conformation which mimics the topology of DNA as it enters or emerges from a nucleosome [32]. AT-rich-DNA-interacting-domain (ARID)-bearing proteins present within SWI/SNF complexes, including SWI1 in yeast [24], *Drosophila* Eld/Osa [59–61], and human p270/BAF250 [62–64], also mediate DNA-binding. While *in vitro* *Drosophila* Eld/Osa protein displays no DNA-binding sequence specificity beyond an apparent preference for AT-rich regions, recessive inactivation of the *eld/osa* gene revealed that *in vivo* the protein influences gene expression selectively, promoting the expression of certain genes while repressing others [59–61,65]. Thus, Eld/Osa proteins may participate in the targetting of SWI/SNF to a subset of promoters *in vivo*, and may then influence whether SWI/SNF chromatin remodelling activity results in the induction or repression of target gene expression. We have cloned and partially characterized a novel human Eld/Osa homologue, hELD/OSA1. We demonstrate that a unique domain in the C-terminus of hELD/OSA1 mediates binding to BRG1, and that Eld/Osa1 is a constitutive component of endogenous SWI/SNF.

MATERIALS AND METHODS

cDNA isolation of human ELD1/OSA1

Gene-specific oligodeoxynucleotide primers (forward: CTTAC-ACGGAAGCATCTGC; reverse: CACAGATGACAGATG-CAACC), derived from overlapping human expressed sequence tag (EST) sequences with homology to *Drosophila* *eld/osa* (Genbank/EMBL accession number AF053091), were used to screen a human fetal brain cDNA library (Rapid-Screen cDNA library panel; Origene Technologies, Rockville, MD, U.S.A.). Automated cycle sequencing (ABI PRISM DNA sequencing kit; PE Applied Biosystems, Boston, MA, U.S.A.), using vector and gene-specific primers, of a positive plasmid revealed an approximately 3 kb cDNA insert. Three rounds of 5'-rapid amplification of cDNA ends (5'-RACE) were used to extend this 3 kb cDNA. PolyA RNA (PolyAtract mRNA isolation system; Promega, Madison, WI, U.S.A.) isolated from Ug37 leukaemia cell line was used as a template for synthesis of first-strand cDNA by AMV reverse transcriptase (Roche, Mannheim, Germany) and thermostable *rTth* reverse transcriptase (Perkin Elmer Cetus, Boston, MA, U.S.A.). A polyA tail was subsequently added using terminal deoxynucleotide transferase (Roche), and 5'-RACE products obtained by PCR using *Taq* precision plus polymerase mixture (Stratagene, Amsterdam, The Netherlands). 5'-RACE products were cloned without further modification into pGEM-T vector (Promega) and sequenced as above. 5'-RACE products were assembled as a single insert in pBluescript II SK (+/–) (Stratagene) by successive rounds of restriction digestion and ligation. Finally a 5482 bp cDNA comprising a 5220 bp open reading frame (ORF) was

obtained which was sequenced in entirety on sense and antisense strands as above.

Mammalian expression constructs

hELD1/OSA1 mammalian expression constructs were generated in pGLOFLAG [derived from pcDNA3.1 (Invitrogen, Leek, The Netherlands) containing bovine globin untranslated regions separated by a FLAG epitope MDYKDDDDKKDP proximal to a multiple cloning site]. Full-length hELD1/OSA1 was cloned in frame with the FLAG-epitope tag. For FLAG-ΔARID, nucleotides between 1535 and 2131 of the human ELD1/OSA1 cDNA, which encode the ARID, were deleted by restriction digestion with *NcoI*. Re-ligation resulted in an in-frame deletion of amino acids 494–693. For FLAG-ΔEHD1 (where EHD stands for Eld/Osa homology domains), nucleotides between 2972 and 4160 encoding the EHD1 were deleted by restriction digestion with *SfiI* and *ClaI* followed by blunting with T4 DNA polymerase. Re-ligation resulted in an in-frame deletion of amino acids 973–1368. For FLAG-ΔEHD2, nucleotides 3623 onwards encoding EHD2 were deleted by digestion with *EcoRI*. Resulting in truncation of the C-terminus of hELD1/OSA1 protein from amino acid 1188. For FLAG-ARID and FLAG-EHD2, cDNA corresponding to amino acids 546–723 and 1197–1740 respectively was amplified by PCR using *Taq* precision plus polymerase mixture and cloned in frame with the FLAG-epitope tag.

Construction of plasmids expressing human BRG1 (pCMV-BRG1) or an ATPase-defective variant of BRG1 (K798R) (pCMV-K798R) fused at their C-termini to an epitope tag (YPYDVPDYA) derived from haemagglutinin protein (HA) was described previously [50]. These two plasmids were a gift from Daniel A. Engel (Department of Microbiology and Cancer Center, University of Virginia School of Medicine, Charlottesville, VA 22908, U.S.A.).

Mouse anti-hEHD2 monoclonal antibody production

A 1 kb PCR product derived from hELD1/OSA1 cDNA encoding the last 330 amino acids of hELD1/OSA1 protein (EHD2) was cloned into pET21, a prokaryote expression vector (Novagen, Madison, WI, USA), and used to generate His-tagged recombinant protein from BL21 *Escherichia coli*. BALB/c mice were immunized with purified EHD2 peptide and hybridomas generated as described previously [67]. Monoclonal antibodies from subclones 5A5, 6H3, and 8B8 were affinity purified from 1 litre of supernatant by binding to HighTrap Protein A-Sepharose HP (Amersham Biosciences, Roosendaal, The Netherlands).

Yeast two-hybrid library screen

Yeast two-hybrid library screens were performed essentially according to the MATCHMAKER two-hybrid system protocol manual (Clontech Laboratories, Palo Alto, CA, U.S.A.). Baits were constructed in yeast expression vector pMD4 (a gift from Dr L. van 't Veer, Division of Diagnostic Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands) encoding the ARID (amino acids 546–723), EHD1 (amino acids 1095–1188), and EHD2 (amino acids 1410–1740) of hELD1/OSA1 protein. *Saccharomyces cerevisiae* HF7c cells transformed with bait constructs were subsequently re-transformed with a human fetal brain MATCHMAKER library (Clontech Laboratories). Library plasmid DNA was isolated from individual HF7c double transformants growing on synthetic nutrient agar LTH plates lacking leucine, tryptophan and histidine,

supplemented with 25 mM 3-amino-1,2,4-triazole (3-AT) and positive for β -galactosidase activity. Library plasmid inserts were sequenced as above using vector-derived primers. Interactions in yeast cells were confirmed by re-transforming single-transformant pMD4-bait yeast strains with isolated library plasmid DNA and assaying double-transformants for their ability to grow on LTH 3-AT plates.

To study the cellular distribution of FLAG-epitope-tagged hELD1/OSA1 proteins, 2 to 5×10^4 COS7 cells in the wells of a 48-well cell culture plate were transfected with $0.5 \mu\text{g}$ of various expression constructs using FuGENE 6 reagent (Roche) according to the manufacturer's recommendations. Cells were washed in PBS 16 – 24 h after transfection, and fixed at -20°C in methanol. Transfected cells were first incubated with hybridoma supernatant or with anti-FLAG-epitope mouse monoclonal antibody (Sigma, #A1205), followed by a horseradish-peroxidase-conjugated rabbit anti-mouse polyclonal antibody (DAKO, Glostrup, Denmark; A/S #P0161). Positive staining was revealed by incubation with 0.2% (w/v) amino ethyl carbonate/ 0.1% (v/v) hydrogen peroxide, in 0.1 M sodium acetate, pH 4.8 , before microscopic examination.

293T cells (5×10^5) were transfected with $0.5 \mu\text{g}$ each of FLAG-epitope-tagged h*ELD1/OSA1* mammalian expression constructs, and/or HA-epitope-tagged h*BRL1* expression constructs, and/or empty vector (pcDNA3.1), where appropriate, using FuGENE 6 reagent. Cells were harvested 16–24 h later and whole cell lysates were prepared in Triton X-100 lysis buffer (20 mM Tris/HCl, pH 8.0, 1% (v/v) Triton X-100, 140 mM NaCl, and 10% (v/v) glycerol) containing protease-inhibitor-cocktail tablets (Roche). Protein extracts were incubated with $1 \mu\text{g}$ anti-FLAG-epitope mouse monoclonal antibody (Sigma, # A1205) and $10 \mu\text{l}$ Protein A-Sepharose (Sigma, # P-3391) for 3 h at 4°C . Beads were then washed three times in wash buffer (20 mM Tris/HCl, pH 8.0, 140 mM NaCl, and 0.5% (v/v) Nonidet P40). Immunoprecipitated proteins were eluted in $1 \times$ sample buffer (50 mM Tris/HCl, pH 6.8, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 0.1% (w/v) Bromophenol Blue, and 10% (v/v) glycerol), resolved by SDS/PAGE and transferred to a PVDF membrane (Immobilon P; Millipore, Eschborn, Germany). The membrane was incubated with anti-HA-epitope mouse monoclonal antibody (12CA5) and subsequently with horseradish peroxidase-conjugated rabbit anti-mouse IgG (Pierce, Rockford, IL, U.S.A.; #31450). Immune reactive proteins were revealed by enhanced chemiluminescence with the ECL[®] plus kit (Amersham Bioscience).

For detection of endogenous hELD1/OSA1 protein, 10^6 sample cells were lysed in $200\ \mu\text{l}$ of $1\times\text{SDS/PAGE}$ sample buffer (see above), vortexed vigorously, denatured by heating at $98\ ^\circ\text{C}$ for 5 min, and cleared by centrifugation for 5 min at $21\,000\ g$. Protein extract ($10\ \mu\text{l}$) was resolved on an 8% (w/v)

The 5482 bp cDNA sequence for human *ELDI/OSA1* is depicted along with the deduced 1740 amino acid sequence of the product of the 5220 bp ORF. The ARID (amino acids 550–665), EHD1 (amino acids 1096–1186), and EHD2 (amino acids 1411–1740) are boxed. One conserved LXXLL motif is underlined.

denaturing polyacrylamide gel, and transferred to a PVDF membrane. hELD1/OSA1 protein was detected by probing the blot, first with 6H3 (or 5A5) anti-EHD2 mouse monoclonal antibody followed by a horseradish peroxidase-conjugated rabbit anti-mouse IgG polyclonal antibody, and finally visualized by enhanced chemiluminescence.

Purification of BRG1-containing SWI/SNF complex

Nuclear extract was purified from mouse brain tissue as previously described [68]. BRG1-containing BAF complexes were immunoaffinity purified from 120 µg of nuclear extract, made up to a total volume of 250 µl with cold RIPA buffer (10 mM Tris/HCl, pH 7.2, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 158 mM NaCl, 5 mM EDTA and protease-inhibitor-cocktail tablets) by incubating with J1 anti-BRG1 rabbit polyclonal antibody [36] bound to Protein A-Sepharose beads (Sigma) overnight at 4 °C. Beads were washed three times with 500 µl of cold RIPA buffer (10 min incubation at 4 °C, rotating between washes) followed by two washes with cold BC100 [20 mM Tris/HCl, (pH 7.9), 100 mM KCl, 1 mM EDTA, 20% (v/v) glycerol, 0.2 mM PMSF] and two washes with cold water. The complex was eluted in 0.1 M glycine, pH 2.5, immediately neutralized with 1/10 vol. of 1 M Tris/HCl, pH 8.0, and separated by electrophoresis through a 7.5% (w/v) reducing and denaturing polyacrylamide gel. Protein in the gel was either silver stained or transferred to a PVDF membrane for immunodetection with anti-EHD2 monoclonal antibody.

Sizing column fractionation

A Superose 6 HR10/30 column (Amersham Biosciences) was equilibrated with buffer BC500 [20 mM Hepes, pH 7.6, 500 mM KCl, 0.2 mM PMSF, 0.2 mM EDTA, 1 mM DTT, and 10% (v/v) glycerol] containing 0.1% (v/v) NP-40. Approximately 0.5 mg of mouse brain nuclear extract was loaded at a flow rate of 0.2 ml/min and 55 fractions of 0.5 ml each were collected. Out of every other fraction, 100 µl was precipitated with 10% (v/v) trichloroacetic acid, resuspended in buffer BC100 (as BC500, with only 100 mM KCl) and SDS loading buffer and resolved by SDS/PAGE (7% gel). Proteins in the gel were transferred to a PVDF membrane, and the elution profile of BRG1, ELD/OSA1 and BAF60a analysed by Western blot using specific antibodies.

RESULTS

Cloning of a human homologue of *Drosophila eld/osa*

A report by Treisman et al. [60] described the cloning of a gene, *eld*, whose recessive inactivation in *Drosophila* produced effects resembling the ectopic expression of Wg. The authors inferred that Eld was a possible downstream antagonist of Wg. *Drosophila eld* encodes a 2713 amino acid, proline- and glutamine-rich nuclear protein that possesses a conserved DNA binding domain, a so-called ARID. Subsequently, it was revealed that *eld* is identical to the trithorax group (TrG) gene *osa* [61]. Other TrG genes include *brahma* and *moira*, and the protein products of all three genes appear to be constitutive components of the *Drosophila* SWI/SNF chromatin-remodelling complex [26,28,59,69].

BLAST [70] analysis revealed two independent clusters of human EST sequences that showed highly significant homology with *Drosophila eld/osa*. We isolated a full-length cDNA clone encompassing one of these clusters, termed by us hELD/OSA1. The 5482 bp sequence (GenBank accession number AF468300) and the deduced amino acid sequence of the corresponding

Figure 2 Alignment of regions of highest conservation among Eld/Osa proteins

(A) ARID, (B) EHD1, and (C) EHD2. Black boxes, majority residues; grey boxes, conservative differences. The ClustalW algorithm was used to align protein sequences using default parameter settings. Accession numbers are as follows: *Drosophila* Eld/Osa (dEld/Osa), AF053091; *C. elegans* ORF products c01g8.7 and c01g8.8, U80439; human p270 protein, AF265208.

Figure 3

(A) Schematic comparison of hELD/OSA1 and *Drosophila* Eld/Osa (dEld/Osa). PQR, proline and glutamine rich region. FLAG-epitope-tagged hELD/OSA1 expression constructs generated during the course of this study. Cellular distribution following transient transfection of COS7 cells and immunocytochemical staining for the FLAG epitope. n, nuclear; c, cytoplasmic. (B) Equivalent expression of hELD/OSA1 constructs and characterisation of anti-EHD2 mouse mAb. 293T cells were transiently transfected with hELD/OSA1 expression constructs shown in (A) (indicated above upper panel) and protein extracted in 1X SDS/PAGE loading buffer. hELD/OSA1 protein was detected by Western blotting with an anti-FLAG-epitope mouse monoclonal antibody (upper panel) or 6H3 mouse monoclonal antibody raised against EHD2 of hELD/OSA1 (lower panel). The 6H3 antibody failed to recognize the ΔEHD2 and ARID proteins confirming its specificity for EHD2. (C) Endogenous hELD/OSA1 protein. Detection of an approximately 189 kDa endogenous protein using 6H3 antibody in protein extracts from the cell lines indicated above the lanes.

5220 bp ORF are depicted in Figure 1. The first methionine residue (at nucleotide position 58) corresponds to an ATG codon within an optimal consensus for translation initiation, as defined by Kozak [71]. This is likely to represent the true translation initiation site of hELD/OSA1 *in vivo*, since examination of the genomic sequence at the 5' end of the human hELD/OSA1 gene (accession number AJ001216) reveals no further in-frame optimal translation start sites or splice acceptor site before an in-frame stop codon is reached. Additionally, this region is rich in CpG dinucleotides, a characteristic of promoter regions. The 1740 amino acid putative product of the hELD/OSA1 ORF (hELD/OSA1 protein) would have an expected molecular mass of 189 kDa and would be comparatively rich in proline and glutamine residues (in total 20% of all residues).

Definition of conserved Eld/Osa protein motifs

Database searches for proteins (or hypothetical ORF products) similar to the hELD/OSA1 protein identified several matches with statistically significant degrees of relationship. Matches fell

into three groups: (i) other Eld/Osa homologues comprising, to date, a hypothetical protein from the *Caenorhabditis elegans* proteome (GenBank/EMBL accession number U80439), *Drosophila* Eld/Osa, and human p270 (GenBank/EMBL accession number AF2652080) which shares 66% overall similarity to hELD/OSA1; (ii) other proteins bearing an ARID including, from among several, yeast SWI1 protein (GenBank/EMBL accession number P09547), a constitutive component of yeast SWI/SNF, mouse bright protein (GenBank/EMBL accession number U60355) and *Drosophila* dead-ringer protein (GenBank/EMBL accession number U62542). ARID-bearing proteins are strongly implicated in the regulation of cell growth, differentiation, and tissue-specific gene expression (see [72] for review and references); (iii) several proteins rich in glutamine and proline residues.

Three conserved regions and other structural features define the group of Eld/Osa orthologues (Figures 1 and 2). All bear an ARID (Figure 2A), which for Eld/Osa proteins may be a misnomer, since while indeed appearing to mediate DNA binding, the isolated ARIDs of *Drosophila* Eld/Osa protein and human p270 demonstrate little or no sequence binding preference *in vitro*

[59,63]. There are two additional regions of conserved amino acid sequence within the C-termini of Eld/Osa proteins, named Eld/Osa homology domains (EHD) 1 and 2 (Figures 2B and 2C respectively). They are separated by more sparsely conserved regions which are nonetheless characteristic in being uncommonly rich in proline and glutamine residues (PQR). Glutamine-rich regions have been implicated in transactivation functions [73]. Also a number of LXXLL motifs can be identified within Eld/Osa proteins. These motifs appear critical for the binding of a variety of nuclear proteins to liganded nuclear hormone receptors [74]. Yeast SWI1 protein resembles Eld/Osa to some degree, since it also possesses ARID and LXXLL motifs and is similarly proline- and glutamine-rich. However, there is no significant degree of similarity between SWI1 and the EHD1 or EHD2 of Eld/Osa proteins.

Expression profile of hELD/OSA1

Consultation of the UniGene database and the Human Gene Map (both accessible in electronic form at the NCBI website: www.ncbi.nlm.nih.gov) for entries derived from the hELD/OSA1 gene revealed its cytogenetic map location to be 6q25.1 between the genes *ESR1* and *TCTEL1*. Furthermore, EST sequences derived from the hELD/OSA1 gene are represented in cDNA libraries isolated from a wide range of tissues including brain, colon, lung, placenta, prostate, testis, tonsil, and uterus, suggesting that the hELD/OSA1 protein is ubiquitously expressed. Immunoblotting protein extracts from a series of human cell lines representing a spectrum of tissue types using a monoclonal antibody, 6H3, raised against EHD2 of hELD/OSA1 revealed an approximately 189 kDa protein in all samples tested (Figure 3C). An equivalent sized endogenous protein was also detected with two other anti-EHD2 monoclonal antibodies (results not shown). Immunocytochemical detection of FLAG-epitope tagged hELD/OSA1, expressed ectopically in COS7 cells, revealed it to be an exclusively nuclear protein (results not shown).

EHD1 and EHD2 of ELD/OSA proteins interact and EHD2 binds BRG1

We sought potential interacting protein partners for hELD/OSA1 through performing a yeast two-hybrid library screen. Reporter yeast were transformed independently with three bait constructs encoding hELD/OSA1 ARID, EHD1, or EHD2, and subsequently with a human fetal brain cDNA library. We failed to isolate any clones interacting with hELD/OSA1 ARID, despite adequate transformation efficiency. From 36 yeast clones isolated from the EHD1 screen, 25 of the recovered library plasmids encoded portions of p270 and 5 plasmids portions of hELD/OSA1 itself. The shortest plasmid insert isolated encoded only the last 314 amino acids of p270, which map to its EHD2. This finding suggests that the EHD1 of Eld/Osa proteins may be capable of mediating an intramolecular association with EHD2, and/or an intermolecular association resulting in homo- or hetero-dimerization of Eld/Osa proteins. Two independent plasmid clones isolated from the EHD2 screen encoded amino acids 476–587 and 509–587 of the human Swi2/Brahma homologue BRG1 (also known as hSnf2 β , GenBank/EMBL accession number U29175) (Figure 4).

hELD/OSA1 and BRG1 interact *in vivo*

Drosophila Eld/Osa protein and human p270 have been isolated in large protein complexes that also contain Swi2 homologues, as

Figure 4 EHD2-interacting yeast two-hybrid clones

Schematic representation of the domain structure of BRG1 [30], and of the two partial BRG1 clones isolated in a yeast two-hybrid library screen using hELD/OSA1 EHD2 as a bait. Domain I is characteristically proline rich; the function of domain II is undetermined, but it encompasses at least a portion of the putative hELD/OSA1 interaction domain; domain III comprises the ATPase domain essential for chromatin-remodelling catalytic activity; the function of domain IV, the bromodomain, is also undetermined but possibly mediates binding to acetylated lysine residues [66,79], which may be important in allowing synergy with HATs. Domains I–IV are conserved in human BRM and *Drosophila* Brm. Only the distal end of domain II, including notably the putative hELD/OSA1 interaction domain, and domains III and IV are also conserved in yeast Swi2/Snf2 protein.

Figure 5 *In vivo* interaction between hELD/OSA1 and BRG1 is mediated by EHD2

Following transient transfection of 293T cells with constructs shown above lanes, protein was extracted in Triton X-100 lysis buffer and subjected to immunoprecipitation (IP) with an anti-FLAG-epitope mouse monoclonal antibody. Co-immunoprecipitation of BRG1 (upper panel) and expression of BRG1 in cell lysates (middle panel) was detected by immunoblotting (IB) with an anti-HA mouse monoclonal antibody. Expression of FLAG-epitope-tagged hELD/OSA1 constructs (arrowheads) in cell lysates detected by immunoblotting with an anti-FLAG-epitope mouse monoclonal antibody (lower panel).

well as varying numbers of conserved accessory proteins (brahma-associated proteins in *Drosophila*, and their mammalian counterparts, BAFs) [44,59,62]. Thus Eld/Osa proteins are considered core components of SWI/SNF complexes. To establish that hELD/OSA1 can also associate with BRG1 *in vivo*, we transiently co-expressed FLAG-epitope-tagged versions of hELD/OSA1 (Figure 3A) with HA-epitope-tagged versions of BRG1 in 293T cells, and attempted to co-precipitate them in an immune complex. The hELD/OSA1 expression plasmids were capable of directing equivalent expression of encoded constructs in 293T cells (Figure 3B). We found that full-length hELD/OSA1 co-precipitated with full-length wild-type BRG1 or an

Figure 6 Anti-EHD2 antibody detects ELD/OSA1 peptide in endogenous SWI/SNF complex

SWI/SNF was immunoaffinity purified from mouse brain nuclear extract (NE) using an anti-BRG1 antibody (J1). A mock immunopurification (IP) was performed using an irrelevant antibody (12CA5). Purified protein was resolved by SDS/PAGE under reducing conditions. (A) Silver-stained gel of BRG1-containing BAF complex immunopurified from mouse brain. MW, molecular mass marker (B) Immunoblot analysis of NE or IP protein from mouse brain using the 6H3 anti-EHD2 mouse monoclonal antibody. (C) Elution profile of BAF subunits from mouse brain NE size-fractionated on Superose 6 HR10/30 column. Odd elution fractions containing proteins or protein complexes with molecular mass in excess of 158 kDa were resolved by SDS/PAGE and then analysed for the presence of BAF190/BRG1, ELD/OSA1, or BAF60a protein by Western blotting.

ATPase-dead form of BRG1 (K798R) (Figure 5; lanes 4 and 5 upper panel, numbering from the left). Deletion of the ARID (Δ ARID) did not appear to impair significantly the interaction between hELD/OSA1 and BRG1 (Figure 5, lane 6, upper panel). hELD/OSA1 lacking either EHD1 or EHD2 (Δ EHD1 and Δ EHD2 respectively) were not extracted in Triton X-100 lysis buffer as efficiently as the other hELD/OSA1 constructs (Figure 5, lanes 7 and 8, lower panel). Immunocytochemical staining of COS7 cells transiently transfected with the Δ EHD2 construct revealed that the protein was largely concentrated in cytoplasmic inclusions (results not shown), suggesting that this protein may be insoluble or improperly folded. The limited extraction of these two proteins possibly accounts for the reduced co-immunoprecipitation of BRG1. EHD2 is sufficient for interaction with BRG1 (Figure 5, lane 10, upper panel). Similarly, performing the immunoprecipitation with an anti-HA-epitope monoclonal antibody followed by immunoblotting with an anti-FLAG-epitope monoclonal antibody revealed the presence of full-length, Δ ARID and EHD2 hELD/OSA1 protein in complex with BRG1, but not Δ EHD1, Δ EHD2, or ARID hELD/OSA1 protein (results not shown).

Endogenous ELD/OSA1 is a core component of mammalian SWI/SNF

BRG1-containing SWI/SNF complex was purified from mouse brain nuclear extract by immunoprecipitation with an anti-BRG1 rabbit polyclonal antibody (see Materials and Methods section). Purified protein was then subjected to SDS/PAGE under reducing conditions. The specificity of the isolation procedure can be judged by comparing silver-stained material purified with the anti-Brg1 reagent to that purified with an irrelevant control antibody (12CA5) (Figure 6A). A small number of discreet bands are observed in brain extract precipitated with the anti-BRG1 antibody that are not apparent in control lanes. These bands correspond to previously identified BAFs. For instance, BAF190 corresponds to BRG1 and BAF250 to p270. A band of approximately 189 kDa is also present in the immune complex.

In parallel, the immunoaffinity purified BRG1-containing BAF complex preparation was probed with 6H3 anti-EHD2 mouse monoclonal antibody (Figure 6B). While the approximately 250 kDa immunoreactive peptide most likely corresponds to p270/BAF250, the approximately 189 kDa species should correspond to mouse ELD/OSA1 protein. The anti-EHD2 antibody probably recognizes an epitope that is highly conserved in mammalian ELD/OSA proteins. An identical pattern of immunoreactivity was obtained using two other anti-EHD2 mouse monoclonal antibodies (results not shown). Size fractionation of mouse brain extract using a Superose 6 HR10/30 column followed by Western-blot analysis of different fractions revealed that ELD/OSA1 protein co-fractionates with BAF190/BRG1 and BAF60a components of mouse SWI/SNF (Figure 6C). Essentially no uncomplexed ELD/OSA1 protein could be detected in mouse brain extracts.

DISCUSSION

BRG1 alone is sufficient to stimulate nucleosomal remodelling *in vitro*, albeit at reduced efficiency compared to whole SWI/SNF complex. Addition of the core SWI/SNF subunits INI1/SNF5, BAF155, and BAF170 to BRG1 reconstitutes chromatin remodelling to near optimal levels [75]. An actin-related protein, BAF53, and β -actin itself have also been isolated as components of human SWI/SNF and shown to stimulate the DNA-dependent ATPase activity of BRG1. These two factors regulate the ability of SWI/SNF to interact with chromatin and the nuclear matrix in response to secondary messenger signalling pathways, such as those dependent on the production of phosphatidylinositides [33]. It is evident, therefore, that SWI/SNF accessory proteins modulate the enzymic activity of BRG1, sometimes in response to signalling molecules. Other subunits present within the complex likely influence the affinity with which SWI/SNF binds chromatin, its processivity as it remodels nucleosomes, and possibly its role as co-repressor or co-activator of transcription. The subunit composition of mammalian SWI/SNF varies significantly between cells of different tissue origin, and within a given cell type more than one species of complex can be isolated [35,36], suggesting that SWI/SNF function may be subject to regulation at multiple levels.

Members of the Eld/Osa protein family also appear to be core components of SWI/SNF. These proteins possibly represent functional homologues of yeast Swi1 and are characterized by possession of an ARID, which mediates DNA binding, and by the presence of two further conserved domains within the C-terminus, conserved LXXLL motifs, and a high frequency of proline and glutamine residues. Within the human genome

two Eld/Osa homologues have thus far been identified, p270 and the *hELD/OSA1* gene described here. While overall, p270 and *hELD/OSA1* are 66 % similar at the amino acid level, the degree of conservation rises to over 90 % within the ARID, EHD1, and EHD2. We assume that the function of these domains, and indeed the specificity of their interactions, is likely to be conserved between Eld/Osa homologues. The intervening PQRs in *hELD/OSA1* are shorter than in p270 accounting largely for the difference in molecular masses of these two proteins. In general PQRs are poorly conserved between Eld/Osa family members and possibly play a scaffolding role only. p270 has been shown to correspond to BAF250, a constitutive component of the most abundant form of mammalian SWI/SNF (complex A) [36,62,64]. We cannot yet completely exclude that the 189 kDa species identified in the endogenous SWI/SNF complex represents an alternative, truncated form of p270/BAF250, or a degradation product. However, a peptide of this size is detected in various cell and tissue extracts independently of the method of protein extraction, and a monoclonal antibody raised against p270 failed to immunoprecipitate a cross-reacting peptide of 189 kDa, while co-purifying other BAF peptides [62,64], suggesting that this peptide is not derived from p270 and should represent Eld/Osa1 protein. Thus *ELD/OSA1* protein appears to be a novel component of mammalian SWI/SNF complexes that is particularly abundant in mouse brain extract, but much less abundant in SWI/SNF complexes isolated from human HeLa cells (data not shown).

By yeast two-hybrid assay and also by co-immunoprecipitation from 293T cells, we determined that the highly conserved EHD2 within the C-terminus of *hELD/OSA1* protein is sufficient for mediating binding to BRG1. However, due to the low efficiency of extraction of *hELD/OSA1* Δ EHD2 protein from transfected cells, it is not possible to conclude that the EHD2 is solely responsible for mediating this interaction. Co-expression of Δ EHD2 did not affect the efficiency with which BRG1 was extracted, implying that they occupy independent subcellular compartments and are therefore unlikely to interact *in vivo*. p270 is reported to interact directly not only with BRG1 but also a trimeric complex of INI1/SNF5, BAF155, and BAF170 [62]. This interaction could possibly also allow an indirect interaction with BRG1. The regions of p270 responsible for these interactions were not mapped, but it is possible that *hELD/OSA1* shares these determinants.

Drosophila Eld/Osa appears to influence the expression of only a minority of genes implicated in the generation of segment polarity during embryogenesis and the determination of homeotic fate of adult tissues [60,61]. Significantly, Eld/Osa in co-operation with Brahma selectively antagonizes Wg signalling through repressing Wg-target gene expression [65]. In contrast, we have recently found that β -catenin and BRG1 homologues interact in both mammalian and *Drosophila* cells, and that the functional consequence of this interaction in both instances is enhancement of Wg/WNT signalling (either increased reporter gene activity or genetic co-operation during *Drosophila* development) [76]. Thus, while SWI/SNF, in co-operation with the transcriptional co-repressor Groucho, mediates repression of Wg target genes *in vivo* in *Drosophila* [65], we find that SWI/SNF is also required for optimal activation of Wg/WNT target genes [76]. These apparently contradictory findings can be resolved by the observation that SWI/SNF *in vitro* can catalyse both forward and reverse nucleosome remodelling reactions [77,78]. Using transient reporter assays and by examining expression of endogenous β -catenin/TCF target genes in mammalian colon-carcinoma-derived cell lines, we have so far been unable to detect either a repressive or stimulatory effect of over-expression of full-length

wild-type *hELD/OSA1* or any mutants derived from it on β -catenin/TCF transcriptional activity (results not shown). Further investigations will thus be required to determine whether mammalian ELD/Osa proteins, like their *Drosophila* counterpart, participate selectively in regulating β -catenin/TCF target gene expression.

A.F.L.H. was supported by Wellcome Trust fellowship 054945 and I.A.O. by a fellowship from the American Cancer Society. This work was also supported by the Association for International Cancer Research, grant 99.025.

REFERENCES

- Lorch, Y., LaPointe, J. W. and Kornberg, R. D. (1987) Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. *Cell* **49**, 203–210.
- Laybourn, P. J. and Kadonaga, J. T. (1991) Role of nucleosomal cores and histone H1 in regulation of transcription by RNA polymerase II. *Science* **254**, 238–245.
- Godde, J. S., Nakatani, Y. and Wolffe, A. P. (1995) The amino-terminal tails of the core histones and the translational position of the TATA box determine TBP/TFIIA association with nucleosomal DNA. *Nucleic Acids Res.* **23**, 4557–4564.
- Adams, C. C. and Workman, J. L. (1993) Nucleosome displacement in transcription. *Cell* **72**, 305–308.
- Blomquist, P., Li, Q. and Wrangé, O. (1996) The affinity of nuclear factor 1 for its DNA site is drastically reduced by nucleosome organization irrespective of its rotational or translational position. *J. Biol. Chem.* **271**, 153–159.
- Zaret, K. S. and Yamamoto, K. R. (1984) Reversible and persistent changes in chromatin structure accompany activation of a glucocorticoid-dependent enhancer element. *Cell* **38**, 29–38.
- Carr, K. D. and Richard-Foy, H. (1990) Glucocorticoids locally disrupt an array of positioned nucleosomes on the rat tyrosine aminotransferase promoter in hepatoma cells. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 9300–9304.
- Reik, A., Schutz, G. and Stewart, A. F. (1991) Glucocorticoids are required for establishment and maintenance of an alteration in chromatin structure: induction leads to a reversible disruption of nucleosomes over an enhancer. *EMBO J.* **10**, 2569–2576.
- Fascher, K. D., Schmitz, J. and Horz, W. (1993) Structural and functional requirements for the chromatin transition at the PHO5 promoter in *Saccharomyces cerevisiae* upon PHO5 activation. *J. Mol. Biol.* **231**, 658–667.
- Munsterkotter, M., Barbaric, S. and Horz, W. (2000) Transcriptional regulation of the yeast PHO8 promoter in comparison to the co-regulated PHO5 promoter. *J. Biol. Chem.* **275**, 22678–22685.
- Then Bergh, F., Flinn, E. M., Svaren, J., Wright, A. P. and Horz, W. (2000) Comparison of nucleosome remodeling by the yeast transcription factor Pho4 and the glucocorticoid receptor. *J. Biol. Chem.* **275**, 9035–9042.
- Ryan, M. P., Stafford, G. A., Yu, L. and Morse, R. H. (2000) Artificially recruited TATA-binding protein fails to remodel chromatin and does not activate three promoters that require chromatin remodeling. *Mol. Cell Biol.* **20**, 5847–5857.
- Ostlund Farrants, A. K., Blomquist, P., Kwon, H. and Wrangé, O. (1997) Glucocorticoid receptor-glucocorticoid response element binding stimulates nucleosome disruption by the SWI/SNF complex. *Mol. Cell Biol.* **17**, 895–905.
- Kouzarides, T. (2000) Acetylation: a regulatory modification to rival phosphorylation? *EMBO J.* **19**, 1176–1179.
- Cheung, W. L., Briggs, S. D. and Allis, C. D. (2000) Acetylation and chromosomal functions. *Curr. Opin. Cell Biol.* **12**, 326–333.
- Peterson, C. L., Dingwall, A. and Scott, M. P. (1994) Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2905–2908.
- Cairns, B. R., Kim, Y. J., Sayre, M. H., Laurent, B. C. and Kornberg, R. D. (1994) A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 1950–1954.
- Gavin, I. M. and Simpson, R. T. (1997) Interplay of yeast global transcriptional regulators Ssn6p-Tup1p and Swi-Snf and their effect on chromatin structure. *EMBO J.* **16**, 6263–6271.
- Hirschhorn, J. N., Brown, S. A., Clark, C. D. and Winston, F. (1992) Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes Dev.* **6**, 2288–2298.
- Matalana, E., Franco, L. and Perez-Ortin, J. E. (1992) Chromatin structure of the yeast SUC2 promoter in regulatory mutants. *Mol. Gen. Genet.* **231**, 395–400.

- 21 Cote, J., Quinn, J., Workman, J. L. and Peterson, C. L. (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265**, 53–60
- 22 Imbalzano, A. N. (1998) SWI/SNF complexes and facilitation of TATA binding protein: nucleosome interactions. *Methods* **15**, 303–314
- 23 Kwon, H., Imbalzano, A. N., Khavari, P. A., Kingston, R. E. and Green, M. R. (1994) Nucleosome disruption and enhancement of activator binding by a human SWI/SNF complex. *Nature (London)* **370**, 477–481
- 24 Peterson, C. L. and Herskowitz, I. (1992) Characterization of the yeast SWI1, SWI2, and SWI3 genes, which encode a global activator of transcription. *Cell* **68**, 573–583
- 25 Yoshinaga, S. K., Peterson, C. L., Herskowitz, I. and Yamamoto, K. R. (1992) Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. *Science* **258**, 1598–1604
- 26 Dingwall, A. K., Beek, S. J., McCallum, C. M., Tamkun, J. W., Kalpana, G. V., Goff, S. P. and Scott, M. P. (1995) The *Drosophila* snr1 and brm proteins are related to yeast SWI/SNF proteins and are components of a large protein complex. *Mol. Biol. Cell* **6**, 777–791
- 27 Elfiring, L. K., Deuring, R., McCallum, C. M., Peterson, C. L. and Tamkun, J. W. (1994) Identification and characterization of *Drosophila* relatives of the yeast transcriptional activator SNF2/SWI2. *Mol. Cell Biol.* **14**, 2225–2234
- 28 Papoulas, O., Beek, S. J., Moseley, S. L., McCallum, C. M., Sarte, M., Shearn, A. and Tamkun, J. W. (1998) The *Drosophila* trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. *Development* **125**, 3955–3966
- 29 Tamkun, J. W., Deuring, R., Scott, M. P., Kissinger, M., Pattatucci, A. M., Kaufman, T. C. and Kennison, J. A. (1992) brahma: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell* **68**, 561–572
- 30 Khavari, P. A., Peterson, C. L., Tamkun, J. W., Mendel, D. B. and Crabtree, G. R. (1993) BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. *Nature (London)* **366**, 170–174
- 31 Muchardt, C. and Yaniv, M. (1993) A human homologue of *Saccharomyces cerevisiae* SNF2/SWI2 and *Drosophila* brm genes potentiates transcriptional activation by the glucocorticoid receptor. *EMBO J.* **12**, 4279–4290
- 32 Wang, W., Chi, T., Xue, Y., Zhou, S., Kuo, A. and Crabtree, G. R. (1998) Architectural DNA binding by a high-mobility-group/kinesin-like subunit in mammalian SWI/SNF-related complexes. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 492–498
- 33 Zhao, K., Wang, W., Rando, O. J., Xue, Y., Swiderek, K., Kuo, A. and Crabtree, G. R. (1998) Rapid and phosphoinositide-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. *Cell* **95**, 625–636
- 34 Bochar, D. A., Wang, L., Beniya, H., Kinev, A., Xue, Y., Lane, W. S., Wang, W., Kaschanchi, F. and Shiekhattar, R. (2000) BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. *Cell* **102**, 257–265
- 35 Wang, W., Xue, Y., Zhou, S., Kuo, A., Cairns, B. R. and Crabtree, G. R. (1996) Diversity and specialization of mammalian SWI/SNF complexes. *Genes Dev.* **10**, 2117–2130
- 36 Wang, W., Cote, J., Xue, Y., Zhou, S., Khavari, P. A., Biggar, S. R., Muchardt, C., Kalpana, G. V., Goff, S. P., Yaniv, M., Workman, J. L. and Crabtree, G. R. (1996) Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. *EMBO J.* **15**, 5370–5382
- 37 Quinn, J., Fyrberg, A. M., Ganster, R. W., Schmidt, M. C. and Peterson, C. L. (1996) DNA-binding properties of the yeast SWI/SNF complex. *Nature (London)* **379**, 844–847
- 38 Holstege, F. C., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S. and Young, R. A. (1998) Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* **95**, 717–728
- 39 Sudarsanam, P., Iyer, V. R., Brown, P. O. and Winston, F. (2000) Whole-genome expression analysis of snf/swi mutants of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 3364–3369
- 40 Neely, K. E., Hassan, A. H., Wallberg, A. E., Steger, D. J., Cairns, B. R., Wright, A. P. and Workman, J. L. (1999) Activation domain-mediated targeting of the SWI/SNF complex to promoters stimulates transcription from nucleosome arrays. *Mol. Cell* **4**, 649–655
- 41 Yudkovsky, N., Logie, C., Hahn, S. and Peterson, C. L. (1999) Recruitment of the SWI/SNF chromatin remodeling complex by transcriptional activators. *Genes Dev.* **13**, 2369–2374
- 42 Cosma, M. P., Tanaka, T. and Nasmyth, K. (1999) Ordered recruitment of transcription and chromatin remodeling factors to a cell c. *Cell* **97**, 299–311
- 43 Natarajan, K., Jackson, B. M., Zhou, H., Winston, F. and Hinnebusch, A. G. (1999) Transcriptional activation by Gcn4p involves independent interactions with the SWI/SNF complex and the SRB/mediator. *Mol. Cell* **4**, 657–664
- 44 Kal, A. J., Mahmoudi, T., Zak, N. B. and Verrijzer, C. P. (2000) The *Drosophila* brahma complex is an essential coactivator for the trithorax group protein zeste. *Genes Dev.* **14**, 1058–1071
- 45 Rozenblatt-Rosen, O., Rozovskaia, T., Burakov, D., Sedkov, Y., Tillib, S., Blechman, J., Nakamura, T., Croce, C. M., Mazo, A. and Canaani, E. (1998) The C-terminal SET domains of ALL-1 and TRITHORAX interact with the INI1 and SNR1 proteins, components of the SWI/SNF complex. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 4152–4157
- 46 Fryer, C. J. and Archer, T. K. (1998) Chromatin remodelling by the glucocorticoid receptor requires the BRG1 complex. *Nature (London)* **393**, 88–91
- 47 Kowenz-Leutz, E. and Leutz, A. (1999) A C/EBP beta isoform recruits the SWI/SNF complex to activate myeloid genes. *Mol. Cell* **4**, 735–743
- 48 Cheng, S. W., Davies, K. P., Yung, E., Beltran, R. J., Yu, J. and Kalpana, G. V. (1999) c-MYC interacts with INI1/hSNF5 and requires the SWI/SNF complex for transactivation function. *Nat. Genet.* **22**, 102–105
- 49 Armstrong, J. A., Bieker, J. J. and Emerson, B. M. (1998) A SWI/SNF-related chromatin remodeling complex, E-RC1, is required for tissue-specific transcriptional regulation by EKLF in vitro. *Cell* **95**, 93–104
- 50 Murphy, D. J., Hardy, S. and Engel, D. A. (1999) Human SWI-SNF component BRG1 represses transcription of the c-fos gene. *Mol. Cell Biol.* **19**, 2724–2733
- 51 Dunaief, J. L., Strober, B. E., Guha, S., Khavari, P. A., Alin, K., Luban, J., Begemann, M., Crabtree, G. R. and Goff, S. P. (1994) The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. *Cell* **79**, 119–130
- 52 Strober, B. E., Dunaief, J. L. and Guha Goff, S. P. (1996) Functional interactions between the hBRM/hBRG1 transcriptional activators and the pRB family of proteins. *Mol. Cell Biol.* **16**, 1576–1583
- 53 Trouche, D., Le Chalony, C., Muchardt, C., Yaniv, M. and Kouzarides, T. (1997) RB and hbrm cooperate to repress the activation functions of E2F1. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 11268–11273
- 54 Zhang, H. S., Gavin, M., Dahiya, A., Postigo, A. A., Ma, D., Luo, R. X., Harbour, J. W. and Dean, D. C. (2000) Exit from G1 and S phase of the cell cycle is regulated by repressor complexes containing HDAC-Rb-hSWI/SNF and Rb-hSWI/SNF. *Cell* **101**, 79–89
- 55 Biggar, S. R. and Crabtree, G. R. (1999) Continuous and widespread roles for the Swi-Snf complex in transcription. *EMBO J.* **18**, 2254–2264
- 56 Pollard, K. J. and Peterson, C. L. (1997) Role for ADA/GCN5 products in antagonizing chromatin-mediated transcriptional repression. *Mol. Cell Biol.* **17**, 6212–6222
- 57 Roberts, S. M. and Winston, F. (1997) Essential functional interactions of SAGA, a *Saccharomyces cerevisiae* complex of Spt, Ada, and Gcn5 proteins, with the Snf/Swi and Srb/mediator complexes. *Genetics* **147**, 451–465
- 58 Bourachot, B., Yaniv, M. and Muchardt, C. (1999) The activity of mammalian brm/SNF2 α is dependent on a high-mobility-group protein I/Y-like DNA binding domain. *Mol. Cell Biol.* **19**, 3931–3939
- 59 Collins, R. T., Furukawa, T., Tanese, N. and Treisman, J. E. (1999) Osa associates with the Brahma chromatin remodeling complex and promotes the activation of some target genes. *EMBO J.* **18**, 7029–7040
- 60 Treisman, J. E., Luk, A., Rubin, G. M. and Heberlein, U. (1997) eyelid antagonizes wingless signaling during *Drosophila* development and has homology to the Bright family of DNA-binding proteins. *Genes Dev.* **11**, 1949–1962
- 61 Vazquez, M., Moore, L. and Kennison, J. A. (1999) The trithorax group gene osa encodes an ARID-domain protein that genetically interacts with the brahma chromatin-remodeling factor to regulate transcription. *Development* **126**, 733–742
- 62 Dallas, P. B., Cheney, I. W., Liao, D. W., Bowrin, V., Byam, W., Pacchione, S., Kobayashi, R., Yaciuk, P. and Moran, E. (1998) p300/CREB binding protein-related protein p270 is a component of mammalian SWI/SNF complexes. *Mol. Cell Biol.* **18**, 3596–3603
- 63 Dallas, P. B., Pacchione, S., Wilsker, D., Bowrin, V., Kobayashi, R. and Moran, E. (2000) The human SWI-SNF complex protein p270 is an ARID family member with non-sequence-specific DNA binding activity. *Mol. Cell Biol.* **20**, 3137–3146
- 64 Nie, Z., Xue, Y., Yang, D., Zhou, S., Deroo, B. J., Archer, T. K. and Wang, W. (2000) A specificity and targeting subunit of a human SWI/SNF family-related chromatin-remodeling complex. *Mol. Cell Biol.* **20**, 8879–8888
- 65 Collins, R. T. and Treisman, J. E. (2000) Osa-containing Brahma chromatin remodeling complexes are required for the repression of wingless target genes. *Genes Dev.* **14**, 3140–3152
- 66 Winston, F. and Allis, C. D. (1999) The bromodomain: a chromatin-targeting module? *Nat. Struct. Biol.* **6**, 601–604
- 67 Barker, N., Huls, G., Korinek, V. and Clevers, H. (1999) Restricted high level expression of Tcf4 protein in intestinal and mammary gland epithelium. *Am. J. Pathol.* **154**, 29–35
- 68 Dignam, J. D., Lebovitz, R. M. and Roeder, R. G. (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11**, 1475–1489
- 69 Crosby, M. A., Miller, C., Alon, T., Watson, K. L., Verrijzer, C. P., Goldman-Levi, R. and Zak, N. B. (1999) The trithorax group gene moira encodes a brahma-associated putative chromatin-remodeling factor in *Drosophila melanogaster*. *Mol. Cell Biol.* **19**, 1159–1170

- 70 Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990) Basic local alignment search tool. *J. Mol. Biol.* **215**, 403–410
- 71 Kozak, M. (1996) Interpreting cDNA sequences: some insights from studies on translation. *Mamm. Genome* **7**, 563–574
- 72 Kortschak, R. D., Tucker, P. W. and Saint, R. (2000) ARID proteins come in from the desert. *Trends Biochem. Sci.* **25**, 294–299
- 73 Seipel, K., Georgiev, O. and Schaffner, W. (1992) Different activation domains stimulate transcription from remote ('enhancer') and proximal ('promoter') positions. *EMBO J.* **11**, 4961–4968
- 74 Heery, D. M., Kalkhoven, E., Hoare, S. and Parker, M. G. (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature (London)* **387**, 733–736
- 75 Phelan, M. L., Sif, S., Narlikar, G. J. and Kingston, R. E. (1999) Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. *Mol. Cell* **3**, 247–253
- 76 Barker, N., Hurlstone, A., Musisi, H., Miles, A., Bienz, M. and Clevers, H. (2001) The chromatin remodelling factor Brg-1 interacts with β -catenin to promote target gene activation. *EMBO J.* **20**, 4935–4943
- 77 Logie, C. and Peterson, C. L. (1997) Catalytic activity of the yeast SWI/SNF complex on reconstituted nucleosome arrays. *EMBO J.* **16**, 6772–6782
- 78 Belikov, S., Gelius, B. and Wrangé, O. (2001) Hormone-induced nucleosome positioning in the MMTV promoter is reversible. *EMBO J.* **20**, 2802–2811
- 79 Dhalluin, C., Carlson, J. E., Zeng, L., He, C., Aggarwal, A. K. and Zhou, M. M. (1999) Structure and ligand of a histone acetyltransferase bromodomain. *Nature (London)* **399**, 491–496

Received 1 November 2001/21 January 2002; accepted 20 February 2002