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SNF2β-BRG1 Is Essential for the Viability of F9 Murine Embryonal Carcinoma Cells

CHIHO SUMI-ICHINOSE,† HIROSHI ICHINOSE,‡ DANIEL METZGER, AND PIERRE CHAMBON*

Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, Collège de France, 67404 Illkirch Cedex, CU de Strasbourg, France

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The yeast and animal SNF-SWI and related multiprotein complexes are thought to play an important role in processes, such as transcription factor binding to regulatory elements, which require nucleosome remodeling in order to relieve the repressing effect of packaging DNA in chromatin. There are two mammalian homologs of the yeast SNF2-SWI2 subunit protein, SNF2α-brm and SNF2β-BRG1, and overexpression of either one of them has been shown to enhance transcriptional activation by glucocorticoid, estrogen, and retinoic acid (RA) receptors in transiently transfected cells. We have investigated here the function of SNF2β-BRG1 in the RA receptor-retinoid X receptor-mediated transduction of the retinoid signal in F9 embryonal carcinoma (EC) cells which differentiate into endodermal-like cells upon RA treatment. The two *SNF2*β-*BRG1* alleles have been targeted by homologous recombination and subsequently disrupted by using a conditional Cre recombinase. We show that F9 EC cells inactivated on both *SNF2*β alleles are not viable and that heterozygous mutant cells are affected in proliferation but not in RA-induced differentiation. Thus, in F9 EC cells, SNF2β-BRG1 appears to play an essential role in basal processes involved in cell proliferation, in addition to its putative role in the activation of transcription mediated by nuclear receptors.

It is well established that transcription is repressed by packaging of DNA in nucleosomes and that remodeling of the chromatin nucleosomal structure accompanies activation of transcription (for reviews, see references 17, 33, 36, 56, and 57). Recent studies have led to the characterization of several multiprotein complexes which may be involved in this remodeling process. Genetic and biochemical studies in the yeast *Saccharomyces cerevisiae* first led to the characterization of a SNF-SWI multiprotein complex, which comprises more than 10 subunits and is required for the proper transcription of a number of inducible genes (for reviews and references, see references 5, 6, 8, 22, 40, and 55). Purified yeast SNF-SWI complex directly alters nucleosome structure and facilitates transcription factor binding to nucleosomal DNA in an ATP-dependent manner (references 12 and 35 and references therein). In this respect, the SNF2-SWI2 subunit protein is particularly interesting, as it contains a domain which is found in a number of DNA-RNA helicases and possesses a DNA-dependent ATPase activity (26). Very recently, Cairns et al. (7) have isolated, on the basis of homology to the SNF-SWI complex, a novel complex, RSC, which also exhibits a DNA-dependent ATPase activity stimulated by both free and nucleosomal DNA and can alter nucleosome structure in vitro. Several subunits of the RSC complex are related to those of the SNF-SWI complex. Interestingly, in contrast to the *SNF2-SWI2*, gene which is not essential, its RSC homolog, the *STH1* gene, is essential for yeast mitotic growth, and the RSC complex may play a wider role than the SNF-SWI complex (7).

Several lines of evidence indicate that higher eukaryotes contain homologs of the yeast SNF-SWI and RSC complexes. Two *Drosophila* homologs of SWI-SNF and RSC proteins, Brahma (brm, a homolog of yeast SNF2) and inr1 (a homolog of yeast SNF5), have been identified and found in a complex similar in size to the yeast complexes (13, 15, 48). brm can suppress mutations in Polycomb, a repressor of several homeotic genes, which is thought to act by condensing chromatin in heterochromatin-like structures (for reviews, see references 16 and 29). A more distantly SNF2-SWI2-related protein, ISWI, has been found in another *Drosophila* multiprotein complex, the nucleosome remodeling factor NURF, which can also remodel nucleosomal structures in vitro (references 50 and 51 and references therein).

In mammals, the best candidates for SNF2-SWI2 function are the human and mouse brm homologs (known as hbrm or hSNF2α and mbrm or mSNF2α) and the human and mouse brahma-related BRG1 (known as hBRG1 or hSNF2β and mBRG1 or mSNF2β) (10, 21, 30, 42). Interestingly, mSNF2β-mBRG1 has been shown to interact with HP1α (27), the mouse homolog of the *Drosophila* heterochromatin protein 1 (HP1), which like Polycomb (see above) is believed to silence transcription by condensing chromatin (16 and references therein). Distinct multiprotein complexes containing either hSNF2α-hbrm or hSNF2β-hBRG1 have recently been purified (53). Both types of complex contain four common subunits, which are homologs of the SNF5, SWI3, and SWP73 polypeptides of the yeast SNF-SWI complex (6), as well as distinct subunits, some of which also have a yeast homolog (54). In addition, it appears that there are multiple SNF2β-BRG1 complexes within the same cell or in different cell lines, which differ from one another by at least one subunit exhibiting some cell or tissue specificity (53, 54). Both partially purified and purified SNF2α-brm and SNF2β-BRG1 complexes possess an ATP-dependent nucleosome disruption activity and can facilitate the binding of activators and TATA binding protein to a nucleosome core (19, 20, 24, 53). Thus, at the present time, it

* Corresponding author. Mailing address: Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, Collège de France, BP 163, 67404 Illkirch Cedex, CU de Strasbourg, France. Phone: 33 388 65 32 13. Fax: 33 388 65 32 03.

† Present address: Department of Pharmacology, School of Medicine, Fujita Health University, Toyoake, Aichi 470-11, Japan.

‡ Present address: Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Aichi 470-11, Japan.

appears that all SNF-SWI-related complexes from yeast to human may possess a core of conserved subunits that includes proteins related to SNF2-SWI2, SWP73, SWI3, and SNF5. Interestingly, all core subunits of the yeast RSC complex are essential for mitotic growth (in contrast to those of the SNF-SWI complex) and appear to be more closely related to their human homologs than to the core subunits of the yeast SNF-SWI complex (7). Additional complexes with chromatin remodeling activity may be present in mammalian cells (37, 52). For instance, the presence in mammalian cells of a close relative of ISWI (see above), SNF2L, suggests the existence of NURF complexes in vertebrates (references 32, 38, and 51 and references therein).

SNF2-SWI2 and SWP73 are required for transcriptional activation by the glucocorticoid receptor (GR) in yeast, and the SWI3 component of the SNF-SWI complex coprecipitates with GR derivatives in yeast extracts (references 6 and 59 and references therein). Similarly, the activities of both the *Drosophila* bicoid factor and *ftz* have been found to be dependent on SWI-SNF in yeast (25, 39). Overexpression of either hSNF2α-hbrm or hSNF2β-hBRG1 in mammalian cells has been shown to enhance transactivation by the GR (30, 53), and an interaction between the retinoblastoma protein and SNF2 proteins is apparently important for stimulation of GR activity (44, 47). The GR stimulation is abolished by mutating the ATP binding motif of hSNF2α-hbrm or deleting its N-terminal region (30). Furthermore, Ostlund Farrants et al. (34) have recently reported that binding of GR to a GR-response element located in a nucleosome enhances the chromatin remodeling activity of human and rat SNF-SWI complexes. Both hSNF2α-hbrm and hSNF2β-hBRG1 are also able to cooperate with the estrogen receptor (ER) and retinoic acid (RA) receptor in transcriptional activation (10), indicating that the SNF2 proteins may act as mediators of the activation functions of nuclear receptors (NRs). Indeed, using the two-hybrid system, we have recently found that the N-terminal regions of hSNF2α-hbrm and hSNF2β-hBRG1 can interact in an agonist-dependent manner with the agonist-dependent activation function AF-2 located within the ligand binding domain of the ER (18). Taken together, these observations suggest that at least part of the transcriptional activation brought about by NR AF-2s involves ligand-dependent remodeling of the chromatin template of target genes by SNF2 complexes (18).

We have tested this hypothesis in murine F9 embryonal carcinoma (EC) cells (45, 46), whose changes in gene expression and differentiation into endodermal cells upon treatment with RA are mediated by two families of NRs, the RA receptors and the retinoid X receptors (for reviews and references, see references 3, 4, 9, and 11). The two *SNF2*β-*BRG1* alleles have been targeted by homologous recombination and subsequently disrupted by using a conditional Cre recombinase (28). We show that F9 EC cells inactivated on both *SNF2*β alleles are not viable and that heterozygous mutant cells are affected in proliferation but not in RA-induced differentiation. Thus, in F9 EC cells, SNF2β appears to play an essential role in basal processes involved in cell proliferation, in addition to its putative role in activation of transcription mediated by NRs.

MATERIALS AND METHODS

Cloning of mouse and human *SNF2*β cDNAs. cDNA probes, 762 and 900 bp in length, corresponding to the 5′ region of the h*SNF2*β cDNA cloned by Chiba et al. (10) and to the 3′ region of the partial m*SNF2*β cDNA cloned by Randazzo et al. (42), respectively, were cloned from a 10-day-old mouse embryo cDNA library constructed in the λZAPII vector. The primers used for synthesis of the 5′ cDNA probe were 5′-GCGGAATTCATATGTCCACTCCAGACCCACCC CTG-3′ and 5′-CTCGGATCCATGAGGCCTGCTGTAATTTGGAGG-3′ (corresponding to nucleotides 58 to 81 and 793 to 818 of h*SNF2*β cDNA [reference 10; EMBL accession no. D26156] and additional sequences for restriction sites), while those used for the 3′ cDNA probe synthesis were 5′-GCGGAATTCA TATGTTCGGCCGTGGTTCTCGCCAC-3′ and 5′-CTCGGATCCATCTTG GCTGGGACGAGCGCCTC-3′ (corresponding to nucleotides 2223 to 2346 and 3098 to 3120 of the partial m*SNF2*β cDNA [42] and additional sequences for restriction sites). Both cDNA probes randomly labeled with [α-^{32}P]dCTP were used to screen 4×10^5 recombinants of the above-described cDNA library.

To clone a full-length h*SNF2*β cDNA, a 713-bp m*SNF2*β cDNA probe encompassing the ATPase-helicase region was generated by PCR from the mouse embryo cDNA library by using the primers 5′-GGCTCTCCAGCTGCAAG GCGA-3′ (primer SN81; nucleotides 732 to 752) and 5′-CAATCCTTGCACG ATGCCGGC-3′ (nucleotides 1425 to 1445) (42). A HeLa cell cDNA library constructed in λZAP II (58) was screened with this probe, yielding a partial 4.2-kb h*SNF2*β cDNA lacking ~300 bp in 5′ and ~500 bp in 3′. The missing 5′ sequence was generated by PCR using the HeLa cell cDNA library and the primers 5′-CCATCGATCCACCATGTCCACTCCAGAC-3′ (nucleotides 58 to 72 in reference 10, together with a *Cla*I site and Kozak sequence immediately upstream of the ATG) and 5′-GGGTCGTCCGACATGCCCTT-3′ (nucleotides 301 to 320 in reference 10). The missing sequence from the 3′ end was similarly generated by using the primer 5′-AGCGAGGTCTTCATCCAGCT-3′ (nucleotides 4501 to 4520 in reference 10) and 5′-GGACTAGTGAGACTGGAATGTC GGGGCT-3′ (nucleotides 5001 to 5020 in reference 10 and a *Spe*I site). A full-length h*SNF2*β cDNA plasmid (phSNF2β) was constructed from these three fragments by using the h*SNF2*β endogenous *Nsi*I and *Nru*I sites and the *Cla*I and *Spe*I sites of the pBluescript II SK+ vector (Stratagene).

Cloning of the mouse *SNF2*β gene and determination of exon-intron boundaries. A mouse genomic library (F9 cells) constructed in λEMBL3 phage was screened with the above-described 713-bp m*SNF2*β cDNA probe to clone the genomic region corresponding to the ATPase-helicase domain. Genomic DNA fragments were subcloned from one positive phage and mapped with various restriction enzymes. The location of each of the exons was determined by Southern hybridization using cDNA fragments as probes, PCR amplification, and genomic DNA sequencing.

Establishment of a F9 cell line expressing Cre-ER. F9 EC cells were cultured as previously described (28). The line F9-C was derived by selection of puromycin-resistant clones obtained after coelectroporation of pCre-ER digested with *Nde*I and pD502 (an expression vector encoding the puromycin resistance gene under the control of the *PGK* promoter; a gift of D. Lohnes) digested with *Sca*I, as described previously (28). Puromycin selection (500 ng/ml) was carried out for 10 days.

Targeting of the *SNF2*β gene in F9 cells. The targeting vector pSNF2β$^{(L:NL)}$ was constructed from the 6.5-kb *Sal*I-*Eco*RI restriction fragment containing exons 1 to 3 of the ATPase-helicase domain of the m*SNF2*β gene (Fig. 1 and 2) and subcloned into pBluescript II SK+ (Stratagene). The oligonucleotides 5′-AGCTATAACTTCGTATAATGTATGCTATACGAAGTTAT-3′ and 5′-AGCTATAACTTCGTATAGCATACATTATACGAAGTTAT-3′, containing a LoxP site, were cloned into the *Hin*dIII site located 1.2 kb upstream of exon 2. pB16-SNF^{L} was obtained by cloning the 5.6-kb *Xba*I-*Eco*RI fragment isolated from the previous plasmid in pB16 (a modified pBluescript II SK+ vector constructed by insertion of the oligonucleotides 5′-TCTAGAGACTGAGAAT TCGTAC-3′ and 5′-GAATTCTCAGTCTCTAGAAGCT-3′ containing *Xba*I and *Eco*RI restriction sites into the *Sac*I and *Kpn*I sites of the polylinker). The 3.2-kb *Eco*RI-*Xba*I fragment containing the thymidine kinase-neomycin resistance gene (TK-Neo) cassette and one LoxP site at the 3′ end, isolated from pHR56S (28), was cloned in the *Bam*HI site of pB16-SNF^{L} after filling in of the ends by Klenow treatment, resulting in pB16-SNF$^{L:NL}$. Finally, the 7.5-kb *Eco*RI fragment containing exons 4 to 8 was inserted into the *Eco*RI site of pB16-SNF$^{L:NL}$.

pSNF2β$^{(L:LHL)}$ (Fig. 2) was constructed as follows. The oligonucleotides 5′-GATCGCGGCCGCAGCTCAGTCGAC-3′ and 5′-GATCGTCGACTGA GCTGCGGCCGC-3′, containing a *Not*I and a *Sal*I restriction site, were cloned in the *Bam*HI site of pB16-SNF^{L}, resulting in pB16-SNF^{L}-NS. The 7.5-kb *Eco*RI genomic DNA fragment containing exons 4 to 8 was inserted in the *Eco*RI site of pB16-SNF^{L}-NS, resulting in pB16b-SNF^{L}-NS. Finally, the 1.9-kb *Not*I-*Sal*I fragment containing a LoxP-flanked hygromycin resistance gene under the control of the *PGK* promoter (PGK-Hygro cassette) was isolated from pLox2 Hygro-a and cloned into pB16b-SNF^{L}-NS digested with *Not*I and *Sal*I. pLox2 Hygro-a was obtained by cloning the 1.8-kb *Bgl*II fragment isolated from pPGKHyg (49) in the *Bam*HI site of pLox2. pLox2 contains two direct-repeated LoxP sites, separated by a *Bam*HI restriction site, and was obtained by cloning the oligonucleotides 5′-CTAGTATAACTTCGTATAATGTATGCTATACG AAGTTATCGGATCCGATAACTTCGTATAATGTATGCTATACGAAGT TATC-3′ and 5′-CCGGGATAACTTCGTATAGCATACATTATACGAAGT TATCGGATCCGATAACTTCGTATAGCATACATTATACGAAGTTAT A3′ in pBluescript II SK+ digested with *Spe*I and *Xma*I.

The F9-C cell line was electroporated with 5 μg of the 14.5- or 12.7-kb *Xba*I fragment, isolated from pSNF2β$^{(L:NL)}$ or pSNF2β$^{(L:LHLa)}$, respectively (Fig. 2). Selection of neomycin- and hygromycin-resistant clones, Cre-ER-mediated excision, and Southern blot analysis were performed as described previously (28). The 5′ and 3′ probes correspond to the 0.9-kb *Bam*HI-*Xba*I fragment containing exon 1 and to the 0.8-kb *Eco*RI-*Xba*I fragment downstream of exon 9, respectively. The primers used for the identification of the genotypes and their locations (Fig. 2) were TB82 (5′-GATCAGCTCATGCCCTAAGG-3′, located

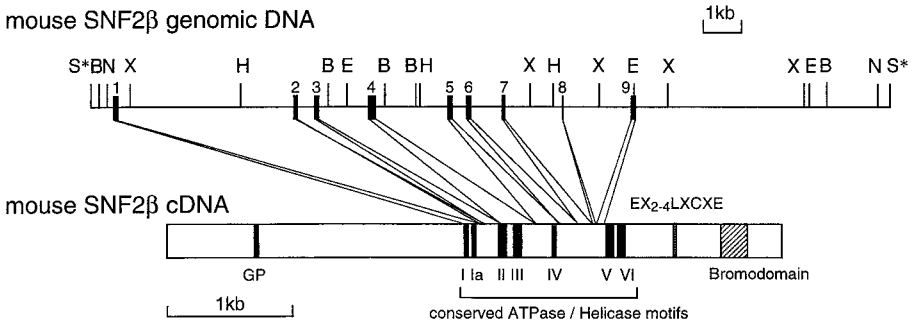

FIG. 1. Organization of the m*SNF2*β gene region encompassing the conserved ATPase-helicase motifs. The nine exons identified in a 20.9-kb genomic clone are indicated as black boxes. A partial restriction enzyme map is given. The m*SNF2*β cDNA (Fig. 3) is represented below the structure of the gene. S, *Sal*I; B, *Bam*HI; N, *Nhe*I; X, *Xba*I; E, *Eco*RI; GP, glycine-proline-rich region; I, Ia, II, III, IV, V, and VI, ATPase-helicase motifs; EX_{2-4}LXCXE, putative retinoblastoma protein interaction site; S*, *Sal*I site not present in genomic DNA.

within the intron between exons 1 and 2, upstream of the first LoxP site), TG57 (5′-GCCTTGTCTCAAACTGATAAG-3′, located within the intron between exons 3 and 4, downstream of the last LoxP site), TH185 (5′-GTCATACTTATG TCATAGCC-3′, located within the intron between exons 3 and 4), and TZ105 [5′-GCCTGCTCTTTACTGAAGGC-3′, located within the *PGK* poly(A) site of the TK-Neo and Hygro cassettes].

Establishment of a rescue cell line. The *SNF2*β cDNA expression vector pSG-hSNF2β was constructed by blunt-end ligation of the 4.9-kb *Acc*I-*Spe*I restriction fragment isolated from plasmid phSNF2β into the *Bam*HI site of pSG5, after T4 polymerase treatment of the DNA fragments. SNF2β$^{-L/L:La}$ cells were coelectroporated with 15 μg of pSG-hSNF2β linearized by *Nde*I and 5 μg of pPGK-hygro linearized by *Pvu*II, and hygromycin-resistant clones were isolated as described above. Integration of the hSNF2β expression cassette was detected by PCR using the primers UI102 (5′-TGACGCAGGTGCTCAA CACG-3′) and UI103 (5′-CGCACTTGATGACGTACTCC-3′), which are specific for the h*SNF2*β cDNA (nucleotides 2735 to 2754 and 3033 to 3052, respectively [10]).

PCR amplification of DNA. Genomic DNA was prepared from F9 cells as described previously (28). Alternatively, for time course experiments (e.g., Fig. 6), cells grown in six-well plates were collected in 100 μl of lysis buffer (50 mM Tris-HCl [pH 8.0], 20 mM NaCl, 1 mM EDTA, 1% sodium dodecyl sulfate, 1 mg of proteinase K per ml) and incubated overnight at 55°C. The lysate was boiled for 5 min and diluted 10 times in water. PCR amplification of genomic DNA was performed in 50-μl reactions containing 67 mM Tris-HCl (pH 8.8), 16 mM $(NH_4)_2SO_4$, 10 mM β-mercaptoethanol, 1.5 mM $MgCl_2$, 1 μl of proteinase K-treated cell extract or 20 ng of genomic DNA, primers (12.5 pmol of each), 0.2 mM deoxynucleoside triphosphates, 10% dimethyl sulfoxide, and 2.5 U of *Taq* polymerase. The amplification conditions were 94°C for 5 min, followed by 35 cycles at 94°C for 30 s and 50°C for 30 s, and finally 1 cycle at 94°C for 30 s, 50°C for 30 s, and 72°C for 5 min.

RNA extraction and semiquantitative reverse transcription (RT)-PCRs were performed as described previously (11).

Western blot analysis. Cells resuspended in 20 mM Tris-HCl (pH 7.5)–2 mM dithiothreitol–20% glycerol–0.4 M KCl–1 mM phenylmethylsulfonyl fluoride–protease inhibitor cocktail (2.5 μg each of leupeptin, pepstatin, chymostatin, antipain, and aprotinin per ml) were lysed by three cycles of freeze-thaw. Equal amounts of protein were loaded on a sodium dodecyl sulfate–8% polyacrylamide gel, separated by electrophoresis, and electroblotted to a nitrocellulose membrane. Western blot analysis was done as described previously (1), using the anti-mSNF2β monoclonal antibody 2E12 (18a) and the anti-SNF2L monoclonal antibody 2F12 (51b), followed by incubation with peroxidase-conjugated goat anti-mouse immunoglobulin G antibody (Jackson ImmunoResearch). The epitope of 2E12 corresponds to the sequence MFGRGSRHRKEVDYSDSLTE (amino acids 1333 to 1352 in mSNF2β), which is identical in mouse and human SNF2β. The epitope of 2F12 corresponds to the sequence KRDFNQFIKA NEKYGRDDID (amino acids 784 to 803 in hSNF2L [32]), which is identical in human and mouse SNF2L (51a). Chemiluminescence detection was performed as instructed by the manufacturer (Amersham).

Analysis of F9 cell growth and differentiation. Cells were seeded in six-well plates at a density of 2.5×10^3 cells/well on day −1. The medium was changed on days 1, 2, and 4, and 1 μM all-*trans* RA or ethanol (as a control) was added from day 1. Cells were counted (11), and their differentiation status was examined at day 5 under light microscopy.

Detection of SNF2β$^{-L/-La}$ cells. SNF2β$^{-L/L:La}$ cells plated at a density of 2.5×10^3 cells/well in six-well plates on day −1 were cultured in medium containing 10^{-7} M estradiol (E2) on days 0 and 1 and then collected every day for 12 days and lysed in 100 μl of lysis buffer. Genomic PCR was performed with primers TB82 and TG57 to amplify the *SNF2*β^{-L} and *SNF2*β^{-La} alleles and with primers TH185 and TG57 to amplify the *SNF2*β$^{L:La}$ allele. The PCR products were probed with oligonucleotides RR211 (5′-AAGCTTGATATCGAATTC CTGC-3′) and TH185 to detect the *SNF2*β^{-La} and *SNF2*β$^{L:La}$ alleles, respectively (see Fig. 6).

RESULTS

cDNA cloning of mouse and human SNF2β. To clone *mSNF2*β (also called m*BRG1* [42]) cDNA encoding the full-length protein, we screened a mouse embryo cDNA library with PCR-generated probes corresponding to the N- and C-terminal regions of the protein (Materials and Methods). Ten positive clones were isolated, subcloned in pBluescript II SK+ (Stratagene), partially sequenced, and mapped with restriction enzymes. A 5,170-base-long cDNA reconstructed from three overlapping DNA fragments contained a 43-base-long 5′ untranslated region followed by a 4,842-base-long open reading frame and a 285-base-long 3′ untranslated region (Fig. 3). This cDNA encodes a 1,614-amino-acid-long protein (181,400 Da) which encompasses the partial m*BRG1*-m*SNF2*β cDNA encoding amino acid residues 595 to 1614 published by Randazzo et al. (42). The amino acid sequences of mSNF2β and hSNF2β-hBRG1 (10, 21) are highly conserved (not shown). An expression vector containing the full-length h*SNF2*β cDNA was also constructed (Materials and Methods) for rescue experiments (see below).

Conditional targeting of the m*SNF2*β gene in F9 cells. We first screened a mouse genomic library with a cDNA probe encompassing the conserved ATPase-helicase motifs II to IV. One phage (with a 20.9-kb insert) contained at least nine exons (Fig. 1). Exon 1 encodes the ATPase motif I (the putative ATP-binding site) and Ia, exon 4 encodes the major part of motif II (the putative ATP hydrolysis site) and the entire motif III, and exon 5 encodes motif IV (Fig. 1 and 3; see Fig. 4 for exon-intron boundary sequences).

To perform a conditional disruption of the m*SNF2*β gene by homologous recombination, we constructed the targeting vector pSNF2β$^{(L:NL)}$, containing the 10.5-kb genomic sequences encompassing exons 2 to 7, in which a LoxP site was inserted in the intron located upstream of exon 2, while a Neo cassette followed by a LoxP site was inserted in the intron located downstream of exon 3 (Fig. 2B). Thus, homologous recombination of a wild-type (WT) allele using pSNF2β$^{(L:NL)}$ should allow the Cre recombinase to excise exons 2 and 3 together with the selection marker cassette, resulting in the deletion of the sequence encoding amino acid residues 814 (threonine) to 872 (lysine) (Fig. 3, from nucleotides 2482 to 2659) and the creation of a frameshift in exon 4 with a stop codon at amino acid position 819. The remaining protein produced from the

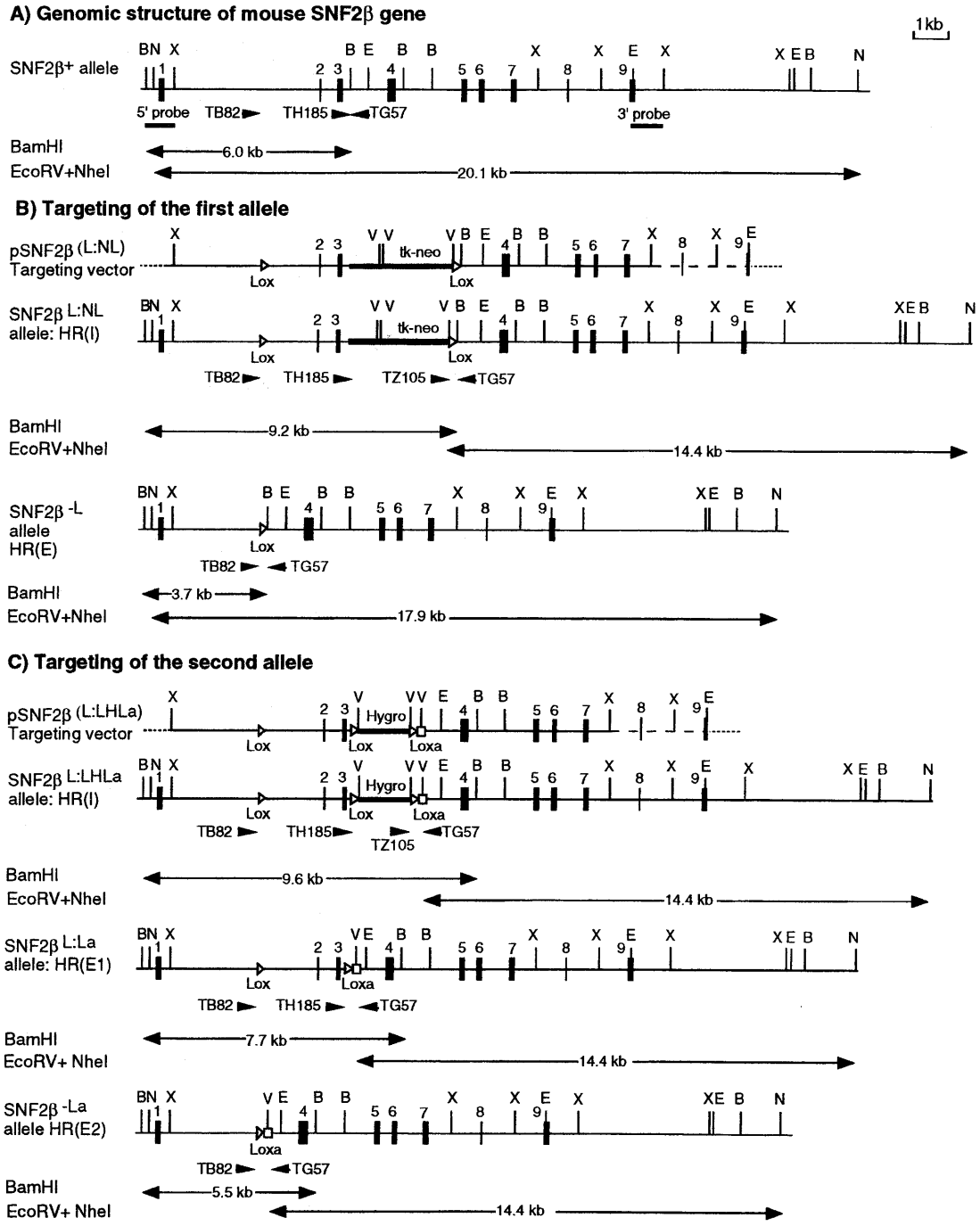

FIG. 2. Strategy for targeting the m*SNF2*β gene. (A) Structure of the m*SNF2*β gene (*SNF2*β^{+} allele). Exons 1 to 9 (Fig. 1) and the 5′ and 3′ probes corresponding to a 0.9-kb *Bam*HI-*Xba*I and a 0.8-kb *Eco*RI-*Xba*I fragment, respectively, are indicated. (B) First allele targeting. The targeting vector pSNF2β$^{(L:NL)}$ is represented. The two LoxP sites (open triangle) and the PGK-TK-Neo cassette (tk-neo) are indicated. The expected genomic maps after homologous recombination [*SNF2*β$^{L:NL}$ allele; HR(I)] and Cre-mediated excision [*SNF2*β^{-L} allele; HR(E)] are shown. (C) Second allele targeting. The targeting vector pSNF2β$^{(L:LHLa)}$ containing the three LoxP sites and the PGK-Hygro cassette (Hygro) are indicated. The 3′ Lox P site (open triangle) and additional pBluescript SKII+ sequences (open square) are indicated (Loxa). The expected genomic maps after homologous recombination (*SNF2*β$^{L:LHLa}$ allele; HR(I)], excision of the resistance marker [*SNF2*β$^{L:La}$ allele; HR(E1)], and exision of both the marker and exons 2 and 3 [*SNF2*β^{-La} allele; HR(E2)] are shown. The horizontal arrowheads in all panels indicate locations of the primers used for PCRs (Fig. 5D). DNA fragments obtained after *Bam*HI digestion and detection with the 5′ probe (Fig. 5B) and after *Eco*RV-*Nhe*I double digestion and detection with the 3′ probe (Fig. 5C) are also indicated. B, *Bam*HI; N, *Nhe*I; X, *Xba*I; E, *Eco*RI; V, *Eco*RV. The solid line in the targeting vectors corresponds to the DNA fragment used for gene targeting. Dotted lines represent plasmid vector sequences.

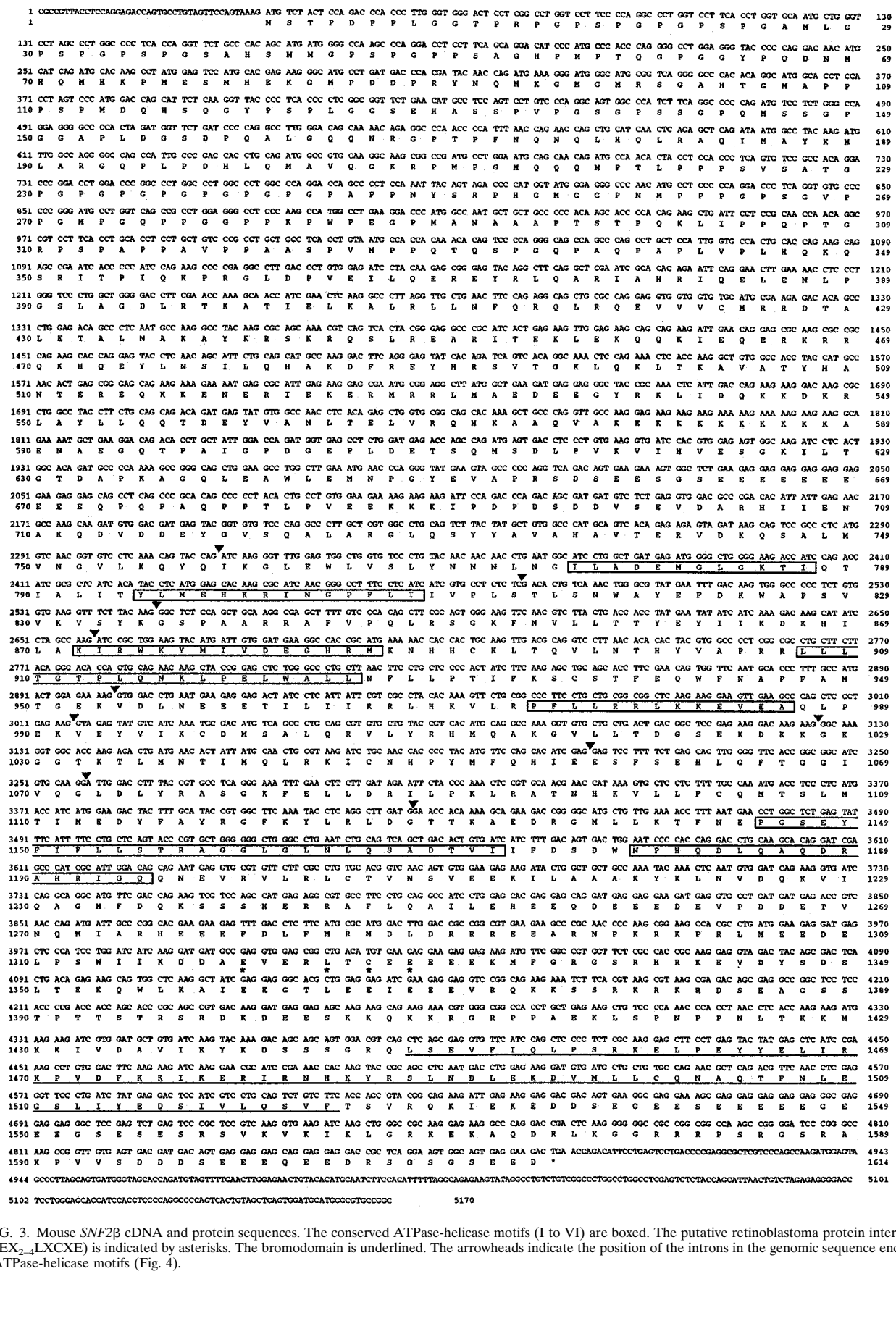

FIG. 3. Mouse *SNF2*β cDNA and protein sequences. The conserved ATPase-helicase motifs (I to VI) are boxed. The putative retinoblastoma protein interaction site ($EX_{2-4}LXCXE$) is indicated by asterisks. The bromodomain is underlined. The arrowheads indicate the position of the introns in the genomic sequence encoding the ATPase-helicase motifs (Fig. 4).

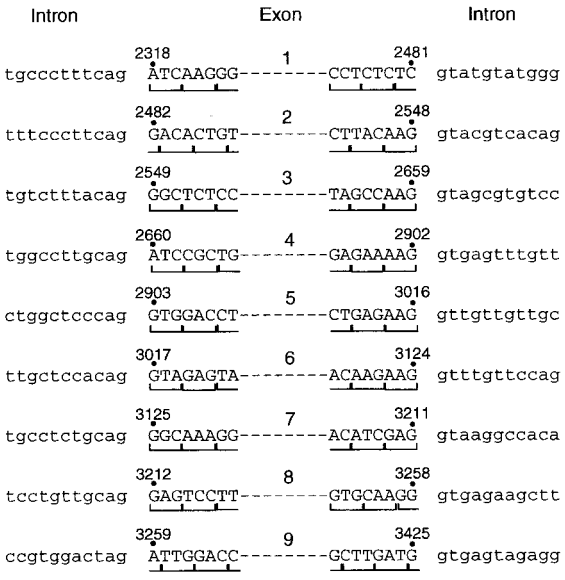

FIG. 4. Exon-intron boundaries of the m*SNF2*β gene region encoding the conserved ATPase-helicase motifs (Fig. 1 and 3). The codons are bracketed. The coordinates of the first and last nucleotides of each exon refer to the cDNA sequence presented in Fig. 3.

disrupted gene will encode a C-terminally truncated protein lacking the conserved motif II (the putative ATP hydrolysis site) and motifs III to VI.

Targeting of the first allele of *SNF2*β in F9 cells was performed by electroporating pSNF2β$^{(L:NL)}$ in the F9-C cell line, which expresses constitutively the ligand-dependent chimeric Cre-ER recombinase (28) (Materials and Methods). Southern blot analysis using 5′ and 3′ probes, both located outside the targeting construct (Fig. 2A), and PCR analysis revealed that 1 of 192 G418-resistant clones had one targeted *SNF2*β allele (*SNF2*β$^{L:NL}$ allele) (Fig. 2B and 5A, Fig. 5B and C [compare lane 2 with lane 1], Fig. 5D [compare lanes 4 to 6 with lanes 1 to 3], and data not shown). This cell line was called SNF2β$^{L:NL/+}$ (for the nomenclature of the different cell lines, see Fig. 5A).

To target the second allele, SNF2β$^{L:NL/+}$ cells were electroporated with the targeting vector pSNF2β$^{(L:LHLa)}$ (Fig. 2C). This vector contains a LoxP site inserted upstream of exon 2 and a LoxP-flanked Hygro cassette inserted in the intron located downstream of exon 3. Targeting the *SNF2*β gene with pSNF2β$^{(L:LHLa)}$ should allow both the Cre-mediated excision of the selection marker, resulting in a modified allele in which exons 2 and 3 are flanked with LoxP sites, and the subsequent Cre-mediated deletion of these exons. Three of the 240 hygromycin-resistant clones isolated had the two *SNF2*β alleles targeted (SNF2β$^{L:NL/L:LHLa}$ cells) (Fig. 5A, Fig. 5B and C [compare lane 3 with lane 2], Fig. 5D [compare lanes 7 to 9 with lanes 4 to 6], and data not shown).

We also established a cell line containing one WT and one inactivated *SNF2*β allele (*SNF2*β$^{-L/+}$) by E2 treatment of SNF2β$^{L:NL/+}$ cells (Fig. 2B and 5A, Fig. 5B [compare lane 4 with lanes 1 and 2], Fig. 5D [compare lanes 10 to 12 with lanes 4 to 6], and data not shown), thus demonstrating that exons 2 and 3 can indeed be deleted by activating the Cre-ER recombinase present in the F9-C line.

To excise the sequences located between the LoxP sites on both alleles, SNF2β$^{L:NL/L:LHLa}$ cells (Fig. 5A) were treated with E2. No clones with both alleles (*SNF2*β$^{-L/-La}$ or *SNF2*β$^{-L/-LHLa}$) inactivated were obtained, but five clones had one inactivated *SNF2*β allele. Among these clones, all possible genotypes for the other allele were present (Fig. 5A); i.e., there was no excision at either the L:NL allele (*SNF2*β$^{L:NL/-La}$, one clone) or the L:LHLa allele (*SNF2*β$^{-L/L:LHLa}$, one clone) or excision of the hygromycin resistance gene only from the L:LHLa allele (*SNF2*β$^{-L/L:La}$, three clones) (Fig. 2 and 5A, Fig. 5B [compare lanes 5 to 7 with lane 3], Fig. 5D [compare lanes 13 to 15, 16 to 18, and 19 to 21 with lanes 7 to 9], and data not shown). We also treated SNF2β$^{-L/L:La}$ cells with E2 and isolated 90 clones. None of them had a *SNF2*β$^{-L/-La}$ genotype (data not shown). Thus, it appears that either one of the two alleles of the m*SNF2*β gene can be independently inactivated, but that inactivation of both *SNF2*β alleles may be lethal.

Inactivation of both *SNF2*β alleles affects F9 cell viability. To demonstrate that inactivation of both *SNF2*β alleles impairs the proliferation of F9 cells, SNF2β$^{-L/L:La}$ cells were treated with E2, and the cell population was genotyped by PCR using DNA extracted over a 12-day period and the primer pairs TH185-TG57 and TB82-TG57, which allow the identification of the L:La allele and of both the −L and −La alleles, respectively (Fig. 2 and 6A). Southern blotting using radiolabeled TH185 for the first series of PCRs and RR211 (which hybridizes to the sequence located at the 3′ end of the La site, sequence a in Fig. 6A) for the second series was used for the detection of the L:La and −La alleles, respectively. The L:La allele signal increased during the first 5 days (due to increasing amount of genomic DNA in the reaction, reflecting cell growth), and then was constant (due to saturation of the PCR). In contrast, although the −La allele signal could be detected 1 day after the E2 treatment, it decreased at day 4 and finally disappeared at day 9 (Fig. 6B). Thus, SNF2β$^{-L/-La}$ cells inactivated on both m*SNF2*β alleles were obtained, but they were severely affected in proliferation and could not be cloned. It therefore appears that the SNF2β complex cannot be functionally substituted by the SNF2α complex which is expressed at low levels in F9 cells (our unpublished results) or by a complex containing SNF2L (see the introduction) which is expressed in F9 cells at levels similar to those found in HeLa or P19 cells (Fig. 7A).

Reduction of the SNF2β protein level impairs the proliferation and the RA-induced antiproliferative response of F9 cells but not their RA-induced differentiation. The SNF2β protein levels of WT and mutant cells were compared by Western blot analysis. These levels were apparently similar in WT (SNF2β$^{+/+}$), SNF2β$^{L:NL/+}$, and SNF2β$^{L:NL/L:LHLa}$ cells and slightly reduced in SNF2β$^{-L/+}$ and SNF2β$^{-L/L:La}$ cells, whereas they were decreased by 60 to 70% in SNF2β$^{-L/L:LHLa}$ and SNF2β$^{L:NL/-La}$ cells (Fig. 7B).

The proliferation rates of SNF2β$^{+/+}$, SNF2β$^{-L/+}$, SNF2β$^{-L/L:LHLa}$, and SNF2β$^{L:NL/-La}$ cells and their abilities to differentiate after RA treatment were analyzed. Whereas the proliferation levels over a period of 5 days were similar for SNF2β$^{-L/+}$ and WT (SNF2β$^{+/+}$) lines, they were reduced in SNF2β$^{-L/L:LHLa}$ and SNF2β$^{L:NL/-La}$ lines (Fig. 8A and B). The antiproliferative effect of RA was also less in SNF2β$^{-L/L:LHLa}$ and SNF2β$^{L:NL/-La}$ cells than in WT and SNF2β$^{-L/+}$ cells (Fig. 8C). In addition, morphological abnormalities were seen in SNF2β$^{-L/L:LHLa}$ and SNF2β$^{L:NL/-La}$ cells cultured for several generations in the absence of RA (data not shown). However, the RA-induced primitive endodermal-like differentiation of SNF2β$^{-L/L:LHLa}$ and SNF2β$^{L:NL/-La}$ cells was similar to that of WT and SNF 2β$^{-L/+}$ cells (Fig. 8D). The expression of the RA-responsive *Hoxa-1* gene was also similarly induced in WT, SNF2β$^{-L/+}$, SNF2β$^{-L/L:LHLa}$, and SNF2β$^{L:NL/-La}$ F9 cells treated with RA for 24 h (Fig. 8E).

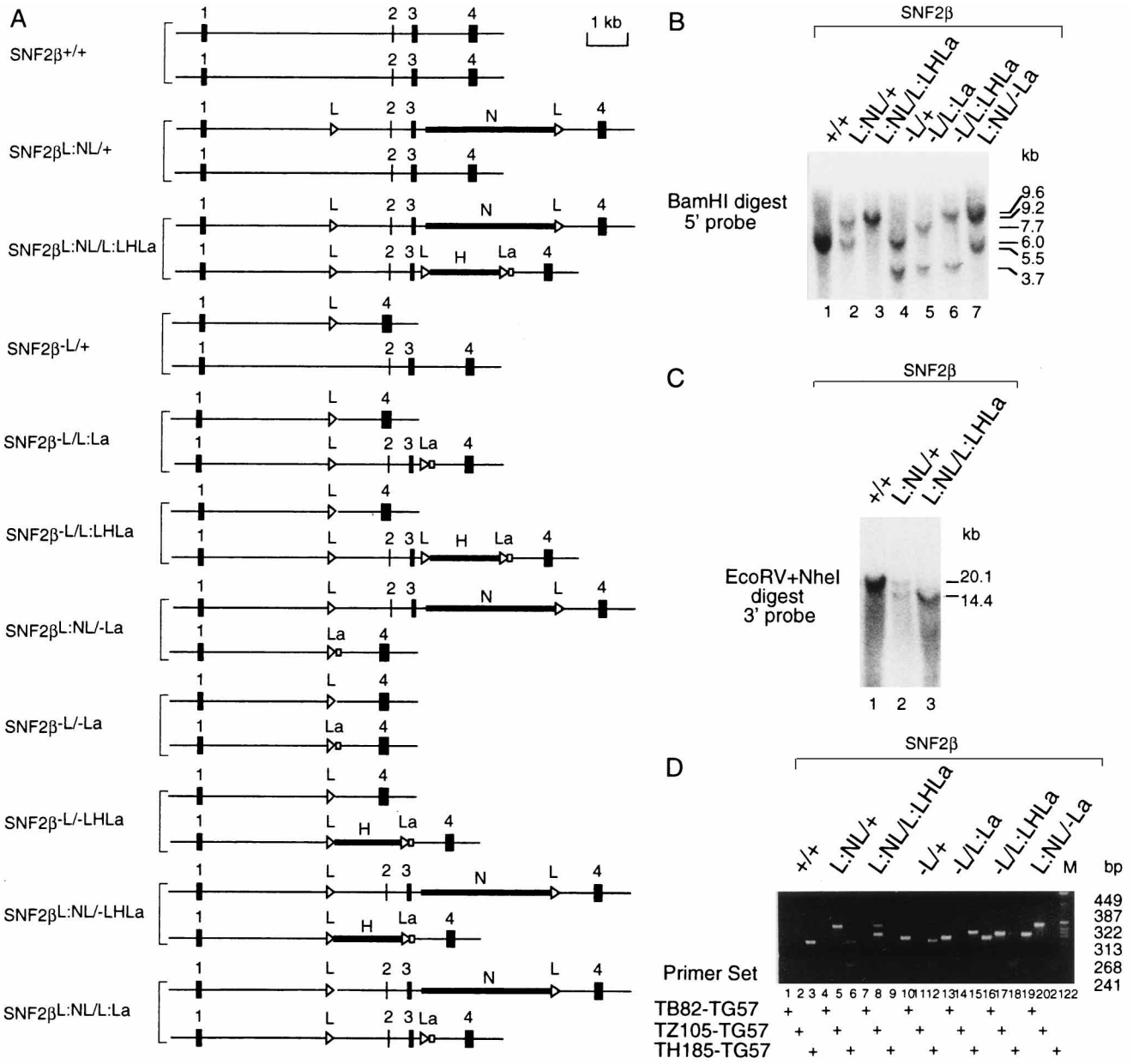

FIG. 5. Nomenclature and characterization of the genotypes of the different F9 cell lines targeted in the *SNF2*β locus by Southern hybridization and genomic PCR analysis. (A) Nomenclature of the different SNF2β mutant cell lines. Exons are represented as boxes (1 to 4), LoxP sites are represented as open triangles, and the additional pBluescript II SK+ sequences are represented as open squares (Fig. 2). The selectable marker genes are also indicated (N, TK-Neo cassette; H, Hygro cassette). (B) Southern blot of *Bam*HI-digested *SNF2*β genomic DNA (as indicated) hybridized with the 5′ probe (Fig. 2). Lane 1, +/+ (6.0 kb); lane 2, L:NL/+ (9.2 and 6.0 kb); lane 3, L:NL/L:LHLa (9.2 and 9.6 kb); lane 4, −L/+ (3.7 and 6.0 kb); lane 5, −L/L:La (3.7 and 7.7 kb); lane 6, −L/L:LHLa (3.7 and 9.6 kb); lane 7, L:NL/−La (5.5 and 9.2 kb). (C) Southern blot of *Eco*RV-*Nhe*I-digested *SNF2*β genomic DNA (as indicated) was hybridized with the 3′ probe (Fig. 2). Lane 1, +/+ (20.1 kb); lane 2, L:NL/+ (14.4 and 20.1 kb); lane 3, L:NL/L:LHLa (14.4 kb). (D) Genomic DNA extracted from the indicated cell lines was analyzed by PCR using three sets of primers as indicated. TB82-TG57 amplifies 268- and 313-bp fragments from the $SNF2\beta^{-L}$ (lanes 10, 13, and 16) and $SNF2\beta^{-La}$ (lane 19) alleles, respectively (Fig. 2B and C and Fig. 6A); TZ105- TG57 amplifies 449- and 322-bp fragments from the $SNF2\beta^{L:NL}$ (lanes 5, 8, and 20) and the $SNF2\beta^{L:LHLa}$ (lanes 8 and 17) alleles, respectively (Fig. 2B and C); TH185-TG57 amplifies 241- and 387-bp fragments from the $SNF2\beta^{+/+}$ (lanes 3, 6, and 12) and $SNF2\beta^{L:La}$ (lane 15) alleles, respectively (Fig. 2A and C and Fig. 6A). The 1-kb ladder DNA (M; GibcoBRL) is shown in lane 22.

Inactivation of both *SNF2*β alleles in an hSNF2β rescue line. To establish that the failure to obtain an F9 cell line lacking both alleles of the *SNF2*β gene was due to an absolute requirement for the SNF2β protein, we constructed a rescue $SNF2\beta^{-L/L:La}$ line expressing constitutively the human *SNF2*β cDNA (called hereafter the $RSNF2\beta^{-L/L:La}$ cell line) by integrating in its genome an expression cassette encoding hSNF2β under the control of the simian virus 40 promoter (Fig. 9A; compare lanes 7 and 8 to lanes 1 and 2 and to lanes 4 and 5). After activation of Cre-ER of this cell line, we obtained one clone in which exons 2 and 3 were deleted on both alleles ($RSNF2\beta^{-L/-La}$ cells) (Fig. 9B [compare lane 4 to lanes 1 to 3], Fig. 9C [compare lanes 10 to 12 to lanes 1 to 3, 4 to 6, and 7 to 9]; see Fig. 2 and Fig. 6A for the sizes of the expected

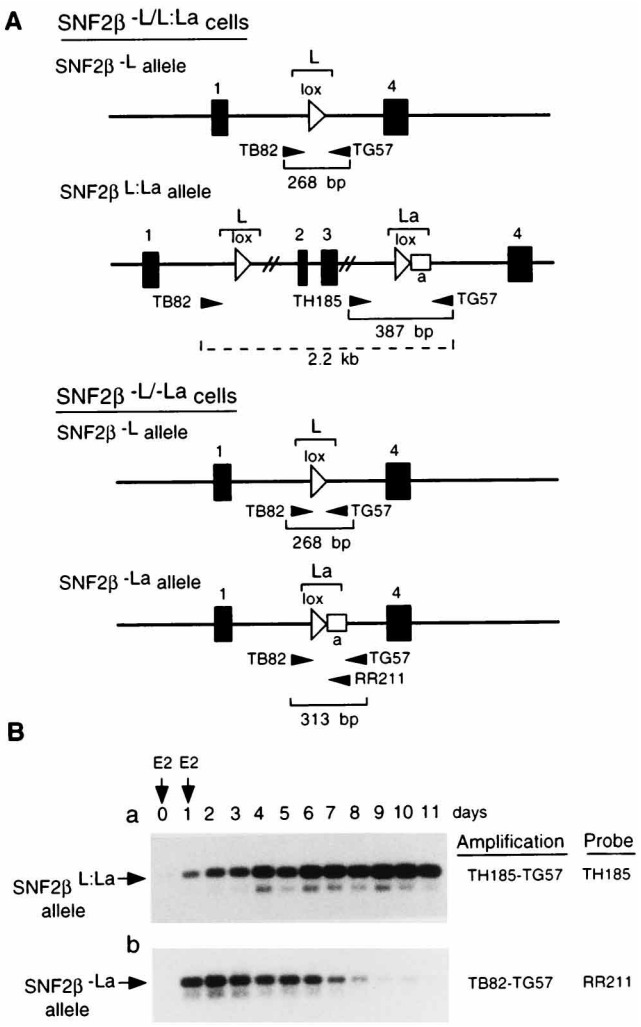

FIG. 6. Detection by PCR amplification of SNF2β$^{-L/-La}$ cells after induction of Cre-ER by E2 in SNF2β$^{-L/L:La}$ cells. (A) The PCR strategy to distinguish the different alleles is outlined. Exons are indicated as numbered boxes. LoxP sites and PCR primers (Materials and Methods) are indicated by horizontal open triangles and by arrowheads, respectively. The lengths of the corresponding amplified fragments are given. (B) Detection of SNF2β$^{-L/-La}$ cells by PCR. SNF2β$^{-L/L:La}$ cells were plated at day −1, treated with 10^{-7} M E2 on days 0 and 1, collected from days 0 to day 11, and stored at −80°C. PCR amplification was carried out on cell lysates (Materials and Methods) with primers TH185 and TG57 (a) and TB82 and TG57 (b), and the PCR products were analyzed by Southern blotting using, as probes, oligonucleotides TH185 (a; exposure time, 2 h) and RR211, which binds specifically the a sequence of the Loxa (La) site (b; exposure time, 16 h).

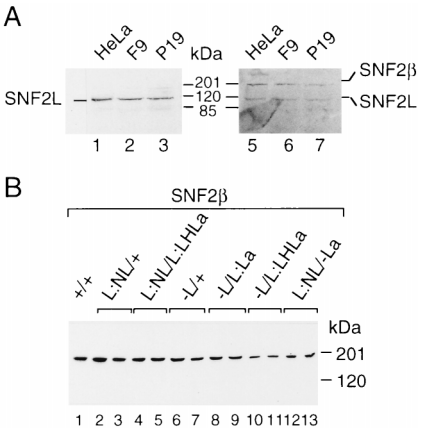

FIG. 7. Western analysis of SNF2β and SNF2L expression. (A) Protein levels of SNF2L and SNF2β in F9, P19, and HeLa cells. The cell extracts were prepared from various cell lines as indicated; 25 μg of each protein extract was loaded per lane and analyzed by Western blotting using the anti-SNF2L monoclonal antibody 2F12, alone (lane 1 to 3) or together with the anti-SNF2β monoclonal antibody 2E12 (lane 4 to 6). (B) Protein levels of SNF2β in WT and mutant F9 cells. A 14-μg aliquot of each protein extract prepared as for panel A was loaded in duplicate and analyzed by Western blotting using the anti-SNF2β monoclonal antibody 2E12.

fragments). RT-PCR assays, using specific primers, clearly showed that this cell line expressed h*SNF2*β RNA but not m*SNF2*β RNA (Fig. 9A; compare lanes 10 and 11 with lanes 7 and 8). Western blot analysis using the monoclonal antibody 2E12, which recognizes an epitope common to mouse and human SNF2β proteins, revealed that the SNF2β levels were similar in the WT and rescued cell lines (Fig. 9D; compare lanes 4 and 5 with lane 1). These results indicate that both *SNF2*β gene alleles can be inactivated, provided the cells express hSNF2β. The growth characteristics of RSNF2β$^{-L/-La}$ cells (increase in cell number, doubling time, and growth inhibition by RA treatment) were similar to those of WT F9 cells (Fig. 9E and data not shown), as well as the RA-induced endodermal-like morphological differentiation (data not shown), indicating that the product of the *SNF2*β gene is indeed necessary for the viability of F9 cells.

DISCUSSION

In this study, we performed conditional disruptions of either one or two alleles of the *SNF2*β-*BRG1* gene in F9 EC cells, using LoxP sites introduced by homologous recombination and an inducible Cre recombinase. The disruption results in a C-terminally truncated protein which, with the exception of motifs I and Ia, lacks all conserved motifs of the SNF2β ATPase domain and therefore should be inactive (30, 43). No F9 cell clone disrupted for the two alleles could be isolated, indicating that SNF2β$^{-/-}$ cells are severely impaired in their proliferation. This was confirmed by a study of the genotype of a SNF2β$^{+/-}$ (SNF2β$^{-L/L:La}$ line) (Fig. 5A) F9 cell population after disruption of the second allele had been induced (Fig. 6). Although SNF2β$^{-/-}$ (SNF2β$^{-L/-La}$) cells could be detected during the 2 days which followed induction of the Cre recombinase by estradiol, their number did not increase with time, and they eventually disappeared after a week, whereas SNF2β$^{+/-}$ cells proliferated normally. Whether the disappearance of the SNF2β$^{-/-}$ cells was due to an arrest of proliferation and/or to the induction of an apoptotic process is unknown. We note, however, that F9 EC cells which have decreased amounts of the SNF2β protein (notably the −L/L:LHLa and L:NL/−La lines, in which one *SNF2*β allele is disrupted, while a resistance gene is inserted in the intron located between exons 3 and 4 of the other allele) have a lower growth rate than WT cells (Fig. 8) and exhibit some morphological abnormalities. In contrast, these cells apparently undergo normal differentiation upon RA treatment. Thus, it appears that there is a more stringent requirement of SNF2β for events related to the proliferation of F9 cells than to the control of transcription of the RA-responsive genes involved in their differentiation. Our data also demonstrate that in F9 cells SNF2β complexes cannot be functionally substituted by SNF2α or by SNF2L complexes.

The crucial need of F9 cells for SNF2β-BRG1 was somewhat unexpected, as the SNF-SWI complex is not required for

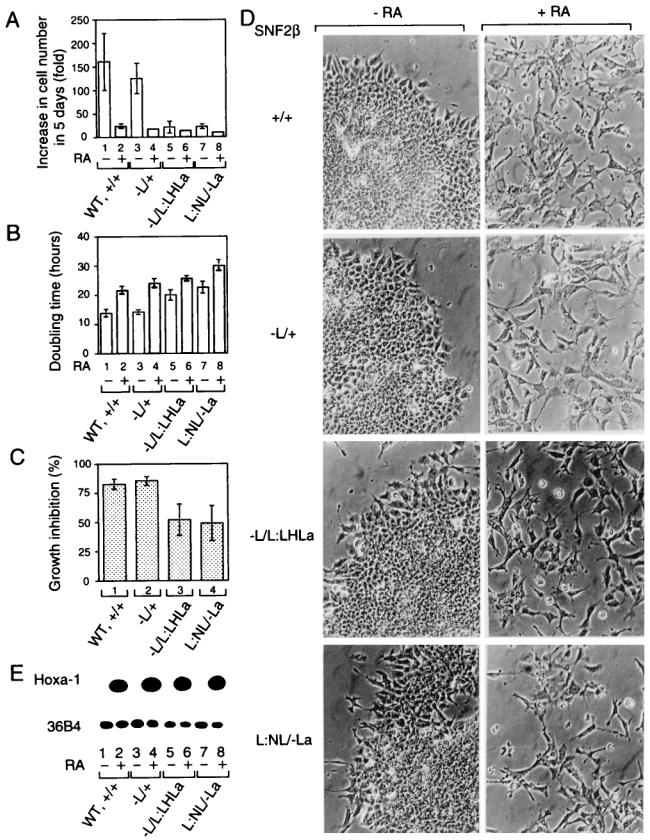

FIG. 8. Comparison of growth characteristics and differentiation of mSNF2β mutant and WT F9 cells. (A) The fold increase in the number of cells during a 5-day period of culture (Materials and Methods) is given for each of the indicated cell lines. The absence (ethanol vehicle) and presence of RA (1 μM) in the culture medium are denoted by − and + RA, respectively. (B) Cell doubling time was calculated from the above increase in cell numbers as described by Kuchler (23). (C) Growth inhibition upon RA treatment. The percentage of growth inhibition corresponds to $(1 - R/C) \times 100$, where R and C represent the ratio between the number of cells present at days 5 and 1 in RA- and ethanol-treated cultures, respectively. In all cases, the bars represent the mean ± standard error of the mean from at least three independent experiments. (D) Differentiation of mutant cells after treatment with RA. Cells cultured for 5 days in the absence (left) or presence (right) of 1 μM RA were photographed under phase-contrast microscopy. (E) Expression of the RA-responsive *Hoxa-1* gene in mutant and WT F9 cells. Total RNA from WT and mutant cells treated with control vehicle (−) or 1 μM RA (+) for 24 h was subjected to RT-PCR analysis for *Hoxa-1*.

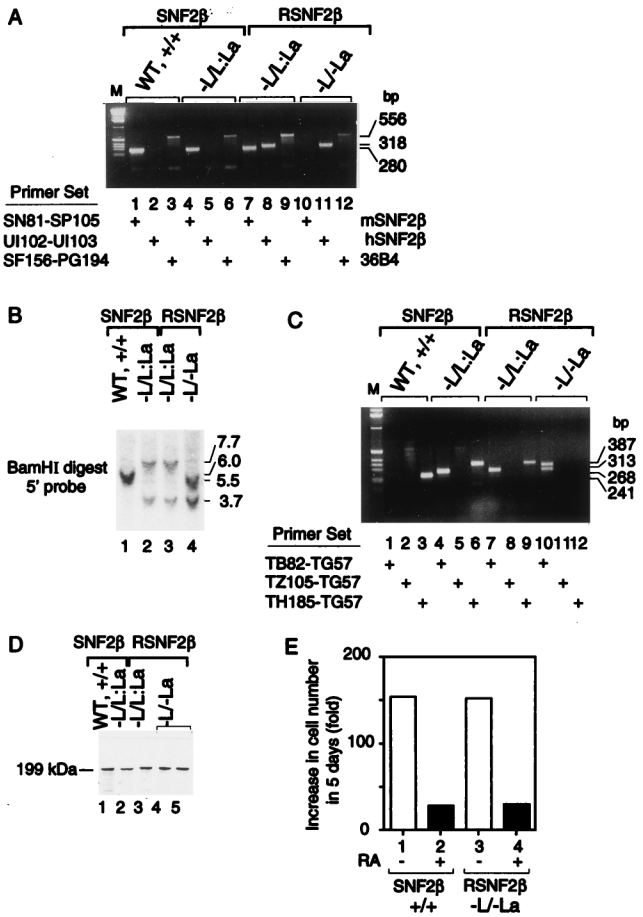

FIG. 9. Inactivation of both *SNF2*β alleles in a cell line expressing hSNF2β. (A) Detection of mouse and human *SNF2*β RNAs by semiquantitative RT-PCR. The primers used to amplify mouse and human *SNF2*β RNAs were SN81 and SP105 (5′-AGCAGGAAGTTAAGCAGGGC-3′) and UI102 and UI103, respectively. The internal control 36B4 RNA was amplified with the primers SF156 (5′-CAGCTCTGGAGAAACTGCTG-3′, nucleotides 290 to 309) and PG194 (5′-GTGTAATCCGTCTCCACAGA-3′, nucleotides 826 to 845) as described previously (2). Lanes 1, 4, and 7, 280-bp fragment from m*SNF2*β RNA; lanes 8 and 11, 318-bp fragment from h*SNF2*β RNA; lanes 3, 6, 9, and 12, 556-bp fragment from 36B4 RNA. The 1-kb ladder DNA (M; GibcoBRL) is shown. (B) Identification of the rescue cell line by Southern hybridization. *Bam*HI-digested genomic DNA of the indicated cell lines was electrophoresed and revealed with the 5′ probe (Fig. 2A and 5B). Lane 1, 6.0-kb fragment; lanes 2 and 3, 3.7- and 7.7-kb fragments; lane 4, 3.7- and 5.5-kb fragments. (C) Identification of rescue cell lines by PCR amplification of genomic DNA. PCR amplification was performed with three sets of primers as indicated. The sizes of the PCR products are given in Fig. 6A and in the legend to Fig. 5D. The 1-kb ladder DNA (M; GibcoBRL) is shown. (D) Detection of SNF2β protein by Western blot analysis. Aliquots of 10 μg of protein extracted from the indicated cell lines were analyzed by Western blotting using the anti-SNF2β monoclonal antibody 2E12 (lanes 4 and 5 are duplicates). (E) Fold increase in the number of cells present after 5 days of culture in the presence and absence of RA for the indicated cell lines. The results correspond to an average (±20%) of two fully independent experiments.

mitotic growth in yeast (see the introduction). However, the recently discovered SNF-SWI-related RSC complex, which is required for yeast mitotic growth, possesses core components which are as similar as or even more closely related to mammalian than to yeast SNF-SWI complex components (see the introduction and reference 7). It is therefore possible that the essential functions played by SNF2β-BRG1 (and presumably by the corresponding complex) in F9 cells are similar to those of the RSC complex in yeast.

The observation that certain mammalian cell lines which efficiently proliferate (e.g., C33A and SW13) contain very little or no SNF2β-BRG1 and SNF2α-brm has suggested that these proteins are not essential for transcription or cell division (31, 47, 53). Moreover, transfection of hSNF2β-hBRG1 and hSNF2α-hbrm into the human carcinoma cell line SW13 induced the formation of nondividing flattened cells, and this formation was dependent on interactions with the retinoblastoma protein (14, 47). These observations, which are in marked contrast with ours, indicate that distinct differentiated cell types have different needs for the multiple mammalian SNF2 complexes (53, 54) (see the introduction). These differential needs may reflect a functional substitution of SNF2 complexes by other mammalian chromatin remodeling complexes (e.g., NURF [see the introduction and reference 53]) in certain cell types. Further genetic studies are clearly necessary to elucidate the physiological role of SNF2β in cellular processes, particularly in those which, besides transcription, also require

remodeling of chromatin. Obviously, conditionally rescued SNF2β$^{-/-}$ F9 lines will be of great help in these studies.

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REFERENCES

1. **Ali, S., Y. Lutz, J. P. Bellocq, M. P. Chenard-Neu, N. Rouyer, and D. Metzger.** 1993. Production and characterization of monoclonal antibodies recognising defined regions of the human oestrogen receptor. Hybridoma **12:**391–405.
2. **Bouillet, P., M. Oulad-Abdelghani, S. Vicaire, J.-M. Garnier, B. Schuhbaur, P. Dollé, and P. Chambon.** 1995. Efficient cloning of cDNAs of retinoic acid-responsive genes in P19 embryonal carcinoma cells and characterization of a novel mouse gene, Stra1 (mouse LERK-2/Eplg2). Dev. Biol. **170:**420–433.
3. **Boylan, J., D. Lohnes, R. Taneja, P. Chambon, and L. J. Gudas.** 1993. Loss of RARγ function by gene disruption results in aberrant *Hoxa-1* expression and differentiation upon retinoic acid treatment. Proc. Natl. Acad. Sci. USA **90:**9601–9605.
4. **Boylan, J., T. Lufkin, C. C. Achkar, R. Taneja, P. Chambon, and L. J. Gudas.** 1995. Targeted disruption of retinoic acid receptor α (RARα) and RARγ results in receptor-specific alterations in retinoic acid-mediated differentiation and retinoic acid metabolism. Mol. Cell. Biol. **15:**843–851.
5. **Cairns, B. R., N. L. Henry, and R. D. Kornberg.** 1996. TFG3/TAF30/ANC1, a component of the yeast SWI/SNF complex that is similar to the leukemogenic proteins ENL and AF-9. Mol. Cell. Biol. **16:**3308–3316.
6. **Cairns, B. R., R. S. Levinson, K. R. Yamamoto, and R. D. Kornberg.** 1996. Essential role of Swp73p in the function of yeast Swi/Snf complex. Genes Dev. **10:**2131–2144.
7. **Cairns, B. R., Y. Lorch, Y. Li, M. Zhang, L. Lacomis, H. Erdjument-Bromage, P. Tempst, J. Du, B. Laurent, and R. D. Kornberg.** 1996. RSC, an essential, abundant chromatin-remodeling complex. Cell **87:**1249–1260.
8. **Carlson, M., and B. C. Laurent.** 1994. The SNF/SWI family of global transcriptional activators. Curr. Opin. Cell Biol. **6:**396–402.
9. **Chambon, P.** 1996. A decade of molecular biology of retinoic acid receptors. FASEB J. **10:**940–954.
10. **Chiba, H., M. Mimatsu, A. Nomoto, and H. Kato.** 1994. Two human homologues of Saccharomyces cerevisiae SWI2/SNF2 and Drosophila brahma are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. Nucleic Acids Res. **22:**1815–1820.
11. **Clifford, J., H. Chiba, D. Sobieszczuk, D. Metzger, and P. Chambon.** 1996. RXRα-null F9 embryonal carcinoma cells are resistant to the differentiation, anti-proliferative and apoptotic effects of retinoids. EMBO J. **15:**4142–4155.
12. **Côté, J., J. Quinn, J. L. Workman, and C. L. Peterson.** 1994. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science **265:**53–60.
13. **Dingwall, A. K., S. J. Beck, C. M. McCallum, J. W. Tamkun, G. W. Kalpana, S. P. Geoff, and M. P. Scott.** 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.
14. **Dunaief, J. L., B. E. Strober, S. Guha, P. A. Khavari, K. Alin, J. Luban, M. Begemann, G. R. Crabtree, and S. P. Goff.** 1994. The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. Cell **79:**119–130.
15. **Elfling, L. K., R. Deuring, C. M. McCallum, C. L. Peterson, and J. W. Tamkun.** 1994. Identification and characterization of *Drosophila* relatives of the yeast transcriptional activator SNF2/SWI2. Mol. Cell. Biol. **14:**2225–2234.
16. **Elgin, S. R. C.** 1996. Heterochromatin and gene regulation in Drosophila. Curr. Opin. Genet. Dev. **6:**193–202.
17. **Grunstein, M.** 1990. Histone function in transcription. Annu. Rev. Cell Biol. **6:**643–678.
18. **Ichinose, H., J.-M. Garnier, P. Chambon, and R. Losson.** 1997. Ligand-dependent interaction between the estrogen receptor and the human homologues of SWI2/SNF2. Gene **188:**95–100.

18a. **Ichinose, H., and Y. Lutz.** Unpublished data.

19. **Imbalzano, A. N., H. Kwon, M. R. Green, and R. E. Kingston.** 1994. Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature **370:**481–485.
20. **Imbalzano, A. N., G. R. Schnitzler, and R. E. Kingston.** 1996. Nucleosome disruption by human SWI/SNF is maintained in the absence of continued ATP hydrolysis. J. Biol. Chem. **271:**20726–20733.
21. **Khavari, P. A., C. L. Peterson, J. W. Tamukun, D. B. Mendel, and G. R. Crabtree.** 1993. BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. Nature **366:**170–174.
22. **Kingston, R. E., C. A. Bunker, and A. N. Imbalzano.** 1996. Repression and activation by multiprotein complexes that alter chromatin structure. Genes Dev. **10:**905–920.
23. **Kuchler, R.** 1977. Development of animal cell populations in vitro, p. 90–113. *In* R. Kuchler (ed.), Biochemical methods in cell culture and virology. Dowden, Hutchinson and Ross Inc., Stroudsburg, Pa.
24. **Kwon, H., A. N. Imbalzano, P. A. Khavari, R. E. Kingston, and M. R. Green.** 1994. Nucleosomal disruption and enhancement of activator binding by a human SWI/SNF complex. Nature **370:**477–481.
25. **Laurent, B. C., and M. Carlson.** 1992. Yeast SNF2/SWI2, SNF5 and SNF6 proteins function coordinately with the gene-specific transcriptional activators GAL4 and Bicoid. Genes Dev. **6:**1707–1715.
26. **Laurent, B. C., I. Treich, and M. Carlson.** 1993. The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. Genes Dev. **7:**583–591.
27. **Le Douarin, B., A. L. Nielsen, J. M. Garnier, H. Ichinose, F. Jeanmougin, R. Losson, and P. Chambon.** 1996. A possible involvement of TIF1α and TIFβ in epigenetic control of transcription by nuclear receptors. EMBO J. **15:**6701–6715.
28. **Metzger, D., J. Clifford, H. Chiba, and P. Chambon.** 1995. Conditional site-specific recombination in mammalian cells using a ligand-dependent chimeric Cre recombinase. Proc. Natl. Acad. Sci. USA **92:**6991–6995.
29. **Moehrle, A., and P. Paro.** 1994. Spreading the silence: epigenetic transcriptional regulation during Drosophila development. Dev. Genet. **15:**478–484.
30. **Muchardt, C., and M. Yaniv.** 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.
31. **Muchardt, C., J.-C. Reyes, B. Bourachot, E. Legouy, and M. Yaniv.** 1996. The hbrm and BRG-1 proteins, component of the human SNF/SWI complex, are phosphorylated and excluded from the condensed chromosomes during mitosis. EMBO J. **15:**3394–3402.
32. **Okabe, I., L. C. Bailey, O. Attree, S. Srinivasan, J. M. Perkel, B. C. Laurent, M. Carlson, D. L. Nelson, and R. L. Nussbaum.** 1992. Cloning of human and bovine homologs of SNF2/SWI2: a global activator of transcription in yeast S. cerevisiae. Nucleic Acids Res. **20:**4649–4655.
33. **Orlando, V., and R. Paro.** 1995. Chromatin multiprotein complexes involved in the maintenance of transcription patterns. Curr. Opin. Genet. Dev. **5:**174–175.
34. **Ostlund Farrants, A. K., P. Blomquist, H. Kwon, and O. Wrange.** 1997. Glucocorticoid receptor-glucocorticoid response element binding stimulates nucleosome disruption by the SWI/SNF complex. Mol. Cell. Biol. **17:**895–905.
35. **Owen-Hughes, T., R. T. Utley, J. Côté, C. L. Peterson, and J. L. Workman.** 1996. Persistent site-specific remodeling of a nucleosome array by transient action of the SWI/SNF complex. Science **273:**513–516.
36. **Paranjape, S. M., R. T. Kamakura, and J. T. Kadonaga.** 1994. Role of chromatin structure in the regulation of transcription by RNA polymerase II. Annu. Rev. Biochem. **63:**265–297.
37. **Pazin, M. J., R. T. Kamakaka, and J. T. Kadonaga.** 1994. ATP-dependent nucleosome reconfiguration and transcriptional activation from preassembled chromatin templates. Science **266:**2007–2011.
38. **Peterson, C. L.** 1996. Multiple SWItches to turn on chromatin? Curr. Opin. Genet. Dev. **6:**171–175.
39. **Peterson, C. L., and I. Herskowitz.** 1992. Characterization of the yeast SWI1, SWI2, and SWI3 genes, which encode a global activator of transcription. Cell **68:**573–583.
40. **Peterson, C. L., and W. Tamkun.** 1995. The SWI-SNF complex: a chromatin remodelling machine? Trends Biochem. Sci. **20:**143–146.
41. **Quinn, J., A. M. Freyberg, R. W. Ganster, M. C. Schmidt, and C. L. Peterson.** 1996. DNA-binding properties of the yeast SWI/SNF complex. Nature **379:**844–847.
42. **Randazzo, F. M., P. Khavari, G. Crabtree, J. Tamkun, and J. Rossant.** 1994. brg-1: a putative murine homologue of the Drosophila brahma gene, a homeotic gene regulator. Dev. Biol. **161:**229–242.
43. **Richmond, E., and C. L. Peterson.** 1996. Functional analysis of the DNA-stimulated ATPase domain of yeast SWI2/SNF2. Nucleic Acids Res. **24:**3685–3692.
44. **Singh, P., J. Coe, and W. Hong.** 1995. A role for retinoblastoma protein in

potentiating transcriptional activation by the glucocorticoid receptor. Nature **374:**562–565.
45. **Strickland, S., and V. Mahdavi.** 1978. The induction of differentiation in teratocarcinoma stem cells by retinoic acid. Cell **15:**393–403.
46. **Strickland, S., K. K. Smith, and K. R. Marotti.** 1980. Hormonal induction of differentiation in teratocarcinoma stem cells: generation of parietal endoderm by retinoic acid and dibutyryl cAMP. Cell **21:**347–355.
47. **Strober, B. E., J. L. Dunaief, S. Guha, and S. P. Goff.** 1996. Functional interactions between the hBRM/hBRG1 transcriptional activators and the pRB family of proteins. Mol. Cell. Biol. **16:**1576–1583.
48. **Tamkun, J. W., R. Deuring, M. P. Scott, M. Kissinger, A. M. Pattatucci, T. C. Kaufman, and J. A. Kennison.** 1992. brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell **68:**561–572.
49. **te Riele, H., E. R. Maandag, A. Clarke, M. Hooper, and A. Berns.** 1990. Consecutive inactivation of both alleles of the pim-1 proto-oncogene by homologous recombination in embryonic stem cells. Nature **348:**649–651.
50. **Tsukiyama, T., and C. Wu.** 1995. Purification and properties of an ATP-dependent nucleosome remodeling factor. Cell **83:**1011–1020.
51. **Tsukiyama, T., C. Daniel, J. Tamkun, and C. Wu.** 1995. ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. Cell **83:**1021–1026.
51a. **vom Baur, E.** Unpublished data.
51b. **vom Baur, E., and Y. Lutz.** Unpublished data.
52. **Wall, G., P. D. Varga-Weisz, R. Sandaltzopoulos, and P. B. Becker.** 1995. Chromatin remodeling by GAGA factor and heat shock factor at the hypersensitive Drosophila hsp26 promoter in vitro. EMBO J. **14:**1727–1736.
53. **Wang, W., J. Côté, Y. Xue, S. Zhou, P. A. Khavari, S. R. Biggar, C. Muchardt, G. Kalpana, S. P. Goff, M. Yaniv, J. L. Workman, and G. R. Crabtree.** 1996. Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. EMBO J. **15:**5370–5382.
54. **Wang, W., Y. Xue, S. Zhou, A. Kuo, B. R. Cairns, R. Tjian, and G. R. Crabtree.** 1996. Diversity and specialization of mammalian SWI/SNF complex. Genes Dev. **10:**2117–2130.
55. **Winston, F., and M. Carlson.** 1992. Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet. **8:**387–391.
56. **Wolffe, A. P.** 1994. Nucleosome positioning and modification: chromatin structures that potentiate transcription. Trends Biochem. Sci. **19:**240–244.
57. **Wolffe, A. P.** 1994. Transcription in tune with histones. Cell **77:**13–16.
58. **Xiao, J. H., I. Davidson, H. Matthes, J. M. Garnier, and P. Chambon.** 1991. Cloning, expression, and transcriptional properties of the human enhancer factor TEF-1. Cell **65:**551–568.
59. **Yoshinaga, S. K., C. L. Peterson, I. Herskowitz, and K. R. Yamamoto.** 1992. Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. Science **258:**1598–1604.