A Tissue-specific, Naturally Occurring Human SNF2L Variant Inactivates Chromatin Remodeling*

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Mammalian genomes encode two initiation switch family chromatin remodeling proteins, SNF2H and SNF2L. In the mouse, SNF2H is expressed ubiquitously, whereas SNF2L expression is limited to the brain and gonadal tissue. This pattern of SNF2L expression suggests a critical role for SNF2L in neuronal physiology. Indeed, SNF2L was shown to promote neurite outgrowth as well as regulate the human engrafted homoeotic genes, important regulators of brain development. Here we identify a novel splice variant of human SNF2L we call SNF2L+13, which contains a nonconserved in-frame exon within the conserved catalytic core domain of SNF2L. SNF2L+13 retains the ability to incorporate into multiprotein complexes; however, it is devoid of enzymatic activity. Most interestingly, unlike mouse SNF2L, human SNF2L is expressed ubiquitously, and regulation is mediated by isoform variation. The human SNF2L+13 null variant is predominant in nonneuronal tissue, whereas the human wild type active SNF2L isoform is expressed in neurons. Thus, like the mouse, active human SNF2L is limited to neurons and a few other tissues.

The mobilization of chromatin is a prerequisite for many nuclear processes requiring access to DNA. Some of these processes include activation (1) and repression of transcription (2), DNA repair (3), heterochromatin formation (4), and others. This mobilization of nucleosomes, termed chromatin remodeling, is catalyzed by the superfamily of ATP-dependent chromatin remodelers, molecular machines that couple the energy released with ATP hydrolysis to nucleosome mobilization (5). In vitro, chromatin remodelers have been characterized by their ability to slide nucleosomes, assemble chromatin, or hydrolyze ATP in a DNA- or nucleosome-dependent manner (6). As a superfamily, chromatin remodelers are defined structurally by the presence of an SNF2 domain, which renders the protein catalytically active. The SNF2 domain is a helicase-like region containing the seven conserved motifs found in classical helicases, including a nucleotide-binding sequence. The SNF2 domain is remarkably conserved with a low tolerance for primary sequence variability. This is likely the consequence of a highly defined three-dimensional structural topology forming the catalytic domain.

The imitation switch (ISWI) proteins compose one family of chromatin remodelers conserved from budding yeast to humans. The ISWI proteins are defined by the presence of the SNF2 helicase and two SANT domains at their C termini (7). They are capable of remodeling and spacing chromatin in vitro. In metazoans, ISWI proteins have been isolated in a variety of multiprotein complexes including Williams syndrome transcription factor-related chromatin remodeling factor (8), WICH (WSTF ISWI chromatin remodeling complex) (9), nucleosome remodeling factor (NURF) (10, 11), nucleolar remodeling complex (12), remodeling and spacing factor (13), chromatin accessibility complex (14), and SNF2H-Cohesin (15). Although the enzymatic activities of these complexes are similar in vitro, it is presumed that in vivo the ISWI-binding partners play a regulatory role in the complex. They appear to direct the ISWI enzyme to specific regions of the genome in a cell cycle-dependent manner (4, 9).

Mammalian genomes encode two ISWI orthologs, SNF2H and SNF2L. The two mammalian ISWI proteins have high sequence homology to each other with ~86% identity. In mouse, the role of SNF2L in neuronal development is suggested by the expression patterns of the two mammalian ISWI proteins. Mouse SNF2H is expressed ubiquitously, whereas SNF2L expression is restricted to the central nervous system and gonadal tissue. The two proteins also exhibit temporal differences in their expression. For example, in neurons SNF2H is expressed in actively dividing embryonic neurons, whereas SNF2L is highly expressed in differentiated postmitotic adult neurons (16). Most interestingly, ectopic expression of wt-SNF2L results in neurite outgrowth in a tissue culture model system. Neurite outgrowth marks a topic expression of wt-SNF2L results in neurite outgrowth in a tissue culture model system. Neurite outgrowth marks a developmental switch from dividing neuroblasts to terminally differentiated neurons. This change in cell morphology is dependent on the chromatin remodeling activity of SNF2L because a SNF2L-K213R enzymatically dead mutant inhibits neurite outgrowth (10).

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†† The abbreviations used are: ISWI, imitation switch; BPTF, bromodomain PHD transcription factor; KAT, reverse transcription; AP, alkaline phosphatase; GR, glucocorticoid receptor; RyR3, ryanodine receptor; NURF, nucleosome remodeling factor; hNURF, human NURF; r, recombinant; IP, immunoprecipitation.
Differences between the two proteins are also suggested by their biochemistry. SNF2H is the catalytic subunit of most of the known ISWI complexes isolated in mammals including WCRF/hACF, chromatin accessibility complex, nucleolar remodeling complex, remodeling and spacing factor, and SNF2H-cohesin (8, 12, 15, 17). Recently, the first SNF2L-associated complex was isolated to homogeneity with a subunit composition reminiscent of the Drosophila NURF complex (10). Termed human NURF (hNURF), this novel human chromatin remodeling complex plays an important role in neuronal physiology. hNURF was shown to regulate the human engrailed (En) genes, which were implicated in brain development. Mice lacking en-1 and/or en-2 display a severe mid-hindbrain malformation and cerebellar dysgenesis (18). Another factor relating the hNURF complex to neuronal physiology is its large subunit, bromodomain PHD transcription factor (BPTF). An isoform of BPTF, called FAC1, was isolated in a screen for proteins accumulating in senile plaques, a pathognomonic feature of Alzheimer’s disease, and BPTF was later found to be highly expressed in other neuropathologies (19–21). In addition, BPTF localizes in vivo in cerebellar and sensory nuclei of the mouse brain, implying a role in the development of these areas (22).

Since the discovery of mRNA splicing by Sharp and co-workers (23), alternative splicing has become a well established form of regulation of protein expression. Alternative splicing is ascribed with creating the enormous genomic diversity observed in higher eukaryotes. One functional consequence of alternative splicing is the generation of regulatory inactive isoforms of proteins. The glucocorticoid receptor (GR) β isoform, lacking the C-terminal ligand binding domain, inhibits the active GR α isoform, possibly through the formation of an inactive heterodimeric complex (24). Likewise, a splice variant of the ryanodine receptor (RyR3) binds to wild type RyR3 and forms a heteromeric channel with reduced calcium sensitivity (25). With regard to splice variant regulation of enzymatic activity, an isoform variant of the interleukin-1 receptor-associated kinase-1 (IRAK1) was shown to include a 30-amino acid inactive heterodimeric complex (24). Likewise, a splice variant of SNF2L isoform 13 includes a nonconserved sequence within the evolutionarily and highly conserved catalytic core domain of SNF2L. We show that the recombinant SNF2L isoform 13 retains the ability to form high molecular weight multiprotein complexes; however, these complexes are enzymatically inactive. Whereas mouse SNF2L expression is restricted to brain and gonadal tissue, human SNF2L is expressed ubiquitously. Most interestingly, the inactive SNF2L isoform 13 is expressed in non-neuronal tissue, thus ensuring that, similar to the mouse, active human SNF2L is found exclusively in brain and a few other tissues. Therefore, mice and humans have evolved two very different approaches to brain-specific expression of the same chromatin remodeler.

MATERIALS AND METHODS

Cell Culture and Transfections—HEK293 cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 1-glutamine, and antibiotics and grown at 37 °C with 5% CO2. SF9 insect cells were maintained in SF-900 (Invitrogen) supplemented with 1-glutamine and baculobiotics (Invitro- gen) and grown at room temperature. HEK293 cells were transfected with FuGENE 6 (Roche Applied Science) according to the manufacturer’s instructions. For stable cell line generation, cells were cotransfected with cDNA of choice along with a puromycin-expressing plasmid for 48 h and transferred to selective media, complete Dulbecco’s modified Eagle’s medium supplemented with puromycin (2.5 μg ml−1). Puromycin-resistant colonies were selected, amplified, and screened for cDNA expression.

Immunoblot Analysis and Antibodies—Proteins were separated by 4–12% gradient Tris-glycine SDS-PAGE (Invitrogen) and transferred to polyvinylidene difluoride membranes in transfer buffer (50 mM Tris, 380 mM glycine, and 10% methanol). Blots were probed with primary antibodies in Tris-buffered saline containing 0.15% Tween 20, followed by alkaline phosphatase (AP)-conjugated anti-rabbit antibodies (Promega). All blots were visualized as described (27). Anti-SNF2L monoclonal antibodies were raised against a recombinant glutathione S-transferase-fused to the N terminus of human SNF2L protein. Anti-exon 13 antibodies were raised against a keyhole limpet hemocyanin-conjugated peptide corresponding to exon 13 of human SNF2L. Polyclonal antibodies were affinity-purified using the peptide as described (27). Anti-FLAG antibodies were purchased from Sigma. Anti-RhAP46 monoclonal antibodies were purchased from Upstate Biotechnology, Inc. Anti-RhAP46 and anti-RhAP46 polyclonal antibodies were purchased from Oncogene.

Purification of SNF2L-associated Complexes—Nuclear extract was prepared from stable WT-SNF2L or SNF2L Δ13 HEK293 cells according to protocol published previously (28). SNF2L-associated complexes were purified as described previously (10).

Restriction Endonuclease-coupled Chromatin Remodeling Assay—Arrays were formed as described (29) by using purified HeLa cell histones resulting in ~50% saturation leading to a 1:1 ratio of cut:uncut DNA. HINDIII digestion of the remaining remodeling complex containing 10 pmol Tris-Cl (pH 7.9), 125 mM KC1, 1 mM ATP, 2.5 mM MgCl2, 1 mM dithiothreitol, 2.5% glycerol, 100 mg ml−1 bovine serum albumin, 0.5 μl of HC-Sail (New England Bioslabs), 0.1 pmol of nucleosomal array (~10,000–50,000 cpm) along with purified proteins or mock elutions, where indicated, were incubated for 90 min at 37 °C. DNA array was then deproteinized by phenol chloroform extraction. Aqueous fractions containing DNA were supplemented with DNA sample buffer followed by electrophoresis on a 1% agarose gel. Gel was placed on a sheet of DE1 chromatography paper (Whatman) and dried by vacuum for 2 h at 60 °C. Dried gel was then analyzed by PhosphoImager (Amersham Biosciences), and bands were quantified by densitometric analysis (29).

Size Exclusion Chromatography—Recombinant FLAG-SNF2L Δ13 or stable cell line immunoprecipitations were fractionated by using a Superdex 75 column (BioRad) eluted (BioRad) in 50 mM sodium phosphate, 0.5 M NaCl, 0.1% Nonidet P-40. 0.3 ml fractions were analyzed by SDS-PAGE followed by immunoblot for SNF2L.

ATPase Assays—Measurement of ATPase activity was performed in 10–μl reactions under the following conditions: 20 mM Tris-Cl, 60 mM KC1, 4% glycerol, 4 mM MgCl2, 1 mM cold ATP, 1 μCi of [γ-32P]ATP, and 1 pmol of each remodeling enzyme. Where indicated, reaction was supplemented with 50 μg of naked DNA or nucleosomes. Reaction was performed at 30 °C for 1 h. Free phosphate and ATP were separated by TLC on PEI-cellulose plates (J. T. Baker Inc.). 1°c bands were visualized by exposure to PhosphoImager cassette (Amerham Biosciences) for densitometric analysis or film (Eastman Kodak).

Splice Variant PCR Assay—Splice variant analysis was performed by using RT-PCR of tissue-specific RNAs (Clontech). Total RNA was reverse-transcribed to cDNA by random priming using random decamers (Ambion) and Superscript II reverse transcriptase (Invitrogen) according to the manufacturer’s protocol. Subsequent PCRs were performed by using specific primers and Taq polymerase (Roche Applied Science). Primer set A annealed to DNA flanking exon 13 resulting in faster or slower migrating bands depending on presence of exon 13: setA-Rev 5′-TTTGGAGGATTTAGTCATGTGTC-3′ setA-For 5′-TTGTGATGCAAACGTGGTGGTCC-3′. Primer set B annealed to exon 13 and exon 12 resulting in an amplicon only in tissues expressing the exon 13 splice variant: setB-For 5′-CTTTGCGAAGATGGACAGATG-3′ setB-Rev 5′-CTTTGGCACAGAATCTGACCTC-3′. PCR products were analyzed by 4% agarose gel electrophoresis followed by ethidium bromide visualization of bands. Sequence confirmation of bands was performed by gel extraction of band (Qiagen), TA cloning of band into the PCR2.1 vector (Invitrogen), and sequencing of vector inserts using M13-forward and M13-reverse sequencing primers.

Expression and Purification of Recombinant SNF2L and SNF2H—For expression of SNF2L and SNF2H, cDNA of SNF2L Δ13 with cloned FLAG epitope was cloned into the pBacnBlue (Clontech) baculovirus expression vectors. FLAG-SNF2H was cloned into the pBacBlue (Invitrogen) expression vector. Viruses expressing the recombinant ISWI proteins were generated according to the manufacturer’s protocol. For protein expression, high titer virus (~108 plaque-forming units) was used to infect adherent SF9 cells at a multiplicity of infection of 10. 72 h post-infection, cells were raised in Insect lysate buffer (20 mM Tris-Cl, 150 mM NaCl, 10% glycerol, 0.2 mM methionine) supplemented with L-glutamine and baculobiotics (Invitrogen) and grown at room temperature. HEK293 cells were transfected with FuGENE 6 (Roche Applied Science) according to the manufacturer’s instructions. For stable cell line generation, cells were cotransfected with cDNA of choice along with a puromycin-expressing plasmid for 48 h and transferred to selective media, complete Dulbecco’s modified Eagle’s medium supplemented with puromycin (2.5 μg ml−1). Puromycin-resistant colonies were selected, amplified, and screened for cDNA expression.
EDTA, 1% Triton X-100 (pH 7.8)), subjected to one freeze-thaw cycle, and Dounce-homogenized. Cell debris was pelleted, and the supernatant was subjected to overnight immunoprecipitation with M2-anti-FLAG antibody-conjugated agarose beads (Sigma). Beads were washed in three consecutive 10 column volume washes containing BC500 supplemented with 0.5% Nonidet P-40, BC750 supplemented with 0.1% Nonidet P-40, and BC1000 supplemented with 0.1% Nonidet P-40. Finally beads were raised in BC100 and subjected to five 1-column volume elutions using BC100 supplemented with 400 μg/ml of FLAG peptide (Sigma). Protein purity was assessed by SDS-PAGE followed by Coomassie Blue staining.

**RESULTS**

**In Silico Identification of a Novel Human SNF2L Splice Variant**—Human SNF2L (GenBank™ accession number NP_620604) was subjected to alignment with other ISWI family members. We compared the amino acid sequences from an evolutionarily diverse cross-section of eukaryotic ISWI proteins including those from *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Xenopus laevis*, mouse, and a second human ISWI ortholog, SNF2H. The conserved regions of the ISWI proteins aligned as expected (data not shown) including the SNF2 helicase and SANT domains. Most interestingly, we identified a 12-amino acid sequence present in human SNF2L but absent in the other ISWI family proteins (Fig. 1A). The amino acid sequence immediately upstream and downstream to the insertion retained high homology with the sequences from the other ISWI family proteins.

We investigated the genomic DNA encoding human SNF2L, and we identified the exonic sequences corresponding to the
nonhomologous insertion and the homologous flanking regions. The insertion is encoded by a single, 36-bp exon corresponding to exon 13 of human SNF2L. The flanking homologous regions of the SNF2L open reading frame reflected exons 12 and 14.

The highly conserved, catalytically active SNF2 helicase is encoded by exons 6–15 (Fig. 1B). Exon 13 incorporation disrupts the primary sequence of the helicase domain, suggesting a possible effect on the enzymatic activity of SNF2L.

We subjected the region surrounding exon 13 to the Jpred secondary structure prediction algorithm (www.compbio.dundee.ac.uk/H11011www-jpred/). Jpred generates a secondary structure prediction based on an alignment of multiple sequences similar to the query sequence, SNF2L in this case. Most interestingly, Jpred predicted a conserved 11-amino acid α-helix composed of exons 12 and 14 of SNF2L. This α-helix is disrupted upon inclusion of exon 13 (Fig. 1A). This structural compromise by exon 13 supports the hypothesis that the SNF2L+13 isoform may have reduced or even absent enzymatic activity.

To confirm further that exon 13 was a bona fide exon, we analyzed the SNF2L putative intron-exon boundary genomic sequence using the Splice Proximal Check program (www.ebi.ac.uk/−thanaraj/SpliceProximalCheck.html). Splice Proximal Check utilizes a discrete set of rules to determine whether a given sequence is a “false site” or a “possible true site” (31). The program confirmed that the DNA immediately 5’ and 3’ to exon 13 are “possible true” splice sites. Indeed, upon sequence inspection, the introns-exon boundaries upstream and downstream of exon 13 contain the standard GT-AG consensus splice sequences (Fig. 1C).

The inclusion of exon 13 into a highly conserved region of SNF2L may represent a regulatory splicing event occurring during SNF2L mRNA maturation. As such, we would expect that within the set of total human transcripts, both isoforms of SNF2L should be expressed. An approximation of total human transcripts is the expressed sequence tag (EST) database. We performed BLAST analysis on the SNF2L mRNA against the EST data base to identify cDNA variants either incorporating or excluding exon 13. As predicted, sequences encoding both SNF2L isoforms are represented within the EST data base (data not shown). The presence of both isoforms further sup-
ports our hypothesis that exon 13 may act as a cis-acting regulatory element in human SNF2L, possibly regulating its enzymatic activity.

A PCR-based Splice Variant Assay—We next asked whether the in silico data could be recapitulated in vivo. We established a PCR-based method for differentiating the transcripts encoding the two isoforms. By using a set of primers (set A) annealing to exons 12 and 14 and another set (set B) annealing to exons 12 and 13 (Fig. 2A), we predicted that we would be able to differentiate the two isoforms by performing RT-PCR on total RNA. We confirmed the validity of this assay by using cDNA constructs encoding either wt-SNF2L (the SNF2L open reading frame lacking exon 13) or SNF2L+13 (the SNF2L open reading frame containing exon 13). By using the set A primers, we note a faster and slower migrating PCR product corresponding to the absence or presence of exon 13, respectively (Fig. 2B, top panel). Representative bands were extracted and sequenced to confirm the presence or absence of the exon 13 splice variant (data not shown). Indeed, the sequence corresponded to that predicted for each amplicon with the slower migrating band including the exon 13 sequences, and the faster migrating band excluding exon 13.

In Vivo Confirmation of a Tissue-specific Exon 13 Splice Variant—We examined total RNA from normal human tissue using the PCR-based spliced variant assay. Previous analysis suggests that in mouse SNF2L is expressed primarily in brain as well as gonadal tissue (16). We predicted a similar expression pattern in human tissues. Tissue-specific human RNA was subjected to reverse transcription followed by conventional PCR by using the primers from set A, set B, and a glyceraldehyde-3-phosphate dehydrogenase loading control primer set.

By using set A, we were able to visualize two differentially migrating bands corresponding to the two exon 13 splice variants (Fig. 2C, top panel). Representative bands were extracted and sequenced to confirm the presence or absence of the exon 13 splice variant (data not shown). Indeed, the sequence corresponded to that predicted for each amplicon with the slower migrating band including the exon 13 sequences, and the faster migrating band excluding exon 13. The set B primers, which anneal to exon 13, further confirm the results of set A (Fig. 2C, middle panel). Tissues containing a prominent amplicon using set B correlate well with tissues generating slower migrating bands using set A. The glyceraldehyde-3-phosphate dehydrogenase controls (Fig. 2C, bottom panel) demonstrated that similar quantities of total RNA were subjected to amplification from the various human tissues.

Contrary to our predictions, these experiments suggested that human SNF2L appeared to have a broad expression pat-
tern consistent with a ubiquitously expressed gene. All tissues inspected exhibit SNF2L transcripts; however, it is noteworthy that only one of the splice variants predominates in each tissue. For example, lung expresses a SNF2L+13 variant, whereas brain expresses the wt-SNF2L variant (Fig. 2C). The tissue specificity of this splice variant led us to ask whether exon 13 regulates the enzymatic activity of SNF2L. Did most tissues express a null isoform of SNF2L, whereas brain expressed the active SNF2L? This would suggest that using alternative splicing, functional human SNF2L recapitulates the brain-specific mouse expression pattern.

Detection of Endogenous SNF2L+13 Protein—Our experiments identified endogenous mRNA transcripts corresponding to both the wt-SNF2L and SNF2L+13 isoforms. However, we wanted to ensure that both isoforms are indeed translated into SNF2L protein. To this end, we generated antibodies against the SNF2L+13 isoform by inoculating rabbits with a peptide corresponding to the exon 13 sequence. The antibodies were subsequently purified by immunoaffinity chromatography by using an exon 13 peptide affinity column. To test the specificity of the antibodies, we subjected purified recombinant isoforms of SNF2L (Fig. 3, lanes 1 and 2) as well as HEK293 transfected and immunoprecipitated FLAG-tagged isoforms of SNF2L (Fig. 3, lanes 3 and 4) to immunoblot with the exon 13 and total SNF2L antibodies. The antibodies demonstrated exquisite specificity detecting only the SNF2L+13 isoforms and not wt-SNF2L.

We then attempted to detect endogenous SNF2L+13 using the exon 13 antibodies. To do this, we electrophoresed hNURF purified from stably expressing FLAG-BPTF HEK293 cells. We demonstrated previously that FLAG-BPTF associates with endogenous SNF2L and RbAP46/48 (10). By using RT-PCR, we found that HEK293 cells express a low amount of the SNF2L+13 isoform (data not shown). Consistent with this, the exon 13 antibodies detected a small amount of SNF2L+13 protein in the purified hNURF (Fig. 3, lane 5). We conclude that the SNF2L+13 transcript is indeed translated into protein.

Exon 13 Severely Affects SNF2L ATPase Activity—To answer the question of whether exon 13 affects SNF2L enzymatic activity, we evaluated the two SNF2L splice variants for ATPase activity. To this end we expressed and purified the two splice variants of recombinant full-length SNF2L (rSNF2L) using a baculovirus expression system. As a control, we generated a recombinant SNF2H (rSNF2H), the second human ISWI splice variant affecting recombinant SNF2L chromatin remodeling activity. Recombinant human ISWI proteins were assayed for restriction enzyme couple chromatin remodeling activity. rWT-SNF2L (A) and rSNF2H (C) were able to remodel chromatin in an ATP-dependent manner (solid line). Chromatin remodeling activity was absent in the −ATP samples (dotted line). As little as 1 pmol of each remodeler demonstrated potent remodeling activity. The SNF2L splice variant, rSNF2L+13, was unable to remodel chromatin even at the highest concentration of 3 pmol (B). Graphs depict the results of representative, reproducible data.

As expected, the ATPase activities in the absence of nucleosome substrate were almost undetectable in the three ISWI proteins as well as the mock IP (Fig. 4B, lanes 1–4). The addition of DNA resulted in a moderate stimulation of the ATPase activity of rSNF2Lwt and rSNF2H compared with mock IP (Fig. 4B, lanes 5, 6, and 8). Most interestingly, rSNF2L+13 exhibited no stimulation upon addition of DNA (Fig. 4B, lane 7). This effect was more pronounced upon addition of nucleosomes. With nucleosomes, rSNF2H and rSNF2Lwt displayed a potent stimulation of ATPase activity compared with mock IP (Fig. 4B, lanes 9, 10 and 12). Again, stimulation of the ATPase activity of rSNF2L+13 remained undetectable (Fig. 4B, lane 11). Densitometric analyses of the ATPase assays performed in triplicate indicated a 4- and 9-fold increase in ATPase activity with rSNF2Lwt and SNF2H, respectively, upon addition of nucleosomes (Fig. 4C). However, rSNF2L+13 displayed no significant quantitative change in activity. We concluded that exon 13 abrogated the ATPase activity of recombinant SNF2L.

Exon 13 Abolishes SNF2L Chromatin Remodeling Activity in Vitro—ISWI family members possess an intrinsic ATP-dependent chromatin remodeling activity. Because exon 13 effectively abolished ATPase activity, we asked whether it would also interfere with the ability of SNF2L to remodel chromatin. To answer this question, we employed the restriction enzyme-mediated chromatin remodeling assay (29). For this assay, we chromatinized a linear fragment of DNA encoding an array of 11 copies of the 5 S nucleosome incorporation sequence. The DNA fragment also encodes a SalI restriction endonuclease site at the dyad symmetric axis. Approximately half of the chromatinized arrays position a nucleosome at the SalI site protecting it from digestion. Addition of a chromatin remodeler slides the nucleosomes, thus releasing the protection of the SalI site and exposing it to digestion. Remodeling is observed as a decrease in the fraction of uncut DNA in an ATP-dependent manner as visualized by agarose gel electrophoresis followed by densitometric quantification.

We tested the remodeling activity of rSNF2Lwt, rSNF2L+13, and rSNF2H across three different concentra-
have been microsequenced and comprise degradation products of either FLAG-SNF2L, FLAG-SNF2L/H11001 or BPTF.

The ATP lanes from the cut band (lane 1). This experiment marks the first demonstration of remodeling activity by a recombinant SNF2L. The rSNF2L variant, however, was unable to remodel chromatin, even at the highest concentrations of remodeler (Fig. 5B). We cannot rule out the possibility that remodeling activity would be detectable at higher concentrations suggesting a hypoactive enzyme rather than a null; however, higher concentrations were not possible per our reaction conditions. Moreover, these would almost certainly involve supra-physiologic concentrations of SNF2L/H11001. The abrogation of ATPase and chromatin remodeling activities by exon 13 are consistent with the hypothesis that humans generate an endogenous null variant of the SNF2L chromatin remodeler.

SNF2L+13 Incorporates into Multiprotein Complexes—At physiological levels, ISWI family members exist in the context of multiprotein complexes rather than as monomeric proteins. The inactive SNF2L splice variant SNF2L+13 could act as a negative regulator of SNF2L complexes by binding to and inactivating the wild type remodeling complex at large. To accomplish this, it is critical that exon 13 does not adversely affect the three-dimensional structure of SNF2L outside of the catalytic core domain. Although our data demonstrated exon 13-mediated abrogation of enzymatic activity, we wanted to ensure that this was because of a localized disruption of the SNF2 helicase domain rather than a protein folding error resulting in a global change in SNF2L three-dimensional topology.

To address this, we compared the abilities of the two SNF2L variants to incorporate into multiprotein complexes in vivo. The assumption was that complex incorporation is an indicator of appropriate global SNF2L folding. Two different stable FLAG-SNF2L-expressing HEK293 cell lines were constructed, one for each splice variant. Nuclear extract was prepared from the two cell lines, subjected to immunoprecipitation with anti-FLAG antibodies, and washed stringently, and FLAG-SNF2L complexes were labeled either wtSN2L.com or SNF2L+13.com.

We analyzed the molecular weights of the native immunoprecipitated complexes by size exclusion chromatography. The observation of high molecular weight native SNF2L would be an indication of incorporation into one or more multiprotein complexes. As a control, we analyzed recombinant SNF2L to assess the elution profile of noncomplexed monomeric SNF2L. Immunoblot analysis of elutions following gel filtration of rSNF2L indicated a native molecular mass of ~230 kDa (Fig.
6A, bottom panel). This is approximately twice the predicted molecular weight of SNF2L, suggesting a homodimer or a nonspherical shape. Immunoblot analysis of the purified wtSNF2L.com following gel filtration indicated the presence of high molecular weight complexes ranging in size from 1 MDa to 230 kDa (Fig. 6A, top panel). The null variant, SNF2L+13.com purified from HEK293 cells, also eluted at very high molecular weights from the gel filtration column in a manner virtually identical to wtSNF2L.com (Fig. 6A, middle panel). These data indicate that SNF2L+13, although lacking enzymatic activity, retains the ability to bind to and incorporate into high molecular weight multiprotein complexes. We conclude that the consequence of exon 13 inclusion is localized to the catalytic domain and does not affect the general folding of SNF2L.

wt-SNF2L and SNF2L+13 Coimmunoprecipitate Similar Associated Polypeptides—The two SNF2L splice variants exist in high molecular weight complexes in vivo suggesting that SNF2L+13 can act as a negative regulator of chromatin remodelers by generating inactive complexes. We wanted to know whether the two isoforms varied in their complex association. To assess this, we subjected the eluted proteins from the two stable cell lines to SDS-PAGE, and we visualized total protein by silver stain analysis. Compared with mock IP (Fig. 6B, lane 1), a number of distinct polypeptides coeluted with both FLAG-WT-SNF2L and FLAG-SNF2L+13 (Fig. 6B, lanes 2 and 3). These included BPTF and RbAP46/48, components of the human NURF chromatin remodeling complex confirmed by Western blot (data not shown), along with two other polypeptides at 148 and 160 kDa. These latter two polypeptides are novel SNF2L-associated proteins, which will be described in a future study. We conclude that, although differing enzymatically, the macromolecular structure of the SNF2L splice variants are virtually identical allowing for incorporation into similar complexes.

SNF2L+13.com Lacks Chromatin Remodeling Activity—In order to act as a negative regulator of the complex, SNF2L+13 must be inactive, incorporate into chromatin remodeling complexes, and subsequently inactivate those complexes. Although we proved the first two requirements, we wanted to analyze the activity of cell-purified SNF2L-containing complexes for enzymatic activity. To this end, we assayed for chromatin remodeling activity in mock immunoprecipitations, wtSNF2L.com, and SNF2L+13.com. As expected, wtSNF2L.com exhibited substantial chromatin remodeling activity relative to mock as evidenced by the decrease in the uncut band (Fig. 6C, lanes 1 and 3, top panel). This activity is ATP-dependent as would be predicted for an ATP-dependent chromatin remodeler (Fig. 6C, bottom panel). In contrast, SNF2L+13.com lacked any measurable chromatin remodeling activity and was comparable with the mock immunoprecipitation (Fig. 6C, lanes 1 and 2, top panel). In effect, SNF2L+13 associated chromatin remodeling complexes lack remodeling activity in a manner consistent with the SNF2L+13 isoform being a negative regulator.

**DISCUSSION**

To our knowledge, this work is the first description of a null isoform of a chromatin remodeling enzyme negatively regulating its associated multiprotein complexes. Previous examples of null isoforms, sometimes called dominant negatives, have been shown to affect their wild type counterparts by altering binding, specificity, or enzymatic activity. These include the GR β transcription factor, the RyR3 calcium channel, and the IRAK1 kinase (24–26). We predict that alternatively spliced null isoforms of enzymes will prove to be a common regulatory mechanism of multiprotein enzymatic complexes.

Here we present a novel splice variant of SNF2L called SNF2L+13. It is the predominant form of SNF2L in most human non-neuronal tissues. SNF2L+13 includes a 12-amino acid insert within the SNF2 helicase catalytic domain interrupting a predicted conserved α-helix. By using recombinant baculovirus-expressed full-length wt-SNF2L and SNF2L+13 proteins, we showed that exon 13 effectively reduced both the ATPase and chromatin remodeling activities to below detectable levels. However, exon 13 does not interfere with the ability of SNF2L+13 to incorporate into hNURF and other multiprotein complexes in vivo. Consistent with observations from the recombinant human SNF2L proteins, cell-purified complexed SNF2L+13, but not wt-SNF2L, lacked the ability to remodel chromatin.

The data presented describe two very different mechanisms for central nervous system expression of the mammalian ISWI family chromatin remodeler, SNF2L. Mice accomplish this by regulating absolute transcript levels in a tissue-specific manner. By Northern blot, one can identify a prominent SNF2L transcript in cortex and cerebellum but not in heart, lung, spleen, or kidney (16). Humans regulate SNF2L expression by alternative splicing. By Northern blot, one detects SNF2L transcript in many tissues (data not shown). However, we show that by RT-PCR, lung, breast, kidney, and ovary express the catalytically inactive SNF2L+13 isoform. The active isoform of SNF2L is expressed in brain and skeletal muscle.

Why does the cell employ two different methods of expressional regulation to reach the same end? We hypothesize that these two methods have very different consequences for the cell. For example, neither mouse nor human lung expresses active SNF2L. Evidence does suggest that human lung expresses the SNF2L-binding partners in the hNURF complex, BPTF and RbAP46/48 (33, 34). At hNURF-responsive promoters in the lung, we theorize that there can be a fully assembled inactive hNURF recruited to and occupying the promoter at the
expense of other active remodelers/modifiers. By blocking out these other remodelers/modifiers, the inactive hNURF may actually repress transcription from these promoters (Fig. 7). At the identical promoter in human brain, we would find a catalytically intact hNURF actively enabling transcription.

We believe that SNF2L and its splice variants may prove to be a prime example of a real, profound difference between mice and humans. These two organisms diverged ~75 million years ago with mice remaining relatively similar to their common mammalian ancestor, whereas humans evolved significant organismal complexity (35). Perhaps the most significant difference lies in the nervous systems of the two species. Humans have evolved very large brains capable of spoken language, tool construction, and the other consequences of intelligence. The gene catalogs for the two organisms do not suggest differences between the organisms. As of September 2002, mice and humans are conservatively predicted to each encode around 22,000 genes (35). The two genomes are almost completely identically hNURF actively enabling transcription.

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A Tissue-specific, Naturally Occurring Human SNF2L Variant Inactivates Chromatin Remodeling

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