The *Drosophila* snr1 and brm Proteins Are Related to Yeast SWI/SNF Proteins and Are Components of a Large Protein Complex

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During most of *Drosophila* development the regulation of homeotic gene transcription is controlled by two groups of regulatory genes, the trithorax group of activators and the Polycomb group of repressors. *brahma (brm)*, a member of the trithorax group, encodes a protein related to the yeast SWI2/SNF2 protein, a subunit of a protein complex that assists sequence-specific activator proteins by alleviating the repressive effects of chromatin. To learn more about the molecular mechanisms underlying the regulation of homeotic gene transcription, we have investigated whether a similar complex exists in flies. We identified the *Drosophila* snr1 gene, a potential homologue of the yeast SNF5 gene that encodes a subunit of the yeast SWI/SNF complex. The snr1 gene is essential and genetically interacts with *brm* and *trithorax (trx)*, suggesting cooperation in regulating homeotic gene transcription. The spatial and temporal patterns of expression of snr1 are similar to those of *brm*. The snr1 and brm proteins are present in a large (>2 × 10^6 Da) complex, and they co-immunoprecipitate from *Drosophila* extracts. These findings provide direct evidence for conservation of the SWI/SNF complex in higher eucaryotes and suggest that the *Drosophila* brm/snr1 complex plays an important role in maintaining homeotic gene transcription during development by counteracting the repressive effects of chromatin.

INTRODUCTION

The specification and maintenance of cell fates is critical to the development of multicellular organisms. One class of genes that plays critical roles in this process, the homeotic genes of the Antennapedia complex (ANT-C) and the bithorax complex (BX-C), encode homeodomain-containing transcription factors that determine the identities of segments along the body axis in *Drosophila* (Duncan, 1987; Kaufman et al., 1990) and in other animals (Kenyon, 1994; Krumlauf, 1994). The transcription of ANT-C and BX-C genes must be regulated precisely during development, as their misexpression can lead to dramatic alterations in cell fate. Relatively early in embryogenesis, the initial patterns of homeotic gene transcription are established by DNA-binding regulatory proteins encoded by segmentation genes (for review, see Harding and Levine, 1988; Ingham, 1988). Later in development, these patterns are maintained by two opposing groups of trans-acting regulatory genes: the Polycomb group of repressors and the trithorax group of activators. The regulation of homeotic gene expression thus consists of two major phases: establishment by segmentation genes and maintenance by Polycomb and trithorax group genes.

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Polycomb group members (including Polycomb, Polycomblike, Posterior sex combs, extra sex combs, poly-homotect, and others) repress the transcription of ANT-C and BX-C genes outside their normal domains of expression (Wedeen et al., 1986; McKeon and Brock, 1991; Simon et al., 1992; Paro, 1993). In contrast, the members of the trithorax group (including trithorax, ash1, ash2, brahma, and others) maintain the transcription of homeotic genes where they are required (Ken nison, 1993). Both groups of regulatory genes are thus required to maintain the determined states of cells during development. Although the mechanisms of action of Polycomb and trithorax group proteins have not been firmly established, some appear to act by influencing chromatin structure. Several of the Polycomb group proteins are thought to form large complexes (Franke et al., 1992) that can affect local higher-order chromatin structure (Messmer et al., 1992; Fauvarque and Dura, 1993; Rastelli et al., 1993). Furthermore, the Polycomb protein contains a short segment, the chromodomain, which is conserved in the Drosophila HP1 protein, a component of heterochromatin (Paro and Hogness, 1991). Based on these and other observations, it has been suggested that Polycomb, together with other Polycomb group proteins, packages inactive homeotic genes into heterochromatin-like complexes early in development, thereby preventing their subsequent transcription (Paro, 1993). In addition to their silencing effect on transcription of the homeotic genes, members of the Polycomb group have also been implicated in regulating some of the earliest zygotic transcriptional events in embryogenesis (Paro and Zink, 1992; Pelegri and Lehmann, 1994).

Recent studies of brahma (brm), a member of the trithorax group, have provided additional evidence that alterations in chromatin structure are critical for the maintenance of homeotic gene transcription. brm mutations strongly suppress mutations in Polycomb and cause developmental defects similar to those arising from the failure to express homeotic genes after embryogenesis (Kennison and Tamkun, 1988; Tamkun et al., 1992; Brizuela et al., 1994). A possible mechanism of action of the brm protein has been suggested by its similarity to a yeast transcriptional activator SWI2/SNF2. Both brm and SWI2/SNF2 contain six blocks of sequence similar to those found in DNA-dependent ATPases and helicases. SWI2/SNF2 is a subunit of a complex that contains at least 10 subunits, including the SW11, SW13, SNF5, and SNF6 proteins, and has a native molecular mass of \(2 \times 10^6\) Da (Cairns et al., 1994; Cote et al., 1994; Peterson et al., 1994). This SW1/SNF complex does not appear to bind DNA directly, but assists a wide variety of DNA-binding regulatory proteins, including GAL4, SW5, and others, to activate the transcription of their target genes (Carlson and Laurent, 1994). Both genetic and biochemical studies have suggested that the SW1/SNF complex contributes to transcriptional activation by overcoming the repressive effects of chromatin on transcription (Hirschhorn et al., 1992; Winston and Carlson, 1992).

Is a Drosophila counterpart of the yeast SWI/SNF complex involved in the maintenance of homeotic gene regulation, perhaps by alleviating the repressive effects of Polycomb group members? Although brm is the closest Drosophila relative of SWI2/SNF2, their functional relationship remains unclear. The DNA-dependent ATPase domains of the brm and SNF2/SWI2 proteins are functionally interchangeable (Elfring et al., 1994); it is thus likely that brm and SWI2/SNF2 play similar roles in transcriptional activation. However, the brm gene is unable to complement a swi2/snf2 null mutation in yeast (Elfring et al., 1994), suggesting that there may be important differences between the two proteins. Consistent with this possibility, the brm and SWI2/SNF2 proteins are not highly related outside the DNA-dependent ATPase domain; these divergent regions are thought to contribute to the functional specificity of SWI2/SNF2 family members by mediating interactions with other proteins.

To further explore the role of brm in homeotic gene regulation, we examined whether the brm protein is part of a Drosophila counterpart of the yeast SWI/SNF complex. We also searched for additional Drosophila relatives of yeast genes encoding components of the SWI/SNF complex. Our initial attempts to identify Drosophila homologues of the yeast SNF5 and SNF6 genes by low-stringency hybridization and by complementation of null mutants were unsuccessful (Dingwall and Scott, unpublished results). As an alternative approach, we searched for Drosophila genes related to ini1, a distant human relative of the yeast SNF5 gene (Kalpana et al., 1994). The ini1 gene was recently identified in a yeast two-hybrid screen for proteins that directly interact with HIV integrase. The ini1 protein activates transcription of a GAL1-lacZ reporter when it is tethered to DNA via a GAL4 DNA binding domain, suggesting that ini1 may also be involved in transcriptional activation (Kalpana et al., 1994). In this report, we describe the identification and characterization of a Drosophila relative of ini1, that we have named snr1, for snf5-related 1. We find snr1 to be an essential gene and that both the snr1 and brm proteins are part of a large complex. Our findings provide direct evidence that a relative of the yeast SWI/SNF complex is present in Drosophila and is involved in regulating the transcription of homeotic and other genes during development.

MATERIALS AND METHODS
Isolation of cDNA Clones and DNA Sequence Analysis
A 1-kb ini1 partial cDNA fragment was labeled by random priming (Sambrook et al., 1989) and hybridized to a Drosophila cDNA library.

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obtained from larval imaginal discos (Brown and Kafatos, 1998). Approximately 500,000 recombinants were screened using low stringency conditions. The filters were incubated for >18 h at 55°C in 5X SSPE, 5X Denhardt’s, 200 μg/ml salmon sperm DNA, 0.5% sodium dodecyl sulfate (SDS), 10% dextran sulfate, and washed three times for 30 min at room temperature in 2X SSC, 0.5% SDS. The full DNA sequence on both strands was obtained using overlapping subclones and sequence-specific primers (Operon, Alameda, CA) either by the dideoxy procedure with the Sequenase kit (United States Biochemical, Cleveland, OH) or by automated sequencing on an Applied Biosystems apparatus (ABI, Columbia, MD). The snrl sequence was used to search the GenBank and EMBL databases for related genes by the FASTDB method (IntelliGenetics, Mountain View, CA). The National Center for Biotechnology Information BLAST electronic mail server was used to identify sequences related to snrl in the GenBank 86.0, EMBL 40.0, PIR 41.1, and SWISS-PROT 30.0 data bases, using the tblastn and blastp programs (Altschul et al., 1990) and the BLOSUM62 matrix (Henikoff and Henikoff, 1992). Alignments were performed using the BESTFIT program (Wisconsin Genetics Computer Group) and Pustell matrix analysis (MacVector 4.1.1 software, IBI, New Haven, CT; Pustell and Kafatos, 1982). The snrl sequence has been deposited into the GenBank database (accession number U28485).

**Pulsed-Field Gel Analyses**

High molecular weight chromosomal DNA from several P-element Drosophila lines was prepared for pulsed-field gel analysis (D. Garza, personal communication). Frozen adult flies (100) of the appropriate genotype were ground to a fine powder with a mortar and pestle. The powder was mixed with 3 ml ice cold nuclear isolation buffer (NIB; 10 mM Tris, pH 8.5; 60 mM NaCl; 10 mM EDTA; 0.15 mM spermine; 0.15 mM spermidine; 0.5% Triton X-100) and dounce homogenized. The suspension was centrifuged at 3000 rpm in an IEC cell centrifuge at 4°C for 15 s. The supernatant containing cell nuclei was removed to prechilled 1.5 ml microcentrifuge tubes and centrifuged at ~3000 rpm for 5 min at 4°C. The supernatant was removed and the nuclei pellet gently resuspended in 500 μl of NIB and centrifuged as before. The nuclei pellet was gently resuspended in 100 μl NIB and warmed briefly to 37°C, and then mixed with 150 μl of 1.2% low melting point agarose, 0.125 M EDTA. The mixture was poured into plug molds and allowed to harden at 4°C. Plugs were prepared for electrophoresis as described (Gemmill et al., 1992). The chromosomal DNA was digested with either NcoI or XbaI (Boehringer Mannheim, Indianapolis, IN), electrophoresed through an 0.8% agarose gel with an 8 s pulse time, then transferred to a Hybond-N (Amersham, Arlington Heights, IL) nylon filter. Hybridization was performed using standard conditions (Sambrook et al., 1989).

**Isolation of DNA, RNA, and Nucleic Acid Blot Analyses**

Chromosomal Drosophila DNA isolated from the P-element excision lines was examined by polymerase chain reaction (PCR) analysis (Rasmussen et al., 1993) using primers generated from the sequence of the snrl cDNA or from the terminal ends of the P-element (IR primer; Rasmussen et al., 1993). Chromosomal DNA used for Southern blots of the P-element excision lines was prepared essentially as described by Roberts (1986). Hybridization of the snrl cDNA to genomic DNA blots was carried out as described above. RNA was isolated and analyzed by Northern blotting as described by Tamkun et al. (1992). The RNA blot was simultaneously hybridized with random-primed cDNA probes for both snrl and brm, using standard conditions.

**Production of Antibodies, Western Blotting, and Immunostaining of Embryos**

A 940-bp SacII–EcoRI fragment of the snrl cDNA (nucleotide 439 to an EcoRI site in the polylinker) was cloned into the T7 promoter vector pATH10 by addition of EcoRI linkers to the SacII site. Induction and purification of inclusion bodies was performed as previously described (Carroll and Laughon, 1987). Rats were injected with 50 μg protein per boost using the Ribi Adjuvant System (Ribi). Whole anti-sera was used at a dilution of 1:250 to 1:500 for localization of the snrl protein in Drosophila embryos and at a dilution of 1:450 or 1:500 for Western immunoblot analyses.

Extracts were prepared from staged Oregon-R embryos for Western analysis. Embryos were dechorionated, washed, and homogenized in (1:1 w/v) 1X sample buffer (2.5% SDS, 10% glycerol, 62.5 mM Tris, pH 6.8). Samples were then boiled for 2 min followed by microcentrifugation for 5 min at room temperature to pellet insoluble material. Samples were electrophoresed through 11% SDS-polyacrylamide gels as above, and transferred by electroblootting to nitrocellulose (Towbin et al., 1979). Filters were blocked for 30 min at room temperature in 1X Tris buffered-saline (TBS; 100 mM Tris, pH7.5, 0.9% NaCl), with 10% nonfat dry milk, 3% BSA, and 4% normal goat serum. Incubation with rat anti-snrl serum was carried out in blocking buffer (without milk) overnight at 4°C. The filters were washed in TBS (0.1% Tween 80) and incubated at room temperature for 30 min with goat anti-rat secondary antibody (Jackson Immuno Research Labs, West Grove, PA) conjugated to horseradish peroxidase at a dilution of 1:10,000. Filters were washed as above and developed with the enhanced chemiluminescence (ECL) method (Amersham).

Embryos used for whole mount antibody detection of snrl protein were fixed and stained as described by Reuter et al. (1990). Antibody-stained embryos were viewed on a Zeiss Axiohot microscope (Thornwood, NY) with Nomarski optics and photographed on Kodak Ektachrome 64 Tungsten film ( Rochester, NY).

**Fly Strains and Genetic Manipulations**

All fly strains were raised at 25°C, unless otherwise noted. The P-element enhancer trap lines, including AS1319, were cytologically mapped by T. Laverty (University of California, Berkeley, CA) and were generously provided as part of the Drosophila Genome Project. During the course of this work, we mapped the lethality of the P-element strain AS1319 to the snrl gene, and for this reason named this allele snrlP1. Excision/transposition of the P-element in AS1319 was induced after the introduction of a stable source of transposase from Firy+ [Δ2,3(998)] (Laski et al., 1986). Twenty females of the genotype snrlP1/TM3, ry506 were mated with 20 males of the genotype snrlP1/TM3, ry506. Male progeny (200) of the genotype snrlP1/TM3, ry506 were then mated with virgin females of the genotype TM3, ry506/TM6B, and ry+ progeny were selected. Eighty independent ry+ progeny were then analyzed by genetic complementation of the lethality associated with the snrlP1 allele. These potential new alleles of snrl were also molecularly characterized by PCR and Southern blot analyses.

Interaction crosses between snrl, brm, and trx were carried out essentially as described (Kennon and Tamkun, 1988; Tamkun et al., 1992), except that crosses were maintained at 23°C. The snrlP1neo stock is a viable excision line obtained as described above, that fully complements the lethality of both snrlP1 and snrlP2.

**Superose 6 Chromatography**

Nuclear proteins were obtained from Drosophila embryos as described by Kamakaka et al. (1991). The nuclear extract was applied to a Sepharose C25 column equilibrated in 50 mM sodium phosphate, pH 7.8, 425 mM NaCl and the excluded protein was concentrated to approximately 4 mg/ml. Eight hundred micrograms of this material was applied to a Superose 6 fast-performance liquid chromatography (FPLC) column, with elution of the protein in 50 mM Nucleic acids, proteins, and nucleotides are often isolated and characterized using gel electrophoresis, blotting, and antibody detection methods. The text describes the isolation and analysis of DNA, RNA, and nucleic acid blots, as well as the production and use of antibodies for protein detection. The methods used include polymerase chain reaction, electrophoresis, and Western blotting. This information is essential for the study of gene expression and protein function in biological systems.
sodium phosphate, pH 7.8, 425 mM NaCl. brm and snr1 proteins in the 0.5-ml fractions were detected by immunoblotting as described above.

**Epitope-tagging of the brm Protein and Immunoprecipitation Assays**

A 14.4-kb BamHI–EcoRI genomic DNA fragment spanning the brm gene (Brizuela et al., 1994) was modified using PCR to create a brm transgene encoding a protein in which the C-terminal two residues of the brm are replaced by the sequence SSSPPYDPVPDYSHSHHHHHH. This tag contains the 9-amino acid epitope of the influenza hemagglutinin (HA) protein, which is recognized by the monoclonal antibody 12CA5(BABCo). The modified fragment was subcloned into the P-element transformation vector CaSpeR and transformed into the germ line of Df(1)w67c2, y embryos as described previously. Five independent transgenic lines were generated and found to complement the recessive lethality of an extreme brm allele. A transgenic line (Df(1)w67c2, y P[+u 9222–3 brm-HA]) homozygous for an insertion of the transgene on the X chromosome was used for the studies described below.

Native protein extracts were prepared from either control (Df(1)w67c2,y) or transgenic (Df(1)w67c2, y P[+u 9222–3 brm-HA]) embryos as follows. Embryos (0–12 h) were dechorionated in 50% bleach for 2 min and washed extensively in 0.7% NaCl, 0.03% Triton X-100. Approximately 0.5 g embryos were homogenized in an equal or greater volume of 40 mM N-2-hydroxyethylpiperezine-N'-2-ethanesulfonic acid (HEPES), pH 7.0, 350 mM NaCl, 0.1% Tween-20, 10% glycerol, 100 μg/ml phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 1 μg/ml pepstatin, and 1 mM benzamidine and centrifuged in a TLA45 Beckman microfuge rotor at 45,000 rpm 30 min. Avoiding the top lipid layer, the supernatant was transferred to new tubes and stored at ~8°C. The 12CA5 monoclonal antibody recognizes the brm protein in transgenic, but not Df(1)w67c2, y, protein extracts by Western blotting.

brm and associated proteins were immunoprecipitated from total embryo extracts using the 12CA5 ascites fluid. Ascites fluid (20 μl) was incubated for 1 h at 4°C with approximately 50 μl of Protein A-Affi-prep beads (Bio-Rad, Richmond, CA) and 130 μl of IP buffer (10 mM HEPES, pH 8.0, 1 mM EDTA, 10% glycerol, 50 mM NaCl); unbound antibody was then removed by washing with IP buffer. Twenty-five microliters of antibody-adsorbed beads was added to 300 μg of embryonic protein extract, brought to 200 μl total volume with IP buffer, and incubated at 4°C with rocking for 2 h. After centrifugation and extensive washing with IP buffer, bound material was eluted with 100 mM glycine, pH 2.75, and neutralized with 1/20 volume of 1 M NaH2PO4, pH 8.0. Unbound and bound proteins were fractionated on a 8% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane with the addition of 0.1% SDS. The upper and lower halves of the Western blot were probed with antibodies against brm and snr1, respectively.

**RESULTS**

**Molecular Cloning of snr1**

A partial ini1 cDNA encompassing the C-terminal two-thirds of the predicted ini1 open reading frame was hybridized to *Drosophila* genomic DNA under conditions of low stringency. A single EcoRI restriction fragment hybridized to the ini1 fragment (our unpublished results), suggesting that only one close relative of ini1 is present in flies. To isolate cDNA clones corresponding to this gene, we screened ~500,000 cDNA clones from a larval imaginal disc library (Brown and Kafatos, 1988) with the ini1 fragment. Eleven clones were isolated and analyzed; each contained an insert of approximately 1.4 kb. Hybridization of cDNA clones to RNA blots of poly(A+) mRNA and total RNA revealed a single 1.4-kb transcript (see Figure 6A), indicating that the cDNA clones are near full-length. The full sequence on both strands was determined for one of the cDNA clones and partial sequence was obtained for four other clones. With the exception of small differences in the length of some of the 5’ ends, all the cDNAs appear to be identical by restriction endonuclease digestion.

The full nucleotide sequence obtained from overlapping clones (Figure 1) encompasses a 1.1-kb open reading frame encoding a 370-amino acid protein with a predicted molecular weight of 43 kDa. The predicted protein coding region beginning at the first AUG (nucleotide position 128) is preceded by a consensus CAAC sequence common among *Drosophila* genes (Cavener, 1987). Stop codons in all three potential reading frames upstream of the predicted initiation codon would prevent use of other upstream AUGs. A consensus polyadenylation signal is located +65 bp from the end of the open reading frame and ~18 bp from the poly(A) tail.

Based on its similarity to the yeast SNF5 gene, we have named this *Drosophila* gene snr1 for snf5-related 1. The predicted snr1 and ini1 proteins are similar in size and highly related over their entire lengths (78% similarity; 65% identity; Figures 2 and 3). In contrast, the snr1 and SNF5 proteins are only distantly related. The 370-residue snr1 protein is much shorter than the 904-residue SNF5 protein, due to the absence of the glutamine-rich and proline-rich segments found at the ends of the SNF5 protein (Figure 2). The glutamine-rich N-terminal region of SNF5 is not essential for SNF5 function (Laurent et al., 1990). The most highly conserved region of snr1 and SNF5 (50% similarity; 41% identity) is a 200-amino acid acidic region including the entire C-terminus of snr1 (Figure 3). This region is also highly conserved between ini1 and snr1 (86% identical). The similarities between snr1, ini1, and SNF5 are restricted to a relatively short segment, suggesting that this region may represent a discrete functional domain. Outside this domain, the snr1 and SNF5 proteins are highly divergent.

A search of the available nucleic acid and protein data bases using both the FASTDB and BLAST programs revealed that snr1 is also significantly related to a *C. elegans* gene (CeSNF5), recently identified as part of the worm genome sequencing project (GenBank #Z32683). The deduced snr1 and CeSNF5 protein sequences are approximately 67% similar and 53% identical over their entire predicted lengths (Figure 4). snr1 is also distantly related to the yeast transcription elongation factor S-II, one of a group of yeast strand-transfer proteins. Although this similarity is intriguing in light of the interaction between HIV integrase and ini1, the resemblance is too limited to conclude that
S-II is functionally related to either snr1 or ini1. In contrast, the fly, worm, and human SNF5-related proteins are strikingly similar over their entire lengths, which suggests they are functional homologues.

We also examined the possibility that snr1, like brm and SWI2/SNF2, is a member of a gene family. Hybridization of both snr1 and ini1 cDNAs to Drosophila genomic restriction fragments revealed no obvious additional fly genes. Low stringency hybridization of the SNF5 gene to yeast genomic DNA also does not reveal other related gene(s) (Dingwall and Scott, unpublished observations). Thus in contrast to the SWI2/SNF2 family (Carlson and Laurent, 1994; Elfring et al., 1994) no evidence has been obtained for a family of genes closely related to SNF5 in either Drosophila or yeast. Although snr1 appears to be the only Drosophila gene closely related to SNF5, the sequence similarity be-

*Figure 1. Nucleotide and predicted amino acid sequence of the snr1 gene. The nucleotide sequence of the longest cDNA is shown. The single predicted open reading frame of 1.1 kb could encode a 370-amino acid protein of approximately 43 kDa, beginning with the ATG located at nucleotide position 127 and ending at position 1239. A consensus polyadenylation signal (shown in bold) is located +65 bp from the end of the open reading frame and –18 bp from the poly(A) tail.*

*Figure 2. The snr1 and ini1 proteins are related to the yeast SNF5 protein. The snr1, ini1, and SNF5 proteins are shown in diagrammatic form highlighting regions of strongest similarity. The predicted snr1 and ini1 proteins show 65% overall homology, with three subregions that vary from 60% to 86% identity. The region of strongest identity (black box) is sufficient for ini1 interaction with HIV integrase.*

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between the snr1 and SNF5 proteins is too limited to conclude that they are functional homologues.

**Genetic Analysis of snr1 Mutants**

In situ hybridization of the snr1 cDNA to salivary gland polytene chromosomes locates snr1 near the base of the right arm of the third chromosome, at cytological position 83A5,6. With the exception of the small subunit of RNA polymerase, no known genes or mutations have been mapped to this region, nor are deficiencies available. A screen for dominant modifiers of homeotic mutations identified a number of previously uncharacterized genes including *brm* (Kennison and Tamkun, 1988), but none map to 83A. Thus, snr1 does not appear to correspond to any previously known gene.

**Figure 3.** Sequence similarities among SNF5-related proteins. A direct sequence alignment of snr1, inil, and SNF5 reveals strong conservation. The full length snr1 and inil deduced protein sequences are shown, whereas only the region of highest similarity to SNF5 is presented. The inil protein contains an 11-amino acid stretch not found in snr1 or SNF5, indicated by the gap between the two 60% identity regions (aa 72 to aa 82); furthermore, this region was not present within some of the inil clones sequenced (Kalpana et al., 1994), suggesting that it may be either an exon unique to inil or that it was included in some cDNA clones as a result of alternate or incomplete processing. The snr1 and inil proteins are also nearly co-linear, with a 200-amino acid portion of SNF5, with the exception of a 17-amino acid stretch. Outside of this region, there is little conservation between the yeast and fly proteins. The snr1 protein is truncated at Gln 131 in both the snr1P5 insertional mutant and in the snr13 lethal excision mutant.

**Figure 4.** Matrix alignment of snr1 with a *Caenorhabditis elegans* SNF5-related protein. A *C. elegans* genomic sequence is predicted to encode a protein of 382 amino acids and shows a strong similarity to the snr1 protein using a Pustell protein matrix (MacVector software, International Biotechnologies). The PAM250 scoring matrix (Pearson, 1990), window size of 15 residues, and a minimum score of 35% was used in the analysis. The alignment indicates that the predicted proteins are nearly co-linear, with an overall similarity of 53% identity. The highest identity between the two proteins is within the 200-amino acid region conserved among all four SNF5-related proteins. The gap in the alignment roughly corresponds to the same region poorly conserved between the fly and human proteins.
To begin a genetic analysis of the *snrl* gene we examined lethal *ry* + P-element transposon insertions located in the vicinity of 83A (Drosophila Genome Project, University of California, Berkeley, CA). Pulsed field gel electrophoresis was used to map four of these lethal insertions relative to the *snrl* gene (our unpublished results). The *snrl* gene is contained within a 250-kb NotI restriction fragment (Figure 5). One of the insertions, in the fly stock AS1319, has a restriction fragment polymorphism within the 240-kb fragment detected with the *snrl* cDNA. Additional restriction enzyme analysis, in combination with PCR using P-element-specific and *snrl*-specific primers, indicates that the AS1319 insertion is located within the *snrl* gene (Figure 5). Genomic sequences flanking the insertion site were obtained by PCR and by the plasmid-rescue technique (Bier et al., 1989). Sequencing revealed that the P-element insertion in AS1319 had occurred within an exon of *snrl* (Figure 5). The *snrl* gene is transcribed in a centromere proximal to distal direction.

The mobilization of the P-element allowed construction of additional mutations by transposon excision (Cooley et al., 1988). A source of P-transposase was introduced and progeny were scored for loss of the *ry* + marker contained within the P-element. One hundred four *ry* - progeny were obtained and 80 of these lines were analyzed using a combination of PCR, Southern blot analysis, and genetic complementation of the lethality of the AS1319 mutant. In every case, *ry* - excision lines that retained portions of the P-element, due to incomplete or imprecise excision, failed to complement the lethality associated with AS1319 (32/80 lines). Twenty-six of the 80 *ry* - lines tested had no remaining P-element sequences and all complemented the lethality of AS1319. This lethality was thus due to an insertion within the *snrl* gene, so we named the allele *snrl*<sup>P1</sup>. We therefore conclude that *snrl* is essential for viability.

The remaining 22 excision lines potentially represent new alleles of *snrl*, because each excision chromosome failed to complement the AS1319 lethality and contained no P-element sequences. Chromosomal DNA surrounding the original insertion site was cloned from two lines (*snrl*<sup>R3</sup> and *snrl*<sup>R10</sup>) using PCR primers specific to *snrl*. In both cases, imprecise excision had generated in-frame translation termination codons at amino acid 131. The truncations effectively eliminate the C-terminal two-thirds of the protein, including the regions of highest sequence similarity between the *snrl*, SNF5, and ini1 proteins (Figure 2). PCR and DNA blot analyses did not reveal any significant deletions of surrounding chromosomal DNA in the 22 lethal excision lines.

Both *snrl*<sup>P1</sup> and *snrl*<sup>R3</sup> mutant homozygotes die during the larval period of development before the

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**Figure 5.** Molecular map of the *snrl* region. Proximal is to the left and distal is to the right. The *snrl* cDNA localizes to the salivary gland polytene chromosome region 83A5.6. A lethal P-element insertion (P1319) was localized within a 250-kb NotI fragment and a 12-kb XbaI fragment by pulsed field gel electrophoresis. EcoRI sites within the 12-kb XbaI fragment are shown. The position of the *snrl* transcript relative to the insertion is shown in the lower half of the diagram. The site of the P-element insertion was chosen as the (0) position within the map. The shaded region of the *snrl* mRNA represents the open reading frame that encodes the *snrl* protein. The *snrl*<sup>P1</sup> and *snrl*<sup>R3</sup> alleles (see text for details) are indicated below the molecular map.
third larval instar stage. No homeotic transformations or other phenotypes are seen in homozygotes, nor are distributions of several homeotic gene products including Ultrabithorax (Ubx) and Antennapedia (Antp) notably altered (our unpublished results). As discussed below, the lack of such phenotypes could be due to a large maternal contribution of snr1 gene products.

To investigate a possible role for snr1 in regulating homeotic gene transcription, we explored whether mutations in snr1, like brm mutations, suppress mutations in Polycomb and enhance the adult phenotypes of trx mutations. Heterozygous brm mutations and deficiencies strongly suppress the transformations seen in heterozygous Polycomb adults by preventing the derepression of homeotic genes (Kennison and Tamkun 1988; Tamkun et al., 1992). In contrast, the loss of one copy of snr1 does not suppress adult Polycomb mutant phenotypes, such as transformations of second and third legs to first leg, wing to haltere, and abdominal segments to more posterior identities. The snr1 product thus does not appear to be limiting under these assay conditions.

We also examined whether snr1 interacts with trithorax group members, including brm and trx. trx encodes an activator of homeotic gene transcription (Mazo et al., 1990; Breen and Harte, 1991) and heterozygous mutant adults sometimes display homeotic transformations of thoracic and abdominal segments due to the decreased expression of ANT-C and BX-C genes (Lewis, 1968; Ingham and Whittle, 1980; Ingham, 1983). Heterozygous mutations in several trithorax group genes, including brm, enhance trx mutant phenotypes, such as the anterior transformation of the fifth abdominal segment (A5) (Shearn, 1989; Tamkun et al., 1992). A snr1 mutation also enhances the abdominal transformations seen in trx heterozygotes (Table 1). Individuals containing mutations in all three genes (snr1, brm, and trx) have even stronger transformations (Table 1). As a control, we used a chromosome from which the lethal P-element insertion in snr1P1 had been excised (snr1P1rec) and fully complemented a snr1 mutation. In contrast to the snr1 mutant, the snr1P1rec chromosome does not interact with trx.

snr1 and brm also interact genetically. Individuals heterozygous for either snr1 or brm mutations are phenotypically wild type (Tamkun et al., 1992; our unpublished results). In contrast, approximately 10% of snr1/brm transheterozygous adults display prothoracic defects, including the loss of the humerus. This phenotype is similar to that resulting from decreased function of brm (Tamkun et al., 1992; Brizuela et al., 1994) or Antennapedia (Abbott and Kaufman, 1986) during larval development. These genetic interactions suggest that snr1 and brm act together, and with trx, to regulate homeotic gene transcription.

**snr1 Expression during Development**

To further explore the function of snr1, we characterized its temporal and spatial expression during development. An RNA blot containing poly(A)+ mRNA from different embryonic stages was probed simultaneously with cDNAs for both snr1 and brm (Figure 6A). The snr1 mRNA appears as a 1.4-kb band, whereas the brm mRNA appears as a 5.5-kb band (Tamkun et al., 1992). The timing and variation in level of the brm and snr1 mRNAs are similar, although not identical. The highest level of mRNA accumulation for both genes occurs in unfertilized eggs and early embryos, indicating maternal contributions of both mRNAs. The mRNA accumulation levels decrease steadily throughout embryogenesis until approximately 16 h post-fertilization, when levels dramatically decrease (Figure 6A). By the end of embryogenesis (16–24 h) little snr1 or brm mRNA is detectable. A low level of mRNA accumulates during larval and pupal stages but little, if any, RNA is found in adult males. Therefore, snr1 is unlikely to provide an essential function to all cells.

A rat polyclonal antiserum was generated against the C-terminal two-thirds of the snr1 protein to examine the developmental accumulation and tissue distribution of the protein. The antibodies were tested for

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**Table 1. Interactions of snr1 with brm and trx**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. males scored</th>
<th>A5 transformation*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>none</td>
</tr>
<tr>
<td>snr1P1rec/trxE2</td>
<td>119</td>
<td>83</td>
</tr>
<tr>
<td>snr1E3/brmE2/txE2</td>
<td>98</td>
<td>36</td>
</tr>
<tr>
<td>snr1P1rec/brmE2/txE2</td>
<td>121</td>
<td>53</td>
</tr>
<tr>
<td>snr1E3/brmE2/txE2</td>
<td>94</td>
<td>2</td>
</tr>
</tbody>
</table>

*Homeotic transformations were scored as the transformation of the A5 abdominal segment into the identity of the A4 segment, indicated by the loss of pigment from the A5 segment. Transformations were considered to be strong if greater than half the segment lacked pigmentation. The penetration for each genotype is expressed as a percentage of males showing transformations.
specificity using protein blots of several bacterially expressed snr1 fusions and by testing both embryos and protein blots of embryonic extracts with pre-immune serum (our unpublished results). Extracts from developmentally staged wild-type embryos, larvae, pupae, and adults were probed with the snr1-specific antisera (Figure 6B). The snr1 protein appears as a 43-kDa band, consistent with the size predicted from the snr1 cDNA sequence and Northern blot analysis. The amount of snr1 protein peaks early in embryogenesis with low levels found throughout larval and pupal development. No snr1 protein is detected in adult males, consistent with the RNA accumulation data.

The distribution of snr1 protein during embryogenesis was determined by whole mount staining with the snr1 antibody (Figure 7). In agreement with the RNA and protein analyses presented above, snr1 protein is detected at the earliest stages of development. The protein is clearly associated with nuclei before cellularization (Figure 7A). Consistent with the localization of yeast SNF5 (Laurent et al., 1990), the snr1 protein is located in the nucleus throughout embryogenesis. The snr1 protein is found in all nuclei of the embryo through the germ band extended stage (Figure 7B). The snr1 protein is located almost exclusively in the central nervous system and brain after retraction of the germ band (Figure 7, C and D). snr1 mRNA is similarly distributed during embryogenesis as determined by in situ hybridization (our unpublished results). The imaginal discs and salivary glands of larvae have a uniform nuclear distribution of the snr1 protein, but there is no observable protein in other tissues (our unpublished results).

The temporal and spatial expression pattern of snr1 mRNA is similar to that observed for brm (Tamkun et al., 1992; Elfring et al., 1994), consistent with their genetic cooperation in regulating homeotic gene transcription. In contrast to the homeotic proteins, which are produced in discrete domains along the anterior-posterior axis, snr1 and brm products are fairly uniformly distributed along the embryo. The spatially and temporally restricted patterns of snr1 expression, like brm, argue against a general role for snr1 in transcription or other cellular processes.

**A High Molecular Weight Complex Contains the snr1 and brm Proteins**

The sequence similarity between snr1 and SNF5 suggests that snr1 might also function in concert with other proteins as part of a Drosophila counterpart of the yeast SWI/SNF complex. To test this possibility, we determined whether snr1 and brm are present in high molecular weight complexes. A soluble nuclear extract from 0–12 h embryos was prepared and fractionated on a Superose 6 FPLC column in moderate strength ionic buffer (425 mM NaCl). Under denaturing conditions, the observed molecular weights of the snr1 and brm proteins are similar to those predicted from their sequence (43 kDa and 185 kDa, respectively). In contrast, under non-denaturing conditions, both snr1 and brm proteins elute from the gel filtration column with an apparent molecular mass of approximately $2 \times 10^6$ daltons (Figure 8). Little, if any, brm or snr1 protein elutes at the position of their deduced monomeric sizes, suggesting that all of the brm and snr1 protein in embryonic extracts is present in a high molecular mass complex(es). The apparent molecular mass for both
proteins is in close agreement with that observed for the yeast SWI/SNF complex (Peterson et al., 1994) and for the human brg1 protein (Khavari et al., 1993; Kwon et al., 1994).

DISCUSSION
The discovery of brm and snr1 in the same large protein complex in Drosophila provides strong evi-
dence for conservation of a SWI/SNF-like complex from yeast to animals. The yeast SWI/SNF complex is required for the transcriptional induction of a diversely regulated set of yeast genes (Winston and Carlson, 1992; Carlson and Laurent, 1994). Genetic and biochemical studies suggest that the SWI/SNF complex is targeted to promoters via interactions with DNA-binding regulatory proteins, where it uses the energy of ATP hydrolysis to overcome the repressive effects of chromatin components, including nucleosomal histones, on transcription (Winston and Carlson, 1992; Cote et al., 1994). The discovery that brm, an activator of Drosophila homeotic genes, is related to the yeast SWI2/SNF2 gene provided an initial insight into molecular mechanisms underlying the action of Polycomb and trithorax group genes. Based on the structural and functional similarities between brm and SWI2/SNF2, one possibility is that brm, together with Drosophila homologues of other yeast SWI/SNF proteins, activates ANT-C and BX-C genes by overcoming the repressive effects of Polycomb group proteins (or other chromatin components) on transcription.

A Counterpart of the Yeast SWI/SNF Complex Is Present in Drosophila

A large number of SWI2/SNF2 and brm-related genes have been identified in both mice and humans (reviewed in Carlson and Laurent, 1994), making it difficult to determine which, if any, of the vertebrate relatives are part of a SWI/SNF-like complex. Based on sequence homology, at least two human genes, brg1 and hbrm, are closely related to brm and to each other (Khavari et al., 1993; Muchardt and Yaniv, 1993). Brg1 and hbrm are each capable of stimulating transcription, suggesting that they may be functional homologues of brm; brg1 is present in a large complex as well (Khavari et al., 1993). An attempt to identify a human brg1 complex yielded two partially purified complexes (Kwon et al., 1994) that potentially are counterparts to the yeast SWI/SNF complex (Imbalzano et al., 1994; Kwon et al., 1994). The subunit compositions of these complexes have not been examined, so their relationships to the yeast complex are presently unclear. The existence of multiple human genes with sequences related to SWI2/SNF2 brings up the possibility of multiple complexes that may or may not be related to the yeast complex.

We searched for Drosophila relatives of other subunits of the yeast SWI/SNF complex and identified snr1, a distant relative of the yeast SNF5 gene. Although SNF5 is an essential component of the yeast SWI/SNF complex (Laurent et al., 1990; Peterson et al., 1994), the biochemical function of the SNF5 protein is unknown. Like the SWI2/SNF2 and SNF5 proteins, brm and snr1 are members of huge (∼2 × 10^6 Da) protein complexes. Using a co-immunoprecipitation assay, we found that the brm and snr1 proteins interact, either directly or indirectly. These data strongly suggest that snr1 and brm are members of a Drosophila counterpart of the yeast SWI/SNF complex. Although the exact composition of this Drosophila complex is unknown, it seems likely that it contains relatives of other subunits of the SWI/SNF complex, including SWI1, SWI3, and SNF6.

The existence of a brm/snr1 Drosophila complex related to the yeast SWI/SNF complex argues for both conservation of function and subunit composition of the complex during evolution. The retention of a relationship between two proteins in a large complex for about a billion years raises many questions, including the following: What functions of the complexes might be common to yeast and fly cells? How have the complexes changed to fulfill requirements specific to a higher eucaryote? What are the molecular mechanisms of complex function? Indeed, the existence of multiple SWI2/SNF2-related proteins in yeast and higher eucaryotes (reviewed in Carlson and Laurent, 1994; Peterson and Tamkun, 1995) and the differences between the SNF5 and snr1 sequences raise questions about the extent to which the properties of the SWI/SNF complex may be extrapolated to the brm/snr1 complex we detect in flies.

Roles of the brm/snr1 Complex during Drosophila Development

The temporal and spatial patterns of transcription of snr1 products set limits on the gene's functions. snr1 RNA and protein are present at highest levels early in embryogenesis and at relatively low levels in larvae and pupae. Neither snr1 RNA or protein is expressed at detectable levels in adult males. snr1 is expressed uniformly early in embryogenesis; in later embryos snr1 RNA and protein is restricted to the central nervous system and brain. There is an approximate correlation between the occurrence of cell division and the expression of snr1; cell divisions cease in most cell types midway through embryogenesis, except in the
nervous system. Cell division occurs at high rates in imaginal discs, where snr1 products are also detectable.

Four specific conclusions can be drawn from the spatial and temporal patterns of snr1 expression. First, consistent with the results of our biochemical studies, snr1 and brm are expressed in similar spatial and temporal patterns during development. Second, both snr1 and brm are expressed throughout development at high levels in all cells where homeotic genes are actively transcribed. Third, the differential transcription of homeotic genes does not result from the differential expression of snr1 and brm; both snr1 and brm are expressed uniformly along the anterior-posterior axis at all developmental stages. Fourth, the restricted embryonic expression patterns of snr1 and brm, plus the absence of detectable levels of either mRNA or protein in adult males, implies that snr1 and brm are not required for all transcriptional activation.

What are the roles of snr1 during Drosophila development? A snr1 mutation strongly enhances the anterior transformation of the fifth abdominal segment seen in trx heterozygotes. The transformation is thought to be due to lowered activation of the BX-C homeotic genes by trx and, apparently, snr1 (Ingham, 1983; Breen and Harte, 1993). The genetic interactions between snr1, brm, and trx, together with the physical association of the snr1 and brm proteins, defines snr1 as a new member of the trithorax group of homeotic gene activators. snr1 homozygotes die as second instar larvae with no discernable pattern defects or homeotic transformations. The lack of pattern defects in snr1 mutant homozygotes is probably due to the high maternal contribution of snr1 gene products. Like snr1, brm is expressed both maternally and zygotically. Individuals lacking zygotic brm activity die as unhatched larvae with no obvious pattern defects. Loss of maternal brm activity blocks oogenesis (Brizuela et al., 1994). The brm/snr1 complex is therefore likely to play an important role in early development. We also anticipate that snr1, like brm, may be required for the activation of a large number of Drosophila genes. Conditional or dominant-negative mutations will be required to elucidate the roles of snr1 and brm in oogenesis and embryogenesis.

**Models for Polycomb Group and brm/snr1 Complex Functions in Light of SWI/SNF Mechanisms**

What is the role of the brm/snr1 complex in homeotic gene regulation? DNA-binding regulatory proteins encoded by segmentation genes define the initial patterns of homeotic gene transcription relatively early in embryogenesis (for review see Harding and Levine, 1988; Ingham, 1988). The maintenance and refinement of these patterns depends on cross-regulatory interactions between homeotic genes, trithorax group genes, and Polycomb group genes. Current models favor the view that the Polycomb group of proteins silence transcription by compacting local regions of chromatin, rendering them inaccessible to the transcription machinery (Paro, 1993; Rastelli et al., 1993). Polycomb complexes containing at least three products of Polycomb group genes (Rastelli et al., 1993) are thought to assemble at specific transcription enhancer elements by interacting with segmentation proteins, such as hunchback, thus defining the transition from establishment to maintenance (Zhang and Bienz, 1992). However, because initiation and maintenance elements are in some cases physically separable, Polycomb group proteins may recognize a specific maintenance element (PRE or Polycomb Response Element; Simon et al., 1993) through associations with an unidentified sequence-specific factor. None of the known Polycomb group proteins exhibit sequence-specific DNA binding, but polyhomeotic, Sut(2)2 and Psc proteins contain potential zinc finger–like motifs and bind DNA nonspecifically in vitro (DeCamillis et al., 1992; Rastelli et al., 1993). The PRE site may act as a nucleation center to recruit additional Polycomb group proteins, which spread out along the chromosome and render genes transcriptionally inactive (Paro, 1993); therefore, the inactive state is heritable through cell divisions.

The trithorax group proteins, or some of them, may block assembly or function of Polycomb group complexes. Support for this model comes from experiments in yeast, where the SWI/SNF complex affects the association of histones with DNA (Hirschhorn et al., 1992; Cote et al., 1994) thereby “opening” chromatin to allow for enhanced binding by activators. The brm/snr1 complex might, by analogy to the yeast SWI/SNF complex, use the energy of ATP hydrolysis to counteract the repressive effects of Polycomb or other chromatin components on the transcription of homeotic genes by creating and/or sustaining a permissive chromatin environment for activators such as trx.

The brm/snr1 complex may be targeted to ANT-C and BX-C genes via interactions with either segmentation gene products or trx, which is thought to bind DNA directly (Kuzin et al., 1994). The product of a segmentation gene, fushi tarazu, requires the SWI/SNF complex to activate transcription in yeast (Peterson and Herskowitz, 1992). Because of the strong genetic interactions between trx, brm, and snr1, the trx protein is a likely candidate for a DNA-binding regulatory protein that requires the brm/snr1 complex for its function in maintaining homeotic gene expression.
Studies of Human SWI/SNF Relatives Suggest Unanticipated Functions for the brm/snr1 Complex

Studies of mammalian homologues of brm and snr1 reveal involvement in cellular processes such as regulation of the cell cycle and viral integration. These functions may or may not be the result of transcriptional regulation by brm/snr1-related proteins. For example an unanticipated function of brg1 is its interaction with the retinoblastoma protein (Rb) in regulating progression of the cell cycle (Dunaie et al., 1994). This suggests an additional role for the brm/snr1 complex in regulating cell division.

Studies of in1, the human homologue of snr1, suggest a possible role in HIV proviral integration (Kalpana et al., 1994). The in1 gene was isolated from a yeast two-hybrid screen by interaction with HIV integrase. Although the normal function of in1 is unknown, when tethered to DNA, in1 is capable of activating transcription of a reporter gene (Kalpana et al., 1994), suggesting that in1, like SNF5, may function in transcription regulation. Biochemical evidence shows the interaction between in1 and HIV integrase to be direct and that in1 protein directly stimulates the integration reaction of integrase in vitro (Kalpana et al., 1994). Like in1, snr1 made in bacteria interacts with HIV integrase in vitro (our unpublished results), suggesting that snr1 and in1 may be capable of interacting with a similar set of proteins. The SNF5 protein contains a 200-amino acid region that is highly similar to parts of snr1, in1, and CeSNF5. This same region is sufficient for in1 association with HIV integrase and may define a conserved domain necessary for protein-protein contacts. The interaction between integrase and in1 probably does not represent a normal function of in1; rather, the virus may have evolved to utilize in1 to assist integration. The integration of the HIV viral genome into the host chromosome may be mediated by a direct interaction with in1, either independently or within a human SWI/SNF-like complex (Kalpana et al., 1994). Consistent with this idea, retroviruses have been shown to integrate preferentially into actively transcribed regions and their consequent open chromatin (Vijaya et al., 1986; Rohde-wold et al., 1987; Shih et al., 1988; Scherdin et al., 1990). Alternatively, the integrase may persist at the site of integration and aid in attracting factors to allow transcription initiation.

The similarities between the yeast SWI/SNF complex and its Drosophila counterpart suggest that they may both be involved in gene regulation, albeit with different targets affected in different systems (Peterson and Tamkun, 1995). The unanticipated functions of the mammalian homologues of snr1 and brm suggest that either the fly and human proteins have evolved to interact with different proteins and/or that there is more than one SWI/SNF-like complex in higher eukaryotes. Although neither possibility can be ruled out, the existence of several brm-related genes in flies and humans is consistent with the idea that there are several SWI/SNF-like complexes (Carlson and Laurent, 1994; Elfring et al., 1994). It seems likely that different complexes containing either snr1 or brm, or both, could act on different target genes, have different levels of activity, or have different types of protein-protein associations. Further biochemical characterization of the Drosophila brm/snr1 complex, its components, and possibly other related complexes, should provide a better understanding of the role of SWI/SNF relatives in patterning events in higher eukaryotes and lead to an elucidation of its role in gene expression and the maintenance of cell fates.

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