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Identification and Characterization of *Drosophila* Relatives of the Yeast Transcriptional Activator SNF2/SWI2

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The *Drosophila brahma* (*brm*) gene encodes an activator of homeotic genes that is highly related to the yeast transcriptional activator SWI2 (SNF2), a potential helicase. To determine whether *brm* is a functional homolog of *SWI2* or merely a member of a family of *SWI2*-related genes, we searched for additional *Drosophila* genes related to *SWI2* and examined their function in yeast cells. In addition to *brm*, we identified one other *Drosophila* relative of *SWI2*: the closely related *ISWI* gene. The 1,027-residue ISWI protein contains the DNA-dependent ATPase domain characteristic of the SWI2 protein family but lacks the three other domains common to brm and SWI2. In contrast, the ISWI protein is highly related (70% identical) to the human hSNF2L protein over its entire length, suggesting that they may be functional homologs. The DNA-dependent ATPase domains of brm and SWI2, but not ISWI, are functionally interchangeable; a chimeric SWI2-brm protein partially rescued the slow growth of *swi2*$^-$ cells and supported transcriptional activation mediated by the glucocorticoid receptor in vivo in yeast cells. These findings indicate that *brm* is the closest *Drosophila* relative of *SWI2* and suggest that *brm* and *SWI2* play similar roles in transcriptional activation.

The homeotic genes of the Antennapedia complex (ANT-C) and the bithorax complex (BX-C) direct the choice between alternative pathways of development in *Drosophila melanogaster* (7, 22). Each homeotic gene in the ANT-C and BX-C encodes a homeodomain class transcription factor that specifies the identity of a particular segment, or group of segments, by regulating the transcription of a battery of downstream target genes (2). The transcription of ANT-C and BX-C genes must be regulated precisely, since alterations in cell fate can result from either inactivation or derepression of homeotic genes. Early in embryogenesis, segmentation genes establish the initial domains of homeotic gene transcription (15, 21, 35, 62). During subsequent development, the spatial patterns of homeotic gene transcription are maintained by two other classes of regulatory genes: the Polycomb group of repressors (32, 52) and the trithorax group of activators (23, 25). The regulation of homeotic gene expression is thus often viewed as consisting of two distinct phases: establishment and maintenance.

The molecular mechanisms underlying the maintenance phase of homeotic gene regulation are not well understood but may involve changes in chromatin structure. Mutations in *Polycomb*, the gene for which the Polycomb group is named, cause widespread alterations in cell fate due to the derepression of multiple ANT-C and BX-C genes (32, 41, 52, 61). Both Polycomb and a *Drosophila* heterochromatin-associated protein, HP1, contain a conserved 37-amino-acid segment, the chromodomain (40), which is required for the association of Polycomb with its target genes (33). On the basis of its similarity to a component of heterochromatin, it has been suggested that the Polycomb protein packages inactive homeotic genes into heterochromatin-like complexes early in development, thereby preventing their subsequent transcription (39).

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brahma (*brm*), a member of the trithorax group of activators, was identified in screens for extragenic suppressors of *Polycomb* mutations (24). *brm* mutations strongly suppress *Polycomb* mutations and cause homeotic transformations similar to those resulting from reduced transcription of homeotic genes following embryogenesis (24, 56). Although the molecular basis of the interactions between *brm*, *Polycomb*, and homeotic genes has not been clearly established, a possible function for the brm protein has been suggested by its striking similarity (56) to the yeast transcriptional activator SWI2 (also known as SNF2, TYE3, and GAM1) (5, 30, 63).

In the yeast *Saccharomyces cerevisiae*, *SWI2* assists a variety of DNA-binding regulatory proteins, including SWI5, GAL4, and others, to activate the transcription of their target genes (14, 28, 42, 43, 54, 64). How does *SWI2* activate transcription? *swi2* mutations are suppressed by mutations in nucleosomal histones (19, 27) and cause alterations in chromatin structure in vivo (19), suggesting that *SWI2* counteracts the repressive effects of chromatin on transcription. The SWI2 protein contains several blocks of sequence related to motifs found in nucleic acid-dependent ATPases and helicases (6, 17, 28, 49). These sequence motifs are clustered in a highly conserved, 500-amino-acid domain which is often referred to as the helicase, or DNA-dependent ATPase, domain. As predicted from its sequence, the SWI2 protein catalyzes the DNA-dependent hydrolysis of ATP in vitro (29) and mutations in the putative ATP-binding site disrupt *SWI2* function in vivo (26, 29). On the basis of these findings, it has been proposed that SWI2 utilizes the energy released by ATP hydrolysis to alter the structure of chromatin in the vicinity of target promoters, perhaps by unwinding DNA (59).

Recent studies have suggested that relatives of the yeast SWI proteins play highly conserved roles in transcriptional activation in higher eukaryotes, including *D. melanogaster* (26, 34, 46, 64). *brm* is the closest *Drosophila* relative of *SWI2* identified to date and is therefore a logical candidate for its functional homolog. Ongoing studies of *SWI2* function in *S. cerevisiae* yeast thus have the potential to significantly increase

our understanding of how homeotic genes are regulated by trithorax and Polycomb group genes. If *brm* and *SWI2* play similar roles in transcriptional activation, it is likely that *brm* activates homeotic genes by assisting one or more DNA-binding regulatory proteins to overcome the repressive effects of chromatin structure on transcription (56). This possibility is particularly attractive given recent proposals that *Polycomb* represses homeotic gene transcription by influencing chromatin structure (33, 39).

An alternative possibility, however, is that brm and SWI2 are structurally related, but functionally distinct, regulatory proteins. Ample precedent for this possibility can be found in recent studies of other *SWI2*-related genes. For example, the yeast STH1 protein is highly related to the SWI2 protein both within and outside the DNA-dependent ATPase domain (31) but cannot substitute for SWI2 in vivo (29). Numerous other members of the SWI2 family of putative helicases are involved in processes other than transcriptional activation: ERCC6, RAD16, and RAD54 are involved in DNA repair or recombination (49, 60); MOT1 may act as a transcriptional repressor (6); and the lodestar protein is required for chromosome segregation during mitosis (12). It is thus clear that functional homology between SWI2, brm, and other proteins cannot be deduced from sequence homology alone.

The discovery that brm and SWI2 are members of a rapidly expanding family of functionally diverse proteins prompted us to reevaluate the relationship between the two proteins. Is *brm* a functional homolog of *SWI2*, or simply one member of a family of *SWI2*-related genes in *D. melanogaster*? To clarify this issue, we have searched for additional *Drosophila* relatives of *brm* and *SWI2* and have studied the function of brm and a related *Drosophila* protein in vivo in *S. cerevisiae*.

MATERIALS AND METHODS

Isolation of DNA, RNA, and nucleic acid blot analyses. Genomic DNA was isolated from adult Oregon R flies as described by Scott et al. (51). To minimize contamination with yeast cells present in the fly media, DNA was isolated from adults maintained overnight on glucose. For Southern blots, 3.75 μg of genomic DNA was digested and fractionated on 0.8% agarose gels. Following UV nicking, denaturation, and neutralization, the DNA was transferred to a nylon membrane (AMF Cuno) and hybridized overnight to 100 ng of ^{32}P-labeled DNA prepared by the random-primer method (10) in 15 ml of hybridization buffer (6× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 5× Denhardt's solution, 0.5 mg of salmon sperm DNA per ml, 0.5% sodium dodecyl sulfate [SDS], 10% dextran sulfate) at 65°C. Following hybridization, the blot was washed under conditions of increasing stringency, beginning with 2× SSC–0.1% SDS at 45°C.

RNA was isolated and analyzed by Northern blotting as described previously (56). To control for even loading, the RNA blot was probed with a radiolabeled fragment from the *rp49* gene (37).

Isolation and sequencing of *ISWI* genomic and cDNA clones. Duplicate lifts of a *D. melanogaster* iso-1 EMBL3 genomic library (56) were hybridized to two nonoverlapping *brm* cDNAs (KRA and 6, spanning nucleotides 2572 to 3494 and 3532 to 5296 of the *brm* mRNA [56], respectively) as described above for Southern blots. One of 50,000 recombinant phage hybridized to both *brm* cDNAs but not to fragments from either an intron or nontranscribed flanking regions of the *brm* gene. To map the position of the *brm*-related gene (*ISWI*) within this phage, Southern blots of phage DNA digested with restriction enzymes were hybridized to radiolabeled *brm* cDNA fragments.

cDNA clones corresponding to the *ISWI* mRNA were isolated by screening λgt10 (44) and λgt11 (57) embryonic cDNA libraries with *ISWI* genomic DNA fragments. The 5′ end of the *ISWI* mRNA was amplified from reverse transcribed Oregon R cDNA by the PCR (47), using primers based on the sequence of an *ISWI* cDNA and the presumptive 5′ untranslated region of the *ISWI* gene. cDNA fragments were subcloned into pGEM plasmid vectors, and directional deletions were generated by using the Erase-a-Base kit (Promega Biotec). Double-stranded plasmid DNAs were prepared by the alkaline lysis method (4) and sequenced (48) by using the Sequenase kit (U.S. Biochemical). Both strands of DNA were sequenced for all reported DNA sequence. Genomic DNA corresponding to all PCR-amplified cDNA fragments was sequenced to check for errors introduced during amplification.

The National Center for Biotechnology Information BLAST electronic mail server was used to identify sequences related to *ISWI* and *brm* in the GenBank 75.0, EMBL 34.0, PIR 35.0, and SWISS-PROT 24.0 data bases, using the tblastn and blastp programs (1) and the BLOSUM62 matrix (18).

In situ hybridization to polytene chromosomes and to embryos. Digoxigenin-labeled DNA fragments were prepared by the random-primer method (10) and hybridized either to salivary gland polytene chromosomes (9) or to whole-mount preparations of fixed embryos (58).

Construction of genes encoding chimeric SWI2-brm and SWI2-ISWI proteins. To introduce *Kpn*I and *Sac*I sites flanking the brm ATPase domain, a subcloned fragment of the *brm* gene was used as a template for PCR, (47), using Vent DNA polymerase (New England Biolabs) and the oligonucleotide primers 5′-AATGGTACCCTCAAGGAA-3′ and 5′-CGGAG CTCTTCGTCATCATC-3′. PCR was also used to introduce *Kpn*I and *Bgl*II sites flanking the ISWI ATPase domain, using the oligonucleotide primers 5′-AGTGGTACCATGCGTGAC TA-3′ and 5′-ATAGATCTCATTTCATCCTT-3′. Both fragments were sequenced to ensure that no errors had been introduced as a result of the amplification process. The amplified *Kpn*I-*Sac*I *brm* and *Kpn*I-*Bgl*II *ISWI* fragments were then substituted for the corresponding fragments of the *SWI2* gene.

Studies of brm and ISWI function in *S. cerevisiae*. *S. cerevisiae* CY329 was used as a tester strain for activation by LexA fusion proteins. This strain is identical to GGY:171 (11) but contains a reporter gene integrated at the *URA3* locus that consists of one *lexA* operator upstream of a *GAL1-lacZ* fusion. This strain was transformed with either the LexA-brm expression plasmid, an expression plasmid that expresses only the LexA DNA-binding domain (amino acids 1 to 82), or a LexA-GAL4 fusion protein as a positive control. Three independent transformants of each were tested for production of β-galactosidase, using a sensitive plate assay (3).

To assay the ability of chimeric SWI2-brm and SWI2-ISWI proteins to support glucocorticoid receptor-mediated transcriptional activation in yeast cells, strain CY62 (*swi2*Δ, isogenic to strain CY26 [43]) was transformed with three plasmids: (i) a *lacZ* reporter plasmid (pΔS26.x) which contains three glucocorticoid response elements that drive expression of β-galactosidase (64); (ii) a plasmid that expresses full-length glucocorticoid receptor, pN795-*LYS2* (a derivative of pG-N795 [64]); and (iii) a high-copy-number expression plasmid that expresses either yeast SWI2, the SWI2-brm chimera, the SWI2-ISWI chimera, or vector alone. Cells were grown in liquid cultures, treated with steroid hormone, and assayed for β-galactosidase activity as described previously (64). Average

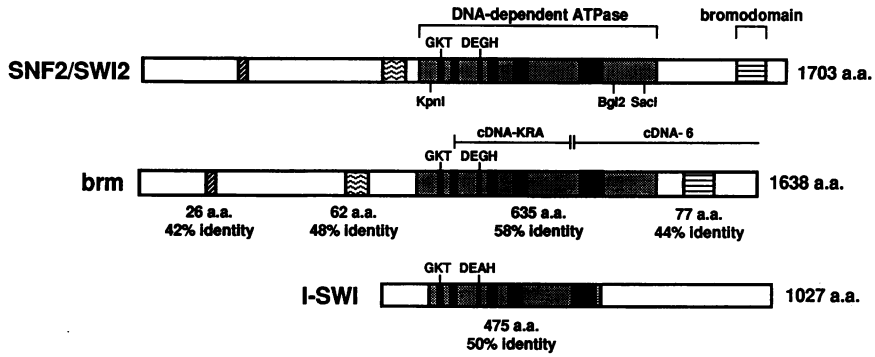

FIG. 1. Similarities between the brm, ISWI, and SWI2 proteins. The complete sequences of the brm and ISWI proteins are compared with the sequence of the SWI2 protein. Conserved domains are represented by shaded boxes (domain I, diagonal lines; domain II, wavy lines; domain III [DNA-dependent ATPase domain], stippled; domain IV [bromodomain], horizontal lines). The length of each domain and its percent identity to SWI2 are shown. Within the DNA-dependent ATPase domain, black boxes represent blocks of sequence (A to F) characteristic of the SWI2 protein family (6, 17). The locations of the bipartite NTP-binding motifs are marked by vertical lines. Positions corresponding to *Kpn*I, *Bgl*II, and *Sac*I sites (in the *SWI2* coding region) used to create SWI2-brm and SWI2-ISWI chimeras are indicated. The regions of the brm protein encoded by the cDNAs (KRA and 6) used to identify *brm*-related genes are marked. a.a., amino acids.

values reported are from at least three independent transformants; variation was less than 10%.

Nucleotide sequence accession number. The GenBank accession number for the sequence reported in this paper is L27127.

RESULTS

Identification and cloning of an additional *Drosophila* member of the *SWI2* family. Four regions of the brm and SWI2 proteins, including the 635-residue DNA-dependent ATPase domain and the 77-residue bromodomain (Fig. 1), are highly related (56). The DNA-dependent ATPase domain is conserved in all members of the SWI2 family of putative helicases; the bromodomain is conserved in two human relatives of brm and SWI2 (26, 34) and several other eukaryotic regulatory proteins (16, 20, 56). The sequences of the brm and SWI2 proteins are not sufficiently related, however, to permit the conclusion that the two proteins are functional homologs; an alternative possibility is that *brm* is a highly related, but functionally distinct, member of a family of *SWI2*-related genes in *D. melanogaster*. As a first step toward distinguishing between these possibilities, we searched for additional *Drosophila* genes related to *brm* and *SWI2*.

To identify new members of the *SWI2* family, we first searched for *Drosophila* genes that cross-hybridize to two nonoverlapping *brm* cDNAs (KRA and 6) which together span most of the DNA-dependent ATPase domain and the entire bromodomain (Fig. 1). A Southern blot of *Drosophila* genomic DNA was hybridized to the *brm* cDNAs and washed under conditions of moderate stringency. In addition to the anticipated strong hybridization signals, a single weak band was detected in each lane, suggesting that at least one other gene related to *brm* is present in the *Drosophila* genome (Fig. 2). When the blot was subsequently washed under conditions of higher stringency, only the strong hybridization signals corresponding to the *brm* gene remained (data not shown). This result suggests that at least one *Drosophila* gene is significantly related to *brm*.

To clone the gene related to *brm*, a *Drosophila* genomic phage library was screened with *brm* cDNA probes (cDNAs 6 and KRA) at low stringency. A phage that hybridized to both *brm* cDNAs, but not to DNA fragments from the largest intron or 3′ untranslated region of the *brm* gene, was selected for further analysis. Both *brm* cDNA probes cross-hybridize to a 5-kb *Eco*RI-*Bam*HI fragment in this phage, suggesting that the fragment contains all or part of the *brm*-related gene. When the *Eco*RI-*Bam*HI fragment was hybridized to the genomic DNA blot described above, the hybridization signals observed verified that we had cloned the *brm*-related gene identified by Southern blotting (Fig. 2).

We also used Southern blotting to search for *Drosophila* genes that cross-hybridize to the *SWI2* gene. Since the DNA-

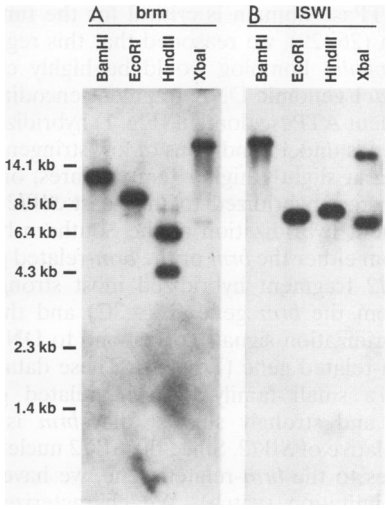

FIG. 2. Identification of a *brm*-related gene in *D. melanogaster*. (A) A Southern blot containing *Drosophila* genomic DNA digested with *Bam*HI, *Eco*RI, *Hin*dIII, or *Xba*I was hybridized to a radiolabeled *brm* cDNA (KRA, nucleotides 2572 to 3494 of the *brm* RNA [56]) that spans the region encoding helicase motifs 2, 3, and 4 and washed under conditions of moderate stringency (1× SSC, 65°C). In addition to the strong hybridization signals corresponding to restriction fragments derived from the *brm* gene, the *brm* probe weakly recognizes one restriction fragment in each digest. Identical results were obtained with use of a nonoverlapping *brm* probe, cDNA 6 (data not shown). (B) Following removal of the *brm* probe by treatment with alkali, the Southern blot was hybridized to a 5-kb *Bam*HI-*Eco*RI restriction fragment from the *ISWI* gene and washed under conditions of high stringency. Note that the *ISWI* probe hybridizes only to the DNA fragments that were weakly recognized by the *brm* cDNA probe.

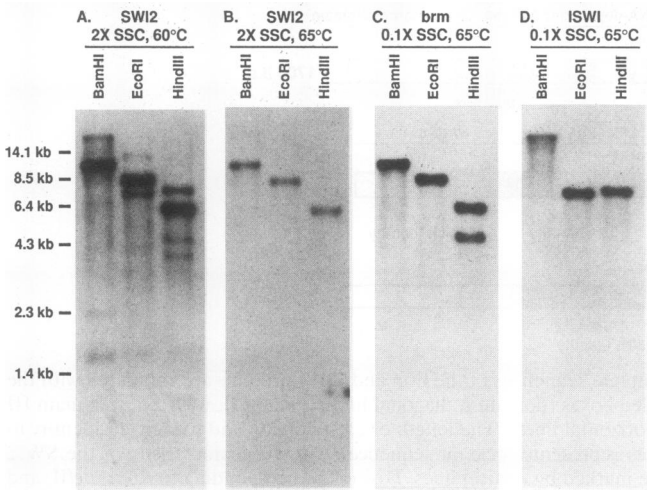

FIG. 3. Identification of *SWI2*-related genes in *D. melanogaster*. (A) A radiolabeled *SWI2* genomic *Kpn*I-*Sac*I fragment (nucleotides 2293 to 4000) spanning the DNA-dependent ATPase domain was hybridized to a Southern blot of *Drosophila* genomic DNA digested with *Bam*HI, *Eco*RI, or *Hin*dIII and washed under conditions of low stringency (2× SSC, 60°C). (B) Following autoradiography, the blot was washed at a higher temperature (2× SSC, 65°C). (C and D) The Southern blot was subsequently stripped and reprobed with a radiolabeled *brm* (cDNA KRA) or *ISWI* (5-kb *Bam*HI-*Eco*RI genomic DNA fragment) probe and washed under conditions of high stringency (0.1× SSC, 65°C). Restriction fragments derived from both the *brm* and *ISWI* genes were recognized by the *SWI2* probes under low-stringency conditions; when the wash temperature was increased, only restriction fragments derived from the *brm* gene were detected.

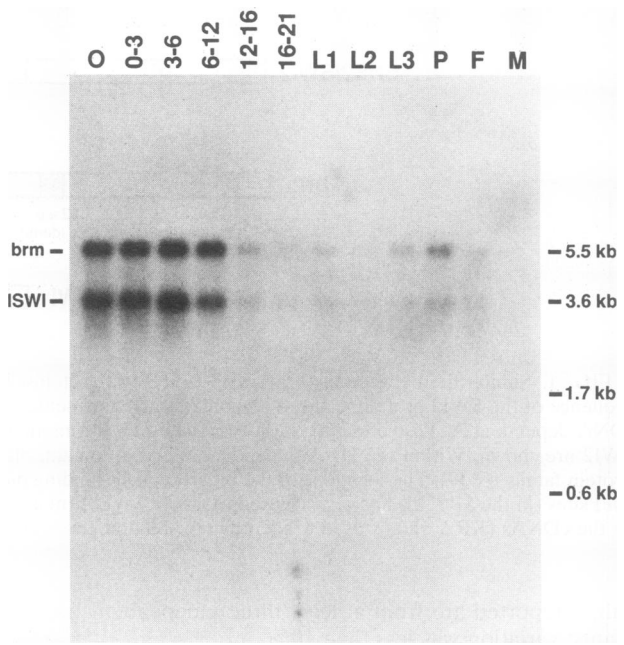

FIG. 4. Developmental expression of the *brm* and *ISWI* mRNAs. A blot containing total RNA isolated from oocytes (O), embryos (0 to 3, 3 to 6, 6 to 12, 12 to 16, and 16 to 21 h), first (L1)-, second (L2)-, and third (L3)-instar larvae, pupae (P), adult females (F), and adult males (M) was hybridized to radiolabeled *brm* (cDNA KRA) and *ISWI* (nucleotides 770 to 1778 in Fig. 5) nucleic acid probes and washed under conditions of high stringency (0.1× SSC, 0.1% SDS). To control for equivalent loading of the mRNA, rRNA was examined by UV shadowing following electrophoresis. As a control for equivalent loading and integrity of the samples, the blot was later hybridized to an *rp49* probe (37) (data not shown).

dependent ATPase domain is critical for the function of the SWI2 protein (26, 29), we reasoned that this region of SWI2 and its *Drosophila* homolog would be highly conserved. A *SWI2 Kpn*I-*Sac*I genomic DNA fragment encoding the entire DNA-dependent ATPase domain (Fig. 1) hybridized to several *Drosophila* genes under conditions of low stringency (Fig. 3A). After washing at slightly higher temperatures, only one *Drosophila* gene cross-hybridized to the yeast *SWI2* probe (Fig. 3B). Subsequent hybridization of the Southern blot to DNA fragments from either the *brm* or the *brm*-related gene showed that the *SWI2* fragment hybridized most strongly to DNA fragments from the *brm* gene (Fig. 3C) and that the next-strongest hybridization signals correspond to DNA fragments from the *brm*-related gene (Fig. 3D). These data confirm the existence of a small family of *SWI2*-related genes in *D. melanogaster* and strongly suggest that *brm* is the closest *Drosophila* relative of *SWI2*. Since the *SWI2* nucleic acid probe also hybridizes to the *brm*-related gene, we have named this gene *ISWI* (imitation switch). We characterized this gene further to examine its relationship to *brm* and *SWI2*.

Molecular characterization of the *ISWI* gene. To identify potential *ISWI* transcripts, the 5-kb *Eco*RI-*Bam*HI genomic DNA fragment from the *ISWI* gene was hybridized to Northern blots containing RNA isolated from *Drosophila* embryos, larvae, pupae, and adults. The fragment hybridized to a single 3.6-kb transcript which, as discussed below, is expressed in a temporal pattern strikingly similar to that of the *brm* RNA (Fig. 4). cDNAs corresponding to the 3.6-kb *ISWI* RNA were isolated from *D. melanogaster* embryonic cDNA libraries, and gaps between nonoverlapping cDNAs were spanned by PCR amplification of cDNA prepared from *Drosophila* embryos. The cDNAs were sequenced on both strands to determine the sequence of the predicted *ISWI* RNA.

The *ISWI* RNA contains an open reading frame of 3,116 nucleotides which, if the first methionine is used to initiate translation, encodes a 1,027-residue protein (Fig. 5). As predicted from our Southern blot analyses, the 119-kDa ISWI protein is highly related to both brm and SWI2 (Fig. 1) and contains all six blocks of sequence characteristic of the SWI2 family (6, 17, 31), including the bipartite nucleoside triphosphate (NTP)-binding site (Fig. 6). However, comparison of the SWI2, brm, and ISWI proteins clearly shows that brm is more closely related to SWI2. The 475-amino-acid DNA-dependent ATPase domain of ISWI is 160 amino acids shorter than the corresponding domains of brm and SWI2 and is less highly conserved. Furthermore, ISWI lacks the other conserved domains common to brm and SWI2, including domains I and II and the C-terminal bromodomain (Fig. 1). Another potentially important difference is that the DEGH sequence found in one of the NTP-binding motifs (the C block, or helicase motif II) of brm, SWI2, and many other family members is replaced by DEAH in the ISWI protein (Fig. 6). These differences suggest that *ISWI* is unlikely to be the *Drosophila* homolog of *SWI2*.

Searches of the current nucleic acid and protein data bases revealed that the ISWI protein is most highly related to the human hSNF2L protein (38). The function of *hSNF2L*, which was identified by serendipity, is not known. The high degree of similarity (70% identity overall) between the ISWI and hSNF2L proteins both within and outside the DNA-dependent ATPase domain (Fig. 7) suggests that they may be functional homologs. Other SWI2 family members closely related to ISWI are, in order of decreasing similarity, the yeast STH1 (31) and SWI2 proteins, the *Drosophila* brm protein, the

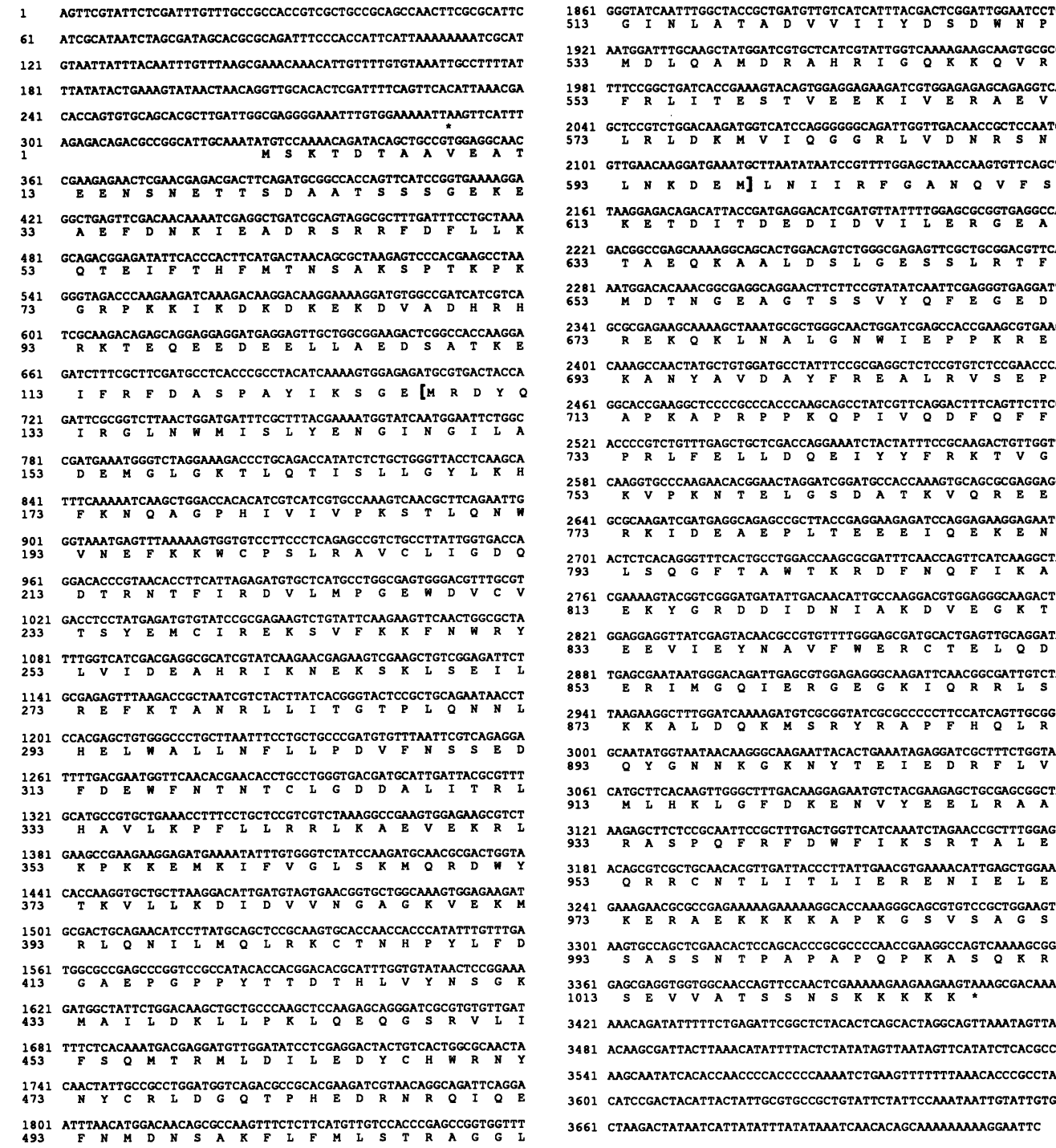

FIG. 5. Complete sequence of the *ISWI* mRNA and the translation of the predicted ISWI protein. Termination codons flanking the translated open reading frame are marked by asterisks. The boundaries of the domain present in the SWI2-ISWI hybrid protein are marked by brackets.

human BRG1 (26) and hbrm proteins (34), and the mouse BRG1 (46) and chd1 (38) proteins.

***brm* and *ISWI* are expressed in similar temporal and spatial patterns during development.** To learn more about the possible functions of *brm* and *ISWI*, we compared their temporal and spatial patterns of transcription. The levels of both *brm* and *ISWI* transcripts are highest in oocytes and early embryos and decrease during late embryonic and larval development (Fig. 4). Very low levels of both transcripts are found in pupae and adult females, and neither is detected in adult males. The spatial distributions of *brm* and *ISWI* transcripts were examined by in situ hybridization to whole-mount preparations of *Drosophila* embryos (Fig. 8). Both *brm* and *ISWI* transcripts are uniformly distributed from the beginning of embryogenesis until germ band retraction; transcripts then gradually become localized to the brain and central nervous system and fall below

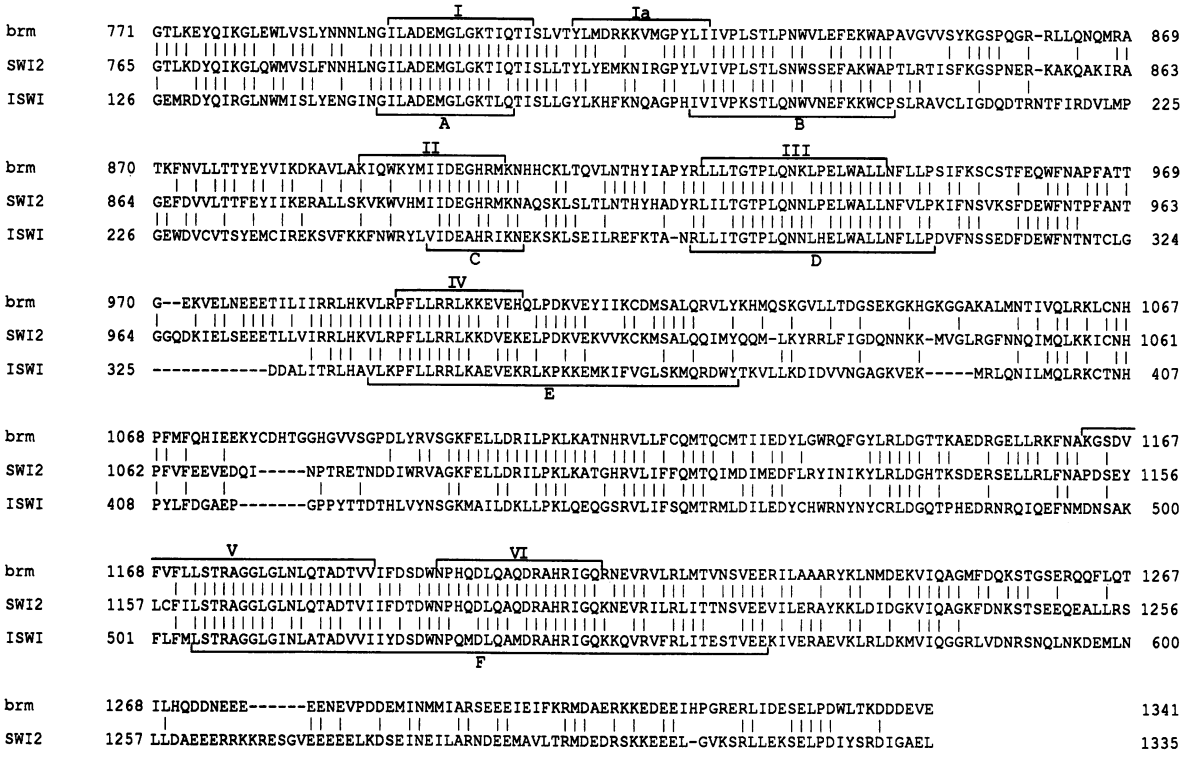

FIG. 6. Sequence alignment of DNA-dependent ATPase domains of the SWI2, brm, and ISWI proteins. The DNA-dependent ATPase (or helicase) domains of SWI2, brm, and ISWI were aligned by using the PIMA algorithm (53). Identities between adjacent sequences are marked by vertical lines. Gaps in the aligned sequences are marked by dashes. The region of each protein shown corresponds to that used to generate SWI2-brm or SWI2-ISWI chimeras. Previously noted motifs (I, Ia, and II to VI) related to those found in DNA helicases (6) are marked above the aligned sequences. Blocks of sequence (A to F) characteristic of the SWI2 protein family (17) are noted below the aligned sequences.

detectable levels near hatching (data not shown). Similar temporal and spatial patterns have been observed for the brm protein (8). *ISWI*, but not *brm*, is expressed at relatively high levels in the embryonic gonads following germ band retraction. The spatially and temporally restricted patterns of *brm* and *ISWI* expression are quite similar and argue against a general role for either gene in transcription or other cellular processes.

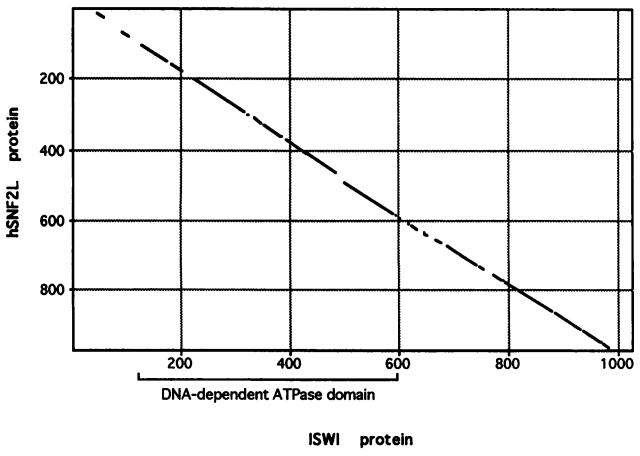

FIG. 7. Structural similarities between the ISWI and hSNF2L proteins. The complete sequences of the ISWI and hSNF2L proteins were compared by a Pustell matrix analysis (45) using MacVector software (International Biotechnologies, Inc.). A protein identity matrix, a window size of 10 residues, and a minimum score of 50% were used for the comparison. Note the high degree of similarity over the entire lengths of both proteins.

Cytological mapping of the *ISWI* gene. Because of its similarity to the *brm* gene, we were interested in determining whether *ISWI* might correspond to any known activator of homeotic genes. To examine this possibility, we mapped the location of the *ISWI* gene in the salivary gland polytene chromosomes by in situ hybridization. The *ISWI* gene is located on the right arm of the second chromosome in region 49BC (data not shown). No previously described activator of homeotic genes, including the loci identified in screens for dominant suppressors of *Polycomb* mutations (24), is located near this region. Further studies will be necessary to determine which, if any, of the previously identified genes in this region correspond to the *ISWI* gene.

In vivo studies of *brm* function in yeast cells. To directly examine the functional relationship between *brm* and *SWI2*, we conducted in vivo studies of *brm* function in yeast cells. Although *SWI2* is not an essential gene, cells lacking *SWI2* grow much more slowly than wild-type cells (36, 43). In addition, many DNA-binding regulatory proteins, including GAL4 (28, 43), SWI5 (3), and glucocorticoid receptor (64), require *SWI2* to activate the transcription of their target genes. To determine whether the highly conserved DNA-dependent ATPase domains of brm and SWI2 are functionally interchangeable, we substituted the DNA-dependent ATPase domain of SWI2 (residues 765 to 1335) with the corresponding region of brm (residues 771 to 1341). This region contains the bipartite NTP-binding site and all other motifs characteristic of the SWI2 protein family (Fig. 2). The SWI2-brm chimera partially rescued the growth defects observed in *swi2*$^-$ cells

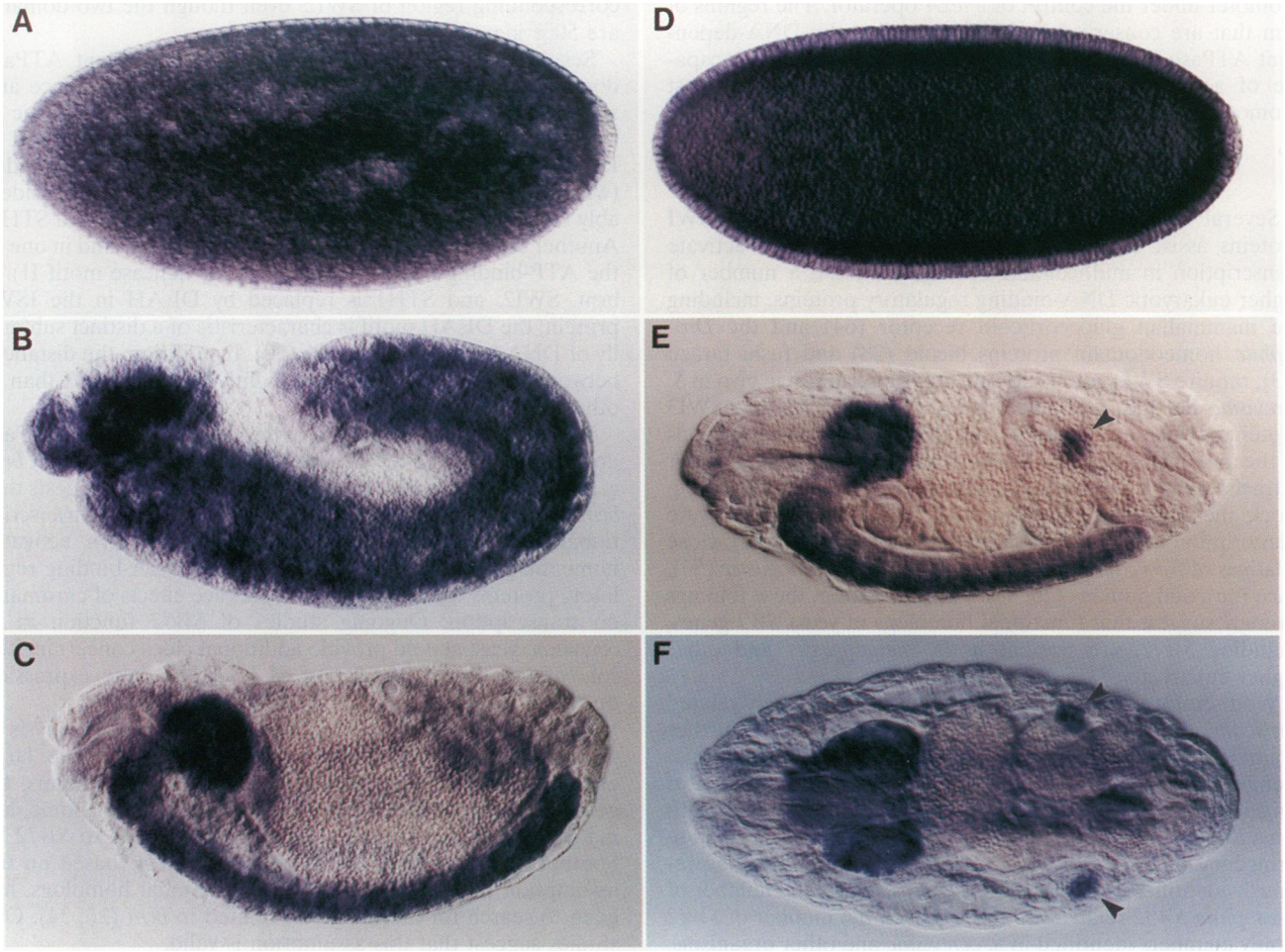

FIG. 8. Distribution of *brm* and *ISWI* mRNAs during embryogenesis. *brm* and *ISWI* transcripts were detected by in situ hybridization of cDNA probes to whole-mount preparations of *Drosophila* embryos. *brm* transcripts are uniformly distributed during the blastoderm (A) and extended germ band (B) stages of embryogenesis. Following germ band retraction, *brm* expression becomes restricted to the ventral nerve cord and brain (C). *ISWI* transcripts are distributed in a similar patterns during most of embryogenesis (D to E). However, unlike *brm*, *ISWI* transcripts are also detected in the embryonic gonads (marked by arrows) following germ band retraction (E, lateral view; F, dorsal view).

(data not shown) and supported transcriptional activation (as measured by β-galactosidase activity) mediated by glucocorticoid receptor in yeast cells: activation of a glucocorticoid response element-linked *lacZ* reporter gene by glucocorticoid receptor was 100% (2,600 Miller units) in the presence of SWI2, 10% for *swi2*$^-$ cells, and 33% in the presence of the SWI2-brm chimera. These data indicate that the function of this domain is highly conserved between brm and SWI2 and strongly suggest that brm, like SWI2, is a DNA-dependent ATPase.

Although the highly related yeast SWI2 and STH1 proteins appear to be functionally distinct (31), their DNA-dependent ATPase domains are functionally interchangeable (29). This result prompted us to examine whether the DNA-dependent ATPase domains of ISWI and SWI2 are also functionally interchangeable. We constructed a gene encoding a chimeric SWI2-ISWI protein in which the DNA-dependent ATPase domain of SWI2 (residues 765 to 1256) is replaced with the corresponding region of ISWI (residues 126 to 600). Unlike the SWI2-brm chimera described above, the SWI2-ISWI chimera failed to support glucocorticoid receptor function in vivo; activation of a glucocorticoid response element-linked *lacZ* reporter gene by glucocorticoid receptor was 9% in the presence of the SWI2 ISWI chimera, as opposed to 10% for *swi2*$^-$ cells (see above). This result is not due to instability of the SWI2-ISWI chimera; epitope-tagged forms of the chimera accumulated to levels equivalent to those of SWI2 as assayed by immunoblotting (data not shown). Thus, the DNA-dependent ATPase domains of SWI2 and ISWI, although highly conserved, are not functionally interchangeable.

A second assay for *brm* function in yeast cells is based on the finding that a fusion protein containing the DNA-binding domain of the LexA protein fused to the N terminus of *SWI2* rescues *swi2* null mutations and activates the transcription of a reporter gene under the control of the *lexA* operator (30). A gene encoding a LexA-brm fusion protein in which the N-terminal 155 residues of brm were replaced by the DNA-binding domain of LexA (residues 1 to 87) was generated by subcloning the *brm* coding region into the high-copy-number yeast expression vector pSH2 (13). This fusion protein, which contains all four of the highly conserved domains common to brm and SWI2, is stable in yeast cells, as evidenced by immunoblotting using an antibody specific for the brm protein (data not shown). However, in contrast to LexA-SWI2 fusion proteins, the LexA-brm fusion protein neither rescued the slow growth of *swi2*$^-$ cells nor activated the transcription of a

promoter under the control of a *lexA* operator. The regions of brm that are conserved in SWI2, including the DNA-dependent ATPase domain and the bromodomain, are thus incapable of activating transcription when tethered near a yeast promoter.

DISCUSSION

Several findings suggest that relatives of the yeast SWI proteins assist DNA-binding regulatory proteins to activate transcription in multicellular eukaryotes. First, a number of higher eukaryotic DNA-binding regulatory proteins, including the mammalian glucocorticoid receptor (64) and the *Drosophila* homeodomain proteins bicoid (28) and fushi tarazu (43), require SWI proteins to activate transcription in vivo in *S. cerevisiae*. Second, antibodies against the yeast SWI1 and SWI3 proteins (which act in concert with SWI2) recognize proteins of the anticipated sizes on Western blots of *Drosophila* nuclear extracts (64). Third, antibodies against the yeast SWI3 protein block the ability of the glucocorticoid receptor to activate transcription in a *Drosophila* in vitro system (64). Finally, close relatives of *SWI2* have been identified in *D. melanogaster* (56), mice (46), and humans (26, 34). Taken together, these findings strongly suggest that functional homologs of yeast *SWI* genes, including *SWI2*, are present in *D. melanogaster* and other higher eukaryotes.

We therefore reasoned that *brm*, or another closely related *Drosophila* gene, is a functional homolog of *SWI2*. To further examine this possibility, we searched for additional *Drosophila* members of the *SWI2* family. We identified a small number of *Drosophila* genes, including *brm* and *ISWI*, that hybridize to yeast *SWI2* DNA probes under conditions of low to moderate stringency. The identification of *ISWI*, together with the previously identified *brm* and *lodestar* genes, brings the number of *Drosophila SWI2* relatives to three. Given the number of *SWI2* family members identified in *S. cerevisiae* and other organisms, it is likely that this number is a minimum estimate. Indeed, at least one additional *Drosophila* genomic DNA fragment appears to hybridize weakly to a *SWI2* DNA probe (Fig. 3). Nonetheless, we have shown that *brm* and *ISWI* are the *Drosophila* genes most similar to the yeast *SWI2* gene.

Comparison of the sequences of the brm and ISWI proteins with the sequence of the SWI2 protein revealed that *ISWI* is unlikely to be a functional homolog of *SWI2*; the ISWI protein contains a highly conserved DNA-dependent ATPase domain, but it lacks the three other domains conserved between brm and SWI2, including the C-terminal bromodomain (16, 56). What is the function of the *Drosophila ISWI* gene? Similarities between the sequence and expression of *ISWI* and *brm* suggest that they may be functionally related. The cytological location of the *ISWI* gene (region 49BC), however, does not correspond to that of any previously identified activators of homeotic (or other) genes. Further studies of *ISWI* will be necessary to determine the role of *ISWI* in transcriptional activation and development.

Since *brm* is the *Drosophila* gene most closely related to *SWI2*, it is the best candidate for its functional homolog. We attempted to confirm this hypothesis by testing the ability of *brm* to substitute for *SWI2* in vivo in *S. cerevisiae*. A SWI2-brm chimera containing the DNA-dependent ATPase domain of the brm protein complemented *swi2* mutations; the chimera partially rescued the slow growth of *swi2* mutants and supported transcriptional activation mediated by the glucocorticoid receptor (see Results). This result suggests that brm, like SWI2, is a DNA-dependent ATPase. In contrast, the DNA-dependent ATPase domain of ISWI cannot substitute for the corresponding region of SWI2, even though the two domains are 50% identical.

Several unusual features of the DNA-dependent ATPase domain of ISWI might explain this result. The sequence and spacing of several of the blocks of sequence characteristic of the SWI2 protein family (6, 17) are not highly conserved in ISWI (Fig. 6). For example, blocks C (helicase motif II) and E (which overlaps helicase motif IV) of ISWI diverge considerably from the corresponding regions of brm, SWI2, and STH1. Another difference is that the DEGH sequence found in one of the ATP-binding motifs (the C block, or helicase motif II) of brm, SWI2, and STH1 is replaced by DEAH in the ISWI protein; the DEAH motif is characteristic of a distinct subfamily of DNA and RNA helicases (50). In addition, the distances between blocks D, E, and F are slightly larger in ISWI than in other SWI2 family members.

The functional conservation of the DNA-dependent ATPase domains of brm and SWI2, together with our finding that *brm* is the closest *Drosophila* relative of *SWI2*, strongly suggests that *brm* and *SWI2* play similar, if not identical, roles in transcriptional activation. It is therefore likely that *brm* activates homeotic genes by assisting one or more DNA-binding regulatory proteins to overcome the repressive effects of chromatin on transcription. Ongoing studies of *SWI2* function in *S. cerevisiae* yeast should provide additional clues concerning the role of *brm* in the regulation of homeotic gene expression during development.

Our work also has important implications for the analysis of genes related to *SWI2* and *brm* in vertebrates. The large evolutionary distance between *S. cerevisiae* and humans, together with the relatively large size of the human genome, has made it difficult to identify human genes related to *SWI2* by Southern blotting (55). An alternative strategy, based on the assumption that *SWI2* and *brm* are functional homologs, has been to search for human genes related to *brm* (26, 34). Our studies suggest that this assumption is valid.

Two human genes, *hbrm* (human *brm*) and *BRG1* (*brm/SWI*-related gene 1) have been independently isolated by screening human cDNA libraries with brm nucleic acid probes (26, 34). The hbrm and BRG1 proteins are highly related to the brm protein over their entire lengths and are therefore excellent candidates for human homologs of brm. The hbrm and BRG1 proteins are also strikingly similar to each other. For example, their DNA-dependent ATPase domains are greater than 90% identical. The existence of two genes highly related to *brm* in humans contrasts sharply to the situation in *D. melanogaster*, in which *brm* is clearly a single-copy gene. This finding suggests that *hbrm* and *BRG1* arose as a consequence of a gene duplication following the divergence of arthropods and chordates. Since no systematic search for human genes related to *SWI2* and *brm* has yet been conducted, it remains possible that additional human members of the *SWI2* family remain unidentified.

Sequence similarities between the *Drosophila* brm and ISWI proteins and their potential human homologs support a recent proposal that regions outside the DNA-dependent ATPase domain contribute to the functional specificity of SWI2 family members (29). Both the hbrm and BRG1 proteins are highly related to brm within and outside the DNA-dependent ATPase domain (26, 34). Similarly, ISWI and hSNF2L are highly conserved over their entire lengths, including the regions flanking the DNA-dependent ATPase domain (Fig. 7). The phylogenetic conservation of the regions flanking the DNA-dependent ATPase domains of brm and ISWI is consistent with the view that these regions are functionally important.

It is therefore notable that with the exception of several short domains, the N-terminal 730 residues of brm and SWI2 are strikingly divergent. Similarly, the least conserved regions of the hbrm and BRG1 proteins are those N terminal to the DNA-dependent ATPase domain. This divergence suggests that *brm* (and its higher eukaryotic relatives) may have evolved to interact with different proteins. Perhaps the N-terminal segment of the brm protein interacts with divergent *Drosophila* relatives of other SNF and SWI proteins or with chromatin components unique to higher eukaryotes such as the Polycomb group of repressors. Further genetic and biochemical studies, including the identification of proteins associated with brm, will be necessary to test this possibility and clarify the functional relationship between the yeast *SWI2* gene and its higher eukaryotic relatives.

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