Genetic Analysis of *brahma*: The Drosophila Homolog of the Yeast Chromatin Remodeling Factor SWI2/SNF2

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Manuscript received July 25, 1997
Accepted for publication September 18, 1997

ABSTRACT

The Drosophila *brahma* (*brm*) gene encodes an activator of homeotic genes related to the yeast chromatin remodeling factor SWI2/SNF2. Here, we report the phenotype of null and dominant-negative *brm* mutations. Using mosaic analysis, we found that the complete loss of *brm* function decreases cell viability and causes defects in the peripheral nervous system of the adult. A dominant-negative *brm* mutation was generated by replacing a conserved lysine in the ATP-binding site of the BRM protein with an arginine. This mutation eliminates *brm* function *in vivo* but does not affect assembly of the 2-MD BRM complex. Expression of the dominant-negative BRM protein caused peripheral nervous system defects, homeotic transformations, and decreased viability. Consistent with these findings, the BRM protein is expressed at relatively high levels in nuclei throughout the developing organism. Site-directed mutagenesis was used to investigate the functions of conserved regions of the BRM protein. Domain II is essential for *brm* function and is required for the assembly or stability of the BRM complex. In spite of its conservation in numerous eukaryotic regulatory proteins, the deletion of the bromodomain of the BRM protein has no discernible phenotype.

THE homeotic genes of the Antennapedia complex (ANTC) and the bithorax complex (BXC) encode transcription factors that specify the identities of body segments in Drosophila (Duncan 1987; Kaufman *et al.* 1990). Since alterations in cell fate result from either the inactivation or derepression of homeotic genes, their transcription must be regulated precisely to ensure normal development. During much of Drosophila development, the transcription of homeotic genes is controlled by two distinct groups of regulatory genes: the Polycomb group of repressors and the trithorax group of activators (Kennison 1993, 1995; Simon 1995). Although the mechanism of action of most Polycomb and trithorax group members is not well understood, several are thought to regulate transcription by altering chromatin structure. For example, the Polycomb (PC) protein contains a short segment—the chromodomain—which is conserved in a component of Drosophila heterochromatin, the HP1 protein (Paro and Hogness 1991). Based on this similarity, it has been proposed that PC represses transcription by packaging inactive homeotic genes into heterochromatin-like complexes. *brahma* (*brm*), a trithorax group gene, was identified in a screen for dominant suppressors of *Pc* mutations (Kennison and Tamkun 1988). A direct connection between the regulation of homeotic gene expression and chromatin was provided by the discovery that BRM is strikingly related to SWI2/SNF2, a chromatin remodeling factor in the yeast *Saccharomyces cerevisiae* (Tamkun *et al.* 1992).

SWI2/SNF2 is a subunit of a 2-MD complex, the SWI/SNF complex, that assists a wide variety of sequence-specific transcription factors to activate the transcription of their target genes (Winston and Carlson 1992; Carlson and Laurent 1994; Peterson and Tamkun 1995). Both BRM and SWI2/SNF2 contain sequence motifs closely related to those found in DNA-stimulated ATPases; these motifs are clustered in a 500–amino acid segment known as the ATPase domain (Henikoff 1993). Purified yeast SWI/SNF complex has ATPase activity and stimulates the binding of GAL4 derivatives and other transcription factors to nucleosomal DNA *in vitro* (Côté *et al.* 1994; Owen-Hughes *et al.* 1996). *swi2/snf2* mutations are suppressed by mutations in nucleosomal histones and cause alterations in chromatin structure in the vicinity of the *SUC2* promoter *in vivo* (Hirschhorn *et al.* 1992, 1995; Prelich and Winston 1993). The SWI2/SNF2 protein thus appears to use the energy of ATP hydrolysis to counteract the repressive effects of chromatin on transcription.

A large number of other eukaryotic proteins are related to BRM and SWI2/SNF2 within the ATPase do-

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main (Eisen *et al.* 1995). Several of these ATPases, including the yeast STH1 and Drosophila ISWI proteins, are subunits of chromatin remodeling complexes that are distinct from SWI/SNF (Peterson 1996; Pazin and Kadonaga 1997). For example, Drosophila ISWI is a subunit of at least three different protein complexes: the 500-kD nucleosome remodeling factor (NURF; Tsukiyama *et al.* 1995), the 670-kD chromatin accessibility complex (CHRAC; Varga-Weisz *et al.* 1997), and the 220-kD ATP-utilizing chromatin assembly and remodeling factor (ACF; Ito *et al.* 1997). The yeast STH1 protein is a subunit of a 1-MD complex known as RSC (remodels the structure of chromatin; Cairns *et al.* 1996). Although SWI/SNF, RSC, NURF, CHRAC, and ACF each catalyze ATP-dependent alterations in chromatin structure *in vitro,* differences in their biochemical properties and subunit composition suggest they are likely to play distinct roles in chromatin remodeling *in vivo.*

Several lines of evidence suggest that BRM and SWI2/SNF2 play similar roles in chromatin remodeling. First, the similarities between the two proteins extend well beyond the ATPase domain and define three additional conserved regions: domain I, domain II, and the bromodomain (Tamkun *et al.* 1992; Figure 1). These similarities are likely to be significant since the regions flanking the ATPase domain are thought to contribute to the functional specificity of SWI2/SNF2 family members by mediating interactions with other proteins (Laurent *et al.* 1993; Treich *et al.* 1995; Treich and Carlson 1997). Biochemical studies of BRM and its putative human homologs, BRG1 (*brm-SWI2/SNF2 related gene 1*; Khavari *et al.* 1993) and HBRM (*human brm*; Muchardt and Yaniv 1993), have provided further evidence that *brm* and *SWI2/SNF2* are functional homologs. BRM is a subunit of a 2-MD complex that contains SNR1, the putative Drosophila homolog of the SNF5 subunit of the yeast SWI/SNF complex (Dingwall *et al.* 1995). The human BRG1 and HBRM proteins are also subunits of 2-MD complexes that contain several proteins related to yeast SWI/SNF proteins (Muchardt *et al.* 1995; Wang *et al.* 1996a,b). Like SWI/SNF, the BRG1 complex facilitates the binding of transcriptional activators to nucleosomal DNA *in vitro* (Kwon *et al.* 1994; Wang *et al.* 1996a). These findings strongly suggest that *brm* and its vertebrate homologs encode catalytic subunits of conserved, higher eukaryotic counterparts of the yeast SWI/SNF complex.

Despite these similarities, there are striking differences in the composition of the yeast SWI/SNF complex and its counterparts in higher eukaryotes. Multiple BRG1 and HBRM complexes are present in human cells, and the subunit composition of these complexes varies in different cell types (Wang *et al.* 1996a). These differences may reflect specialized roles of the BRG1 and HBRM complexes in development or other processes. Indeed, studies of BRG1 have revealed potential roles in viral integration, cell cycle control, and cancer (Kalpana *et al.* 1994; Dunaief 1994; Strober *et al.* 1996).

What have previous genetic studies of *brm* revealed about the role of chromatin remodeling factors in multicellular eukaryotes? *brm* is an essential gene that is expressed both maternally and zygotically (Tamkun *et al.* 1992; Brizuela *et al.* 1994). Loss of maternal *brm* function blocks oogenesis; individuals homozygous for extreme *brm* alleles die as late embryos with no obvious pattern defects (Brizuela *et al.* 1994). Since it has not been possible to generate embryos lacking both maternal and zygotic *brm* function, the exact role of *brm* in embryonic development is not clear. Information concerning the role of *brm* after embryogenesis has been derived primarily from the analysis of hypomorphic *brm* alleles. Individuals trans-heterozygous for certain combinations of *brm* alleles survive to adulthood and exhibit developmental abnormalities similar to those arising from reduced expression of ANTC and BXC genes, including the transformation of first legs to second legs and the fifth abdominal segment to a more anterior identity (Brizuela *et al.* 1994). Because the effect of complete loss of *brm* function had not been examined, it was unclear whether *brm* is also involved in other processes.

To clarify the role of *brm* in Drosophila development, we examined the spatial and temporal expression of the BRM protein and used mosaic analysis to determine the null phenotype of *brm* mutations. As an alternative approach, we used site-directed mutagenesis to generate dominant-negative *brm* mutations and investigate the functions of evolutionarily conserved domains within the BRM protein.

MATERIALS AND METHODS

Stocks: Flies were raised on a cornmeal-molasses-yeast-agar medium containing Tegosept and propionic acid at 25°. Mutations and chromosome aberrations are described in Lindsley and Zimm (1992) unless otherwise noted. brm^{1} and brm^{2} are described in Kennison and Tamkun (1988) and Brizuela *et al.* (1994). *Df(3L)th102* deletes polytene chromosome region 72A1;72D12, including *brm.* The FLP and FRT stocks (Xu and Rubin 1993), *UAS-lacZ* reporter 4-2-4B (Brand and Perrimon 1993), and IJ3 and 69B *GAL4* insertion lines (Brand and Perrimon 1993) used in this study were obtained from the Bloomington Stock Center (Indiana University, Bloomington, IN). The e16E *GAL4* insertion line is described in Harrison *et al.* (1995). *y w P[ry*$^{+}$*, hsFLP]12* was generously provided by T.-B. Chou and N. Perrimon.

Production of antibodies against the BRM protein: Polyclonal rabbit antisera were raised against glutathione *S*-transferase (GST) fusion proteins containing amino acids 1504–1638 or 505–775 of the BRM protein (Figure 1). The first fusion protein was produced by subcloning a 423-bp *Sau3A* fragment from *brm* cDNA6 (Tamkun *et al.* 1992) into the *Bam*HI site of pGEX3X (Pharmacia LKB, Piscataway, NJ). To produce the second fusion protein, the polymerase chain reaction (Saiki *et al.* 1988) was used to amplify an 816-bp fragment from *brm* cDNA 1′ (Tamkun *et al.* 1992) subcloned in pGEM7ZF

using a T7 promoter primer and a primer (5′-TGGAATTCC TTGAGCGTA-3′) that converts nucleotides 2377–2382 of the *brm* cDNA into an *Eco*RI site. The amplified fragment was cloned into the *Eco*RI site of pGEX-KG (Guan and Dixon 1991). GST-BRM fusion proteins were expressed in *Escherichia coli*, purified on glutathione-agarose columns, and used to immunize rabbits as described in Harlow and Lane (1988). Immune sera were affinity purified on columns containing either GST or GST-BRM fusion proteins coupled to Affigel 10 or Affigel 15 resins (Bio-Rad, Richmond, CA) according to the manufacturer's instructions. Antibodies were eluted with high salt, dialyzed, and assayed for specificity by Western blotting.

Rabbit antibodies against the BRM protein were used to stain whole-mount preparations of 0–22-hr Drosophila embryos as described in Carroll and Scott (1985). 12CA5 mouse monoclonal antibody (Babco, Berkeley, CA) was used to detect BRM proteins bearing an epitope tag from the influenza hemagglutinin protein (HA; Wilson *et al.* 1984; Kolodziej and Young 1991). Goat or mouse anti-rabbit secondary antibodies conjugated to horseradish peroxidase (Bio-Rad) were used to detect the bound primary antibody. To localize the BRM protein in larval tissues, third instar larvae were dissected, fixed, and stained as described by Pattatucci and Kaufman (1991).

Clonal analysis of *brm* mutations: Clones of homozygous mutant *brm* tissue were generated in heterozygous larvae by mitotic recombination using the FLP-FRT technique (Golic 1991; Xu and Rubin 1993). As an internal control, we compared the frequency, size, and phenotype of *brm* clones in the presence and absence of a wild-type *brm* transgene carried on the second chromosome ($P[w^+, brm^+]21A$). The FRT at the base of 3L at position 80B was recombined onto the brm^2 *st cp in ri* p^p chromosome. *y w P[ry*$^+$*, hsFLP]12/Y*; $P[w^+, brm^+]21A$/+; *P[ry*$^+$*, y*$^+$*](66E) P[ry*$^+$*, FRT](80B)*/+ males were crossed to *y w*; brm^2 *P[ry*$^+$*, FRT](80B)/TM3, Ser* virgin females, and second instar larvae were exposed to a 90-min heat shock to induce expression of FLP recombinase. Body parts of experimental *[y w P[ry*$^+$*, hsFLP]12/y w*; *P[ry*$^+$*, y*$^+$*](66E) P[ry*$^+$*, FRT](80B)/*brm^2 *P[ry*$^+$*, FRT](80B)* and control (*y w P[ry*$^+$*, hsFLP]12/y w*; $P[w^+, brm^+]21A$/+; *P[ry*$^+$*, y*$^+$*](66E) P[ry*$^+$*, FRT](80B)/*brm^2 *P[ry*$^+$*, FRT](80B)]* adults were dissected in 70% ethanol, mounted in methyl salicylate and Canada balsam, and examined by bright-field microscopy. Clones of homozygous brm^2 tissue were identified in the adult cuticle by the loss of the *y*$^+$ gene on the *P[ry*$^+$*, y*$^+$*](66E) P[ry*$^+$*, FRT](80B)* chromosome.

Site-directed mutagenesis and generation of transgenic strains: Two *brm* genomic DNA fragments were used as the templates for site-directed mutagenesis. The first was a 14.4-kb *Bam*HI-*Eco*RI fragment (BR14.4) that spans the entire *brm* gene (Tamkun *et al.* 1992). The second was a modified version of this DNA fragment (BR14.4T) that encodes a BRM protein with a C-terminal tag from the influenza HA protein followed by six histidines (Dingwall *et al.* 1995). P-element transgenes encoding either untagged ($P[w^+, brm^+]21A$) or tagged ($P[w^+, brm^+]92C$) BRM protein rescue the recessive lethality of extreme *brm* alleles (Brizuela *et al.* 1994; Dingwall *et al.* 1995).

To generate the $brm^{\Delta 1446\text{-}1517}$ mutation lacking the bromodomain, we PCR-amplified fragments from a subcloned 3.6-kb *Bam*HI-*Eco*RI fragment of BR14.4 using primers that hybridize to either the T7 or SP6 promoters flanking the cloning site and the mutagenic primers 5′-CGGACAGAGCTCGTCCAT-3′ or 5′-ACTCGAGAGCTCTGCAAAAG-3′. These primers introduce a *Sac*I site at the site previously occupied by the bromodomain. After cleavage with *Sac*I, the amplified fragments were ligated together, substituted for the 3.6-kb *Bam*HI-*Eco*RI genomic DNA fragment in BR14.4, and subcloned in the *P*-element transformation vector pCaSpeR (Pirrotta 1988) to generate $P[w^+, brm^{\Delta 1446\text{-}1517}]$.

The ATP-binding site mutation brm^{K804R} and domain II deletion $brm^{\Delta 549\text{-}610}$ were generated by site-directed mutagenesis (Kunkel *et al.* 1985) of a 1.3-kb *Bam*HI-*Eco*RI fragment from BR14.4 using the mutagenic oligonucleotides 5′-TGAATG GTTCTACCCAAAC-3′ and 5′-GGTCGTCCTTGTGCTGGT GATTCATCACGGC-3′, respectively. The mutant fragments were used to replace the corresponding wild-type fragments within BR14.4T. The resulting fragments were subcloned in pCaSpeR to generate $P[w^+, brm^{K804R}]$ and $P[w^+, brm^{\Delta 549\text{-}610}]$. Transgenic strains bearing the $P[w^+, brm^{\Delta 1446\text{-}1517}]$, $P[w^+, brm^{K804R}]$ or $P[w^+, brm^{\Delta 549\text{-}610}]$ transgenes were generated by *P*-element–mediated transformation as described in Tamkun *et al.* (1991). To control against errors introduced during PCR amplification or site-directed mutagenesis, all relevant regions were sequenced on one strand (Sanger *et al.* 1977) before transformation.

Functional analysis of *brm* transgenes: An insertion of $P[w+, brm^{K804R}]$ on the second chromosome ($P[w^+, brm^{K804R}]$17D) was tested for the ability to rescue the hemizygous lethality of brm^2. *w/Y*; $P[w^+, brm^{K804R}]17D$/+; *Df(3L)th102, h ri Sb ca*^{2}/+ males were mated to virgin *w/w*; brm^2/*ln(3LR)DcxF, ru h D* females and the progeny were scored for *w*; $P[w^+, brm^{K804R}]17D$/+; brm^2/*Df(3L)th102, h ri Sb ca*^{2} adults. Two independent insertions of the $P[w^+, brm^{\Delta 1446\text{-}1517}]$ transgene on the second chromosome (22-1 and 13-1) were tested for the ability to rescue brm^2 mutations. For both insertions, *w;* $P[w^+, brm^{\Delta 1446\text{-}1517}]$/+; brm^2 *st cp in ri* p^p/*TM3, Sb* virgin females were crossed to *w*; $P[w^+, brm^{\Delta 1446\text{-}1517}]$/+; brm^2 *st cp in ri* p^p/*TM3, Sb* males, and the progeny were scored for $P[w^+, brm^{\Delta 1446\text{-}1517}]$/+; brm^2 *st cp in ri* p^p/brm^2 *st cp in ri* p^p adults.

Two independent insertions of the $P[w^+, brm^{\Delta 549\text{-}610}]$ transgene on the *X* chromosome (24-1 and 4-3) and a single insertion on the second chromosome (3-3) were tested for the ability to rescue the hemizygous lethality of brm^2. For both insertions on the *X* chromosome, *w* $P[w^+, brm^{\Delta 549\text{-}610}]$*/Y*; *Df(3L)th102, h ri Sb ca*^{2}/+ males were crossed to *w*; brm^2 *st cp in ri* p^p/*TM3, Ser* virgins, and the progeny were scored for *w* $P[w^+, brm^{\Delta 549\text{-}610}]$/*w*; *Df(3L)th102, h ri Sb ca*^{2}/brm^2 *st cp in ri* p^p adults. To test the ability of four copies of the $P[w^+, brm^{\Delta 549\text{-}610}]$ transgene to rescue the hypomorphic brm^1 allele, *w* $P[w^+, brm^{\Delta 549\text{-}610}]4\text{-}3$ $P[w^+, brm^{\Delta 549\text{-}610}]24\text{-}1/Y$; *mwh* brm^1 *st cu sr* e^s *ca/ln(3LR)CxD* males were crossed to *w* $P[w^+, brm^{\Delta 549\text{-}610}]4\text{-}3$ $P[w^+, brm^{\Delta 549\text{-}610}]24\text{-}1$/+; *mwh* brm^1 *st cu sr* e^s *ca*/*ln(3LR)CxD* females and the progeny were scored for *w* $P[w^+, brm^{\Delta 549\text{-}610}]4\text{-}3$ $P[w^+, brm^{549\text{-}610}]24\text{-}1$*; mwh* brm^1 *st cu sr* e^s *ca/mwh* brm^1 *st cu sr* e^s *ca* progeny.

As a positive control for all of the above experiments, we showed that a wild-type *brm* transgene ($P[w^+, brm^+]21A$) was able to rescue the homozygous and hemizygous lethality of brm^1 and brm^2. No homozygous or hemizygous *brm* adults were observed in the progeny of the above crosses in the absence of a rescuing *brm* transgene.

Generation and analysis of a GAL4-responsive brm^{K804R} transgene: Using *brm* cDNA clones and the BR14.4T *brm* genomic DNA fragment, we created a 6.7-kb DNA fragment that contains the coding sequence for the HA-tagged BRM protein flanked by 66 nucleotides of 5′ genomic DNA and ~1.3 kb of 3′ genomic DNA, including the *brm* polyadenylation site. This fragment extends from the *Pst*I site 66 nucleotides upstream of the *brm* initiation codon to the *Bam*HI site at coordinate −22 of a chromosome walk through the *brm-arl* region (Tamkun *et al.* 1992), but lacks the introns within this region of genomic DNA. A 0.76-kb *Nsi*I/*Hin*dIII genomic DNA fragment within this fragment (which encodes residues 682–937 of BRM) was replaced with the corresponding fragment of the brm^{K804R} transgene described above. The resulting fragment was placed under the control of the GAL4 *UAS* and minimal

hsp70 promoter in the *P*-element transformation vector pUAST (Brand and Perrimon 1993). A homozygous viable insertion of this transgene ($P[w^+, UAS_{GAL}hsp70:brm^{K804R}]2\text{-}2$) on the third chromosome was generated by *P*-element transformation, as described in Tamkun *et al.* (1991).

To induce expression of the transgene, homozygous *y Df(1)w67c2*; $P[w^+, UAS_{GAL}hsp70:brm^{K804R}]2\text{-}2$ virgins were crossed to males bearing the *GAL4* insertions 69B, IJ3, or e16E. As a negative control in these experiments, virgin females homozygous for a *P*-element insertion bearing a *UAS-lacZ* reporter gene (4-2-4B; Brand and Perrimon 1993) were crossed to homozygous 69B males in parallel. To determine the lethal phase and phenotype of individuals expressing the BRM^{K804R} protein under the control of the 69B *GAL4* insertion, eggs were collected on grape juice plates for 3 hr at either 20° or 25°. 36 hr later, the number of unhatched eggs were counted to score embryonic lethality, and first instar larvae were transferred to vials at the same temperature. The number of pupae and adults per vial were counted to assess larval and pupal lethality. At least 150 embryos were collected at each temperature for both the control and experimental crosses.

Electrophoresis and Western blotting: SDS-polyacrylamide gel electrophoresis and Western blotting were performed as described previously (Tsukiyama *et al.* 1995). Drosophila embryos, larvae, pupae, or adults were homogenized in boiling electrophoresis sample buffer (62.5 mm Tris-HCl, pH 6.8, 2% SDS, 0.72 m β-mercaptoethanol, 10% glycerol), boiled for an additional 10 min, and centrifuged at 10,000 rpm for 10 min at room temperature. Embryos were dechorionated in 50% sodium hypochlorite before homogenization. Protein was quantitated using the Bio-Rad protein assay, and 30 μg total protein extract was loaded per lane.

Gel filtration chromatography: To prepare nondenatured protein extracts, 0–12-hr embryos were dechorionated and homogenized in an equal volume of ice-cold extraction buffer (50 mm Hepes, pH7.6, 385 mm NaCl, 0.1% Tween 20, 0.1 mm EGTA, 1.1 mm $MgCl_2$, 100 μg/ml PMSF, and 1 μg/ml each aprotinin, leupeptin, chymostatin and Pepstatin A) using a Dounce homogenizer. The homogenate was centrifuged for 1 hr at 55,000 *g*. After addition of glycerol (to 10% v/v), the supernatant was aliquoted and frozen in liquid nitrogen. Before gel filtration chromatography, extracts were passed over a G-25 column and eluted in column buffer (50 mm sodium phosphate, pH 7.8, 400 mm NaCl, 0.05% Tween 20, 0.1 mm EGTA, 1.0 mm $MgCl_2$, 0.1 mm DTT, 10% glycerol, and 1 μg/ml each aprotinin, leupeptin, chymostatin, and Pepstatin A). 2 mg of extract was diluted to 200 μl with column buffer and fractionated on a Superose 6 10/30 FPLC column (Pharmacia) equilibrated in column buffer. 0.5-ml fractions were collected and analyzed by Western blotting.

RESULTS

Developmental expression of the BRM protein: Previous studies revealed that the expression of *brm* RNA is both temporally and spatially regulated (Tamkun *et al.* 1992; Elfring *et al.* 1994). *brm* RNA is expressed at high levels throughout the early embryo but becomes restricted to the ventral nerve cord and brain by stage 15 of embryogenesis. No *brm* transcripts are detected between stage 16 of embryogenesis and hatching. Low levels of *brm* RNA are observed in larvae, pupae, and adult females, and no *brm* RNA has been detected in adult male flies. Based on the restricted pattern of *brm* RNA expression, it has been proposed that *brm* is unlikely to play a general role in transcription (Tamkun *et al.* 1992; Elfring *et al.* 1994). To further examine this

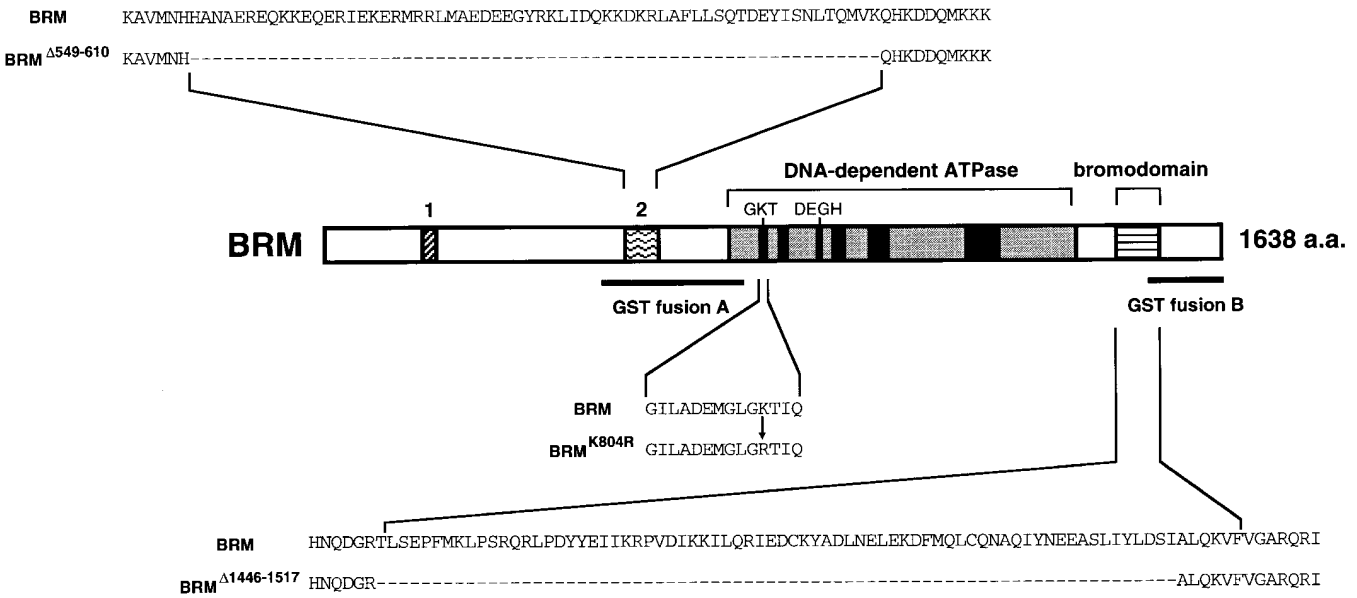

Figure 1.—Schematic comparison of wild-type and mutant BRM proteins. The four regions that are highly conserved in BRM and its putative yeast and human homologs are represented by shaded boxes [domain I, diagonal lines; domain II, wavy lines; domain III (DNA-dependent ATPase domain), stippled; domain IV (the bromodomain), horizontal lines]. The positions of the bipartite ATP-binding site (GKT and DEGH) and other highly conserved blocks of sequence within the ATPase domain (Henikoff 1993) are marked by black boxes. The sequences altered in the $BRM^{\Delta 549\text{-}610}$, BRM^{K804R}, and $BRM^{\Delta 1446\text{-}1517}$ proteins are compared to the sequence of the BRM protein. Regions of the BRM protein contained in the GST-BRM fusion proteins used as immunogens are underlined.

issue, we characterized the expression of BRM protein during Drosophila development.

Rabbit polyclonal antisera were raised against different GST-BRM fusion proteins (Figure 1). One of the fusion proteins contains a region extending from within the bromodomain to the C terminus of BRM (residues 1504–1638). The other fusion protein contains a region of BRM (residues 505–776) that spans domain II and extends into the ATPase domain. Whole-sera and affinity-purified antibodies against both fusion proteins, but not preimmune sera, detect a 200-kD protein on Western blots of Drosophila extracts (Figure 2). This molecular weight is very close to that predicted for BRM based on its sequence (~185 kD). These antibodies (but not preimmune sera) also detect the BRM protein in whole-mount preparations of both embryos and larvae (Figure 3).

Unlike *brm* RNA, BRM protein is present at all stages of development, as revealed by Western blotting (Figure 2). BRM protein is expressed at relatively high levels throughout embryogenesis and in pupae; lower amounts of BRM are present in larvae and adult flies. We investigated the level of BRM protein in developing embryos by quantitative Western blotting using a GST-BRM fusion protein as a standard. Approximately equivalent immunoreactivity was observed with 2 ng of purified fusion protein and protein extracted from 40 3–6-hr embryos (data not shown). Since ~6,000 nuclei are present at this stage of development, we estimate that there are at least 100,000 molecules of BRM protein per nucleus at its peak stage of expression. This level of expression corresponds to approximately one molecule of BRM protein per 20 nucleosomes, contrasting sharply with the relatively low abundance of SWI2/SNF2 in yeast cells (approximately several hundred molecules per nucleus; Côté *et al.* 1994).

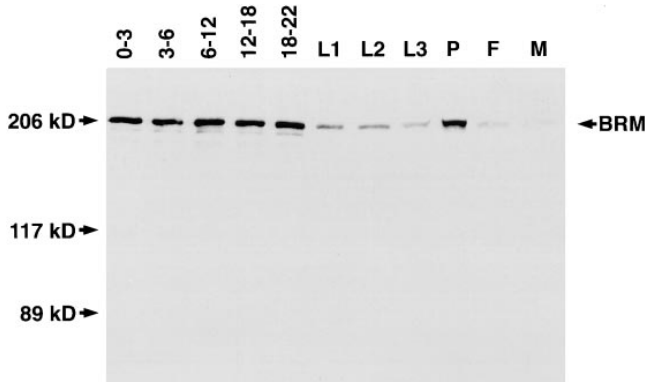

Figure 2.—Developmental expression of the BRM protein. Thirty micrograms each of protein extracted from *Drosophila melanogaster* embryos (0–3, 3–6, 6–12, 12–18, and 18–22 hr); first (L1), second (L2), and third (L3) instar larvae, pupae (P), adult males (M), and females (F) were electrophoresed on an 8% SDS-polycacrylamide gel and analyzed by Western blotting. BRM protein levels are highest in embryos and pupae; much lower levels of BRM are present in larvae and adults.

We also examined the spatial expression of BRM protein by immunostaining whole-mount preparations of embryos and larvae. BRM protein is present at similar levels in nuclei throughout the early embryo (Figure 3A). The BRM protein continues to be expressed ubiquitously during the remainder of embryogenesis, although its levels are somewhat enriched in the ventral nerve cord and brain in late embryos (Figure 3, B and C). In late third instar larvae, BRM protein is expressed at relatively uniform levels in nuclei of the imaginal discs (Figure 3, D–F) and other diploid and polytene tissues, including the polytene nuclei of the salivary gland (data not shown). Thus, in contrast to the previously reported patterns of *brm* RNA expression, the BRM protein is ubiquitously expressed throughout the developing organism.

Somatic clonal analysis of *brm* mutations: Since *brm* mutations are recessive lethal mutations, we used somatic clonal analysis to analyze the role of *brm* after embryogenesis. Homozygous clones of *brm* mutant tissue marked with *yellow* were generated in second instar larvae using the FLP-FRT technique (Golic 1991; Xu and Rubin 1993). A strong *brm* allele, brm^2, was used for the majority of these experiments; evidence that brm^2 is a protein null allele is presented below. As an internal control for effects on cell viability or division, we compared the frequency, size, and phenotype of clones of *brm* mutant tissue in the presence or absence of a wild-type *brm* transgene ($P[w^+, brm^+]21A$) on the second chromosome.

A total of 716 mutant clones were scored and compared with 1396 clones generated in control siblings (Table 1). The size and frequency of the clones in control and experimental individuals revealed that loss of *brm* activity has dramatic effects on cell viability in the imaginal discs; the size and frequency of experimental clones in the head and thoracic segments were significantly reduced relative to the controls (Table 1). In contrast, the frequency and size of control and experimental clones in the abdomen were similar (Table 1). These data indicate that *brm* is essential for the development of imaginal tissues but not abdominal histoblasts. It is also possible that sufficient *brm* RNA or protein persisted after clone induction to allow the development of the abdominal segments.

Examination of the phenotype of brm^2 clones revealed unanticipated defects in the adult peripheral nervous system. The mechanosensory bristles of brm^2 clones in the head, thoracic, and abdominal segments were either duplicated, stunted, or fused (Table 1; Figure 4A). In many cases, the sockets were also malformed, absent, or duplicated (Figure 4A). These defects were not observed in clones generated in individuals bearing a brm^+ transgene, indicating that they are caused by the brm^2 mutation and not another mutation on the brm^2-

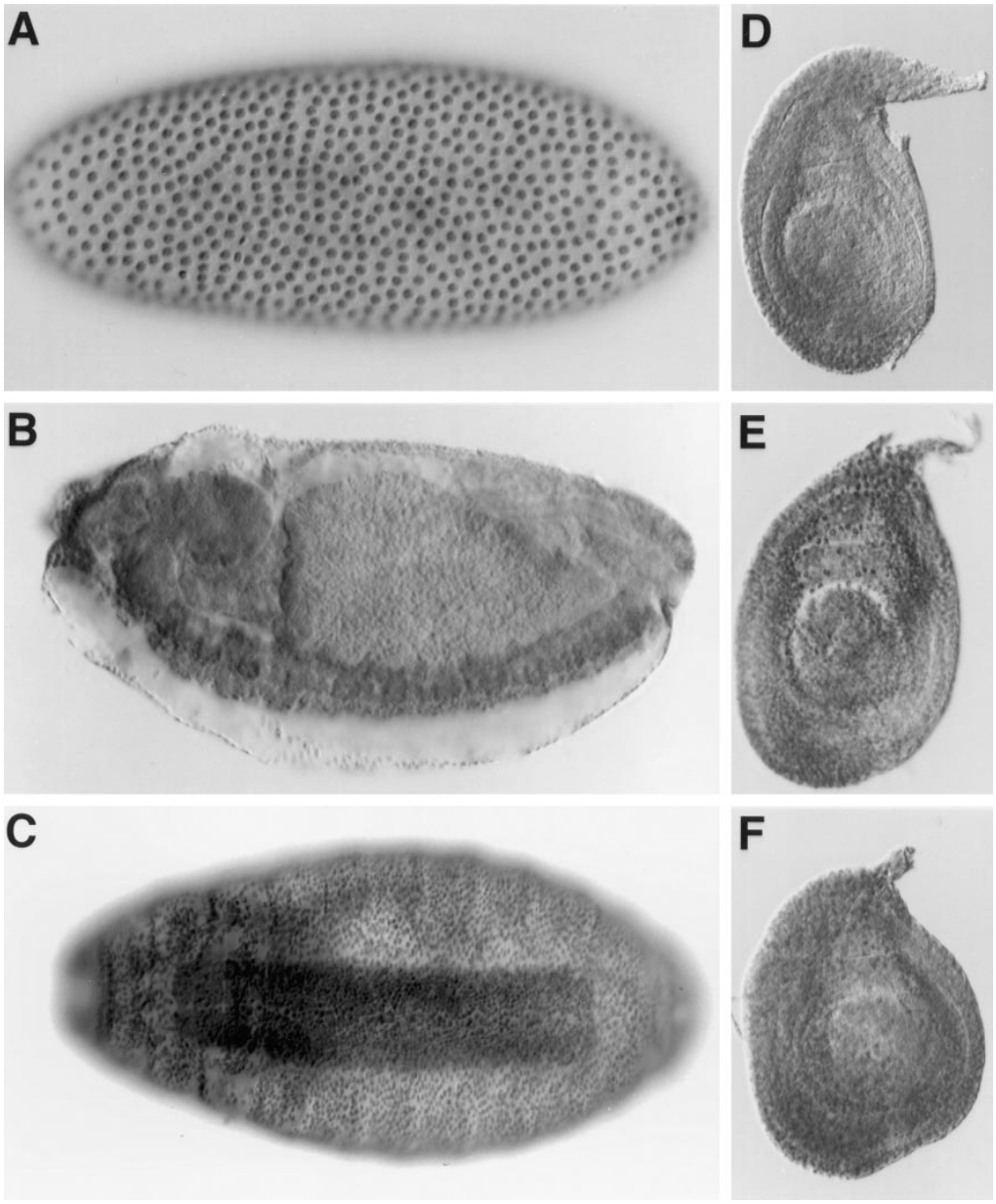

Figure 3.—Expression of the BRM protein in embryos and imaginal discs. Whole-mount preparations of Drosophila embryos or larvae were stained with affinity-purified antibodies directed against the BRM protein. (A) Late syncytial embryo. (B) Lateral view of stage 15 embryo. (C) Ventral view of stage 17 embryo. (D) Prothoracic leg disc. (E) Mesothoracic leg disc. (F) Metathoracic leg disc. The BRM protein is expressed in nuclei throughout the developing embryo and larva.

FRT chromosome. No homeotic transformations were observed in experimental clones in any of the abdominal segments. The small number and size of experimental clones in the head and thoracic segments did not allow us to score alterations in cell fate arising from loss of *brm* activity in these segments.

Generation and analysis of a dominant-negative *brm* mutation: As an alternative method for studying the role of *brm* in Drosophila development, we generated and analyzed dominant-negative *brm* mutations. This approach was based on previous studies of the yeast *SWI2/SNF2* and human *BRG1* genes, which showed that mutations in the ATP-binding site of both proteins eliminate their activity without affecting their ability to interact with other proteins (Khavari *et al.* 1993; Peterson *et al.* 1994; Côté *et al.* 1994). The mutant proteins thus have dominant-negative effects on transcription when expressed in yeast or human cells. We reasoned that similar *brm* mutations could be valuable tools for analyzing *brm* function *in vivo.*

We previously showed that a transgene encoding a BRM protein bearing an epitope tag from the influenza virus HA protein at its C terminus could rescue the recessive lethality of *brm* mutations (Dingwall *et al.*

TABLE 1

Somatic clonal analysis of *brm²*

Structure	No. of clones/structures scored		Frequency		Average clone size		Percent of abnormal bristles	
	Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt.
Head	36/30	1/37	1.20	0.03	4.7	1	0	0
Dorsal thorax	71/40	9/53	1.78	0.17	12.5	2.7	0	75
Wing	76/58	7/68	1.31	0.10	5.5	2.1	0	0
Leg 1	172/97	7/97	1.77	0.07	4.65	1.6	0	55
Leg 2	270/79	12/107	3.42	0.11	5.18	1.4	0	65
Leg 3	202/100	8/100	2.02	0.08	4.2	1.25	0	50
A2	126/66	118/79	1.91	1.49	3.18	2.97	0	91
A3	148/66	125/79	2.24	1.58	3.44	3.40	0.2	96
A4	148/66	142/79	2.24	1.80	3.49	2.77	0	95
A5	149/66	137/79	2.26	1.73	3.45	2.80	0	95
A6	106/66	103/79	1.61	1.30	3.0	2.1	0	92
A7	39/66	46/79	0.59	0.58	2.69	1.33	0	95
A8	36/66	1/79	0.55	0.01	2.0	3.0	0	100

Somatic clones of homozygous *brm²* marked with *y* were generated in the presence (control) or absence (expt.) of a *brm⁺* transgene as described in materials and methods. The clone size was determined by counting the number of bristles per clone.

1995). We used site-directed mutagenesis to create a mutation in this transgene that replaces the conserved lysine (amino acid 804) in the ATP-binding site of the BRM protein with an arginine (Figure 1). Transgenic strains bearing homozygous viable insertions of the mutant transgene on either the *X* or second chromosome (*P[w⁺, brm^{K804R}]22D* and *P[w⁺, brm^{K804R}]17D*, respectively) were generated by *P*-element–mediated transformation. The *brm^{K804R}* transgene is expressed at levels comparable to a *brm⁺* transgene, as assayed by probing Western blots of proteins extracted from *P[w⁺, brm^{K804R}]22D* or *P[w⁺, brm⁺]92C* embryos with a monoclonal antibody against an HA epitope tag (data not shown). However, the *P[w⁺, brm^{K804R}]17D* transgene was unable to rescue the hemizygous lethality of *brm²*, indicating that the ATP-binding site of *brm* is critical for its function *in vivo*.

To determine whether the *brm^{K804R}* mutation interferes with the assembly of the BRM complex, we examined the native molecular weight of the BRM^{K804R} protein in embryos using gel filtration chromatography. Proteins extracted from *P[w⁺, brm^{K804R}]22D* or *P[w⁺, brm⁺]92C* embryos were fractionated on a Superose 6 FPLC column, and the eluted fractions were assayed

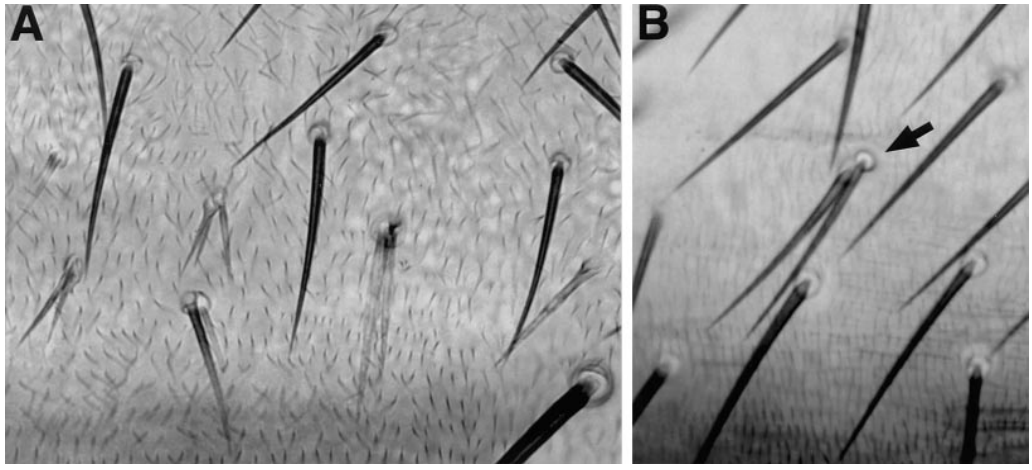

Figure 4.—Bristle defects associated with *brm* mutations. (A) Somatic clone of homozygous *brm²* tissue in an abdominal segment marked with *yellow*. Mechanosensory bristle defects associated with loss of *brm* function include malformation and loss or twinning of shafts and/or sockets. (B) Expression of the BRM^{K805R} protein under the control of the 69B GAL4 driver causes twinning of mechanosensory bristles. Both the socket and shaft are duplicated in a bristle marked by an arrow in an abdominal segment of a *P[w⁺, UAS$_{GAL}$hsp70:brm^{K804R}]2-2/P[w⁺, hsp70:GAL4]69B* adult.

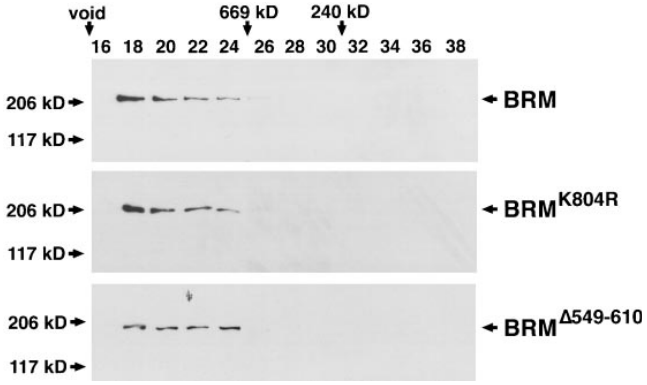

Figure 5.—Gel filtration analysis of wild-type and mutant BRM proteins. Whole embryo extract (2 mg) was applied to a Superose 6 gel filtration column; 0.5-ml fractions were collected and assayed for BRM protein by Western blotting using a monoclonal antibody against an HA epitope at the C-terminus of the BRM proteins. The elution volumes of protein standards are marked by arrows. The BRM and BRM^{K804R} proteins elute from the column with apparent native molecular weights of ~2 MD. Note that the average molecular weight of the BRM$^{\Delta 549-610}$ protein is slightly smaller than that of BRM protein.

for BRM protein by Western blotting (Figure 5). The apparent native molecular weights of the BRM^{K804R} and BRM proteins are identical (~2 MD), indicating that the BRM^{K804R} protein is efficiently incorporated into the BRM complex.

Since BRM^{K804R} is incorporated into the BRM complex, it should have dominant-negative effects on *brm* function *in vivo*. To test this possibility, we examined the effect of varying the relative dosage of the *brm^{K804R}* transgene and the wild-type *brm* gene from 0:2 to 2:1 (Table 2). As anticipated, the BRM^{K804R} protein interferes with the function of the endogenous BRM protein. Individuals bearing one or two copies of the *brm^{K804R}* transgene are viable, but frequently exhibit partial transformations of haltere to wing, as evidenced by an increase in haltere size and the appearance of ectopic bristles on the capitellum (Figure 6). Approximately one third of *P[w$^+$, brm^{K804R}]22D* adults exhibit this transformation, which is presumably caused by the decreased expression of the *Ultrabithorax* (*Ubx*) gene. Increasing the ratio of BRM^{K804R} to BRM to 2:1 was lethal. This effect was revealed when we tried to generate adult flies heterozygous for *brm^2* or a *brm* deficiency [*Df(3L)th102*] that had two copies of a *brm^{K804R}* transgene (*P[w$^+$, brm^{K804R}]17D*) on the second chromosome (Table 2). Thus, the *brm^{K804R}* mutation behaves as an antimorphic allele of *brm*.

We next examined the effect of expressing high levels of the BRM^{K804R} protein in restricted temporal and spatial patterns using the GAL4 system of Brand and Perrimon (1993). A gene encoding HA-tagged BRM^{K804R} protein was placed under the control of a GAL4-regulated promoter in the vector pUAST (Brand and Perrimon 1993), and a homozygous viable insertion of the dominant-negative transgene on the third chromosome (*P[w$^+$, UAS$_{GAL}$hsp70:brm^{K804R}]2-2*) was generated by *P*-element–mediated transformation. Expression of this trangene was induced by crossing this transformant to strains that express GAL4 in a variety of different patterns. Expression of the dominant-negative BRM protein in patterns identical to the segmentation genes *hairy* (using the *GAL4* insertion IJ3; Brand and Perrimon 1993) or *engrailed* (using the *GAL4* insertion e16E; Harrison *et al.* 1995) had no effect on embryonic viability or segmentation (data not shown). The lack of an embryonic phenotype resulting from embryonic expression of the BRM^{K804R} protein may be caused by the high maternal expression of wild-type BRM protein, which is sufficient to allow embryogenesis to proceed to near completion in the absence of zygotic *brm* function. Expression of the BRM^{K804R} protein in imaginal tissues after embryogenesis led to greatly reduced viability. For example, expression of BRM^{K804R} under the control of the *GAL4* insertion 69B (Brand and Perri-

TABLE 2

Genetic interactions between a *brm^{K804R}* transgene and a *brm* deficiency

Genotype	Ratio *brm^{K804R}:brm$^+$*	No. of individuals	Phenotype
y Df(1)w67c2; P[w$^+$, brm^{K804R}]17D/+; + / +	1:2	112	Normal
y Df(1)w67c2; P[w$^+$, brm^{K804R}]17D/+; Df(3L)th102, h ri Sb ca^2/+	1:1	68	Partial haltere to wing transformation
y Df(1)w67c2; P[w$^+$, brm^{K804R}]17D;+/+	2:2	50	Partial haltere to wing transformation
y Df(1)w67c2; P[w$^+$, brm^{K804R}]17D; Df(3L)th102, h ri Sb ca^2/+	2:1	0	Lethal

The genotypes refer to progeny from the mating of *y Df(1)w67c2; P[w$^+$, brm^{K804R}]17D/P[w$^+$, brm^{K804R}]17D* virgin females to *y Df(1)w67c2/Y; P[w$^+$, brm^{K804R}]17D/+; Df(3L)th102, h ri Sb ca^2/+* males. Individuals bearing one or two copies of the *brm^{K804R}* transgene were distinguished by their eye color.

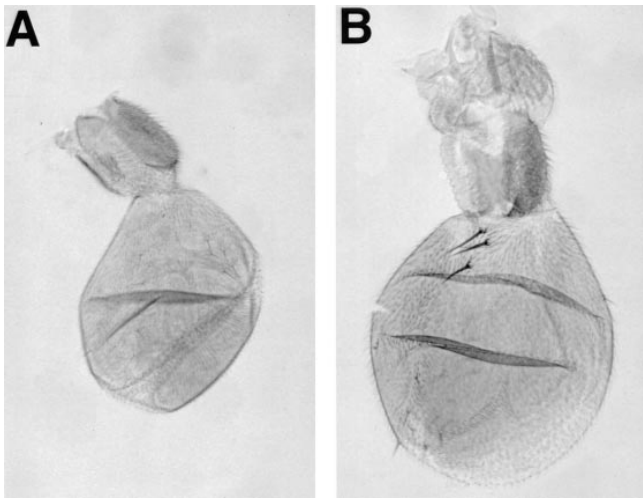

Figure 6.—Expression of the BRM^{K804R} protein in imaginal discs causes homeotic transformations. (A) Wild-type haltere. (B) Haltere from a *P[w$^+$, brm^{K804R}]22D* male. A partial transformation of haltere to wing is evident from the increased size of the haltere and appearance of ectopic bristles on the capitellum.

mon 1993) caused high levels of lethality (>99%). At 25° or higher, the majority of individuals expressing BRM^{K804R} pupated but failed to differentiate.

The *GAL4* system is inherently temperature sensitive; higher levels of induction are observed at elevated temperatures (Brand *et al.* 1994). When the temperature at which crosses were maintained was reduced, we could increase the number of individuals expressing the BRM^{K804R} that survived to adulthood. This allowed us to score adult phenotypes resulting from expression of BRM^{K804R} under the control of the 69B *GAL4* insertion. Individuals reared at 20° displayed partial transformation of first leg to second leg, as evidenced by a reduction in the number of sex comb teeth on the first leg (data not shown). This phenotype is also seen in adults trans-heterozygous for hypomorphic *brm* alleles (Brizuela *et al.* 1994), and it is presumably caused by decreased expression of the *Sex combs reduced* (*Scr*) gene. Adults reared at 20° also displayed twinning of mechanosensory bristles (Figure 4B), a phenotype similar to that observed in clones of *brm^2* tissue.

Expression of the BRM^{K804R} protein induced by the 69B driver at 20° also had dramatic effects on the size and morphology of the wing; mutant wings were reduced in size, and the L5 and the posterior cross-vein (PCV) were usually absent (Figure 7, A and B). Defects in the campaniform sensilla, a class of sensory organs important for flight, were also observed with high frequency because of expression of the BRM^{K804R} protein in imaginal discs (Figure 7). These defects fell into four classes: missing sensilla, duplication or triplication of sensilla, transformation of sensilla into bristles, and the appearance of ectopic sensilla (Table 3; Figure 7). Ectopic sensilla and bristles were observed most frequently on the L3 vein. Three sensilla (L3-1, L3-2, and L3-3) and no bristles are normally found on this vein. By contrast, approximately one-half of mutant wings displayed one or two additional sensilla on L3. Ectopic bristles were observed on this vein in approximately one-fifth of mutant wings.

Functional analysis of evolutionarily conserved domains of the BRM protein: Comparison of the sequences of the BRM protein and its putative homologs in yeast and humans have suggested that they contain at least four functional domains. The largest of these domains, the ATPase domain, is highly conserved in all members of the SWI2/SNF2 family of ATPases (Eisen *et al.* 1995). The regions flanking this domain are thought to contribute to the functional specificity of individual SWI2/SNF2 family members by mediating interactions with other proteins. Three short segments outside the ATPase domain—domain I, domain II, and the bromodomain—are conserved in the yeast SWI2/SNF2 protein, the Drosophila BRM protein, and the human BRG1 and HBRM proteins (Tamkun *et al.* 1992; Khavari *et al.* 1993; Muchardt and Yaniv 1993). The conservation of these domains from yeast to humans suggests that they are critical for the function of the BRM protein. To test this possibility, we examined the consequences of deleting two of these conserved regions of the BRM protein: domain II and the bromodomain.

The bromodomain is dispensable for *brm* function: To investigate the function of the bromodomain, we created a transgene encoding a BRM protein lacking amino acids 1446–1517, including the 46–amino acid segment (residues 1461–1505), which is most highly conserved in other bromodomains (Figure 1). Two independent, homozygous, viable insertions of this transgene on the second chromosome (*P[w$^+$, brm$^{\Delta 1446\text{-}1517}$]22-1* and *P[w$^+$, brm$^{\Delta 1446\text{-}1517}$]13-1*) were tested for the ability to rescue *brm* mutations. Surprisingly, the bromodomain appeared to be dispensable for zygotic *brm* function; both *brm$^{\Delta 1446\text{-}1517}$* transgenes were found to rescue the hemizygous lethality of an extreme *brm* allele, *brm^2. P[w$^+$, brm$^{\Delta 1446\text{-}1517}$]; brm^2/Df(3L)th102* individuals are viable, phenotypically normal, and fertile.

If the BRM protein consists of independent functional domains, interallelic complementation could account for the ability of *P[w$^+$, brm$^{\Delta 1446\text{-}1517}$]* to rescue the *brm^2* mutation. This concern was justified, since *brm^2* had not been shown to be a protein null allele, and previous genetic studies have suggested that BRM may act as a multimer (Brizuela *et al.* 1994). To investigate this possibility, we examined whether *brm^2* is a protein null allele by Western blotting. The difference in size between BRM$^{\Delta 1446\text{-}1517}$ and BRM is sufficient for the two proteins to be resolved by electrophoresis on low-percentage SDS-polyacrylamide gels (Figure 8, lane 3). Polyclonal antibodies against two different regions of the BRM protein (residues 505–776 and 1504–1638)

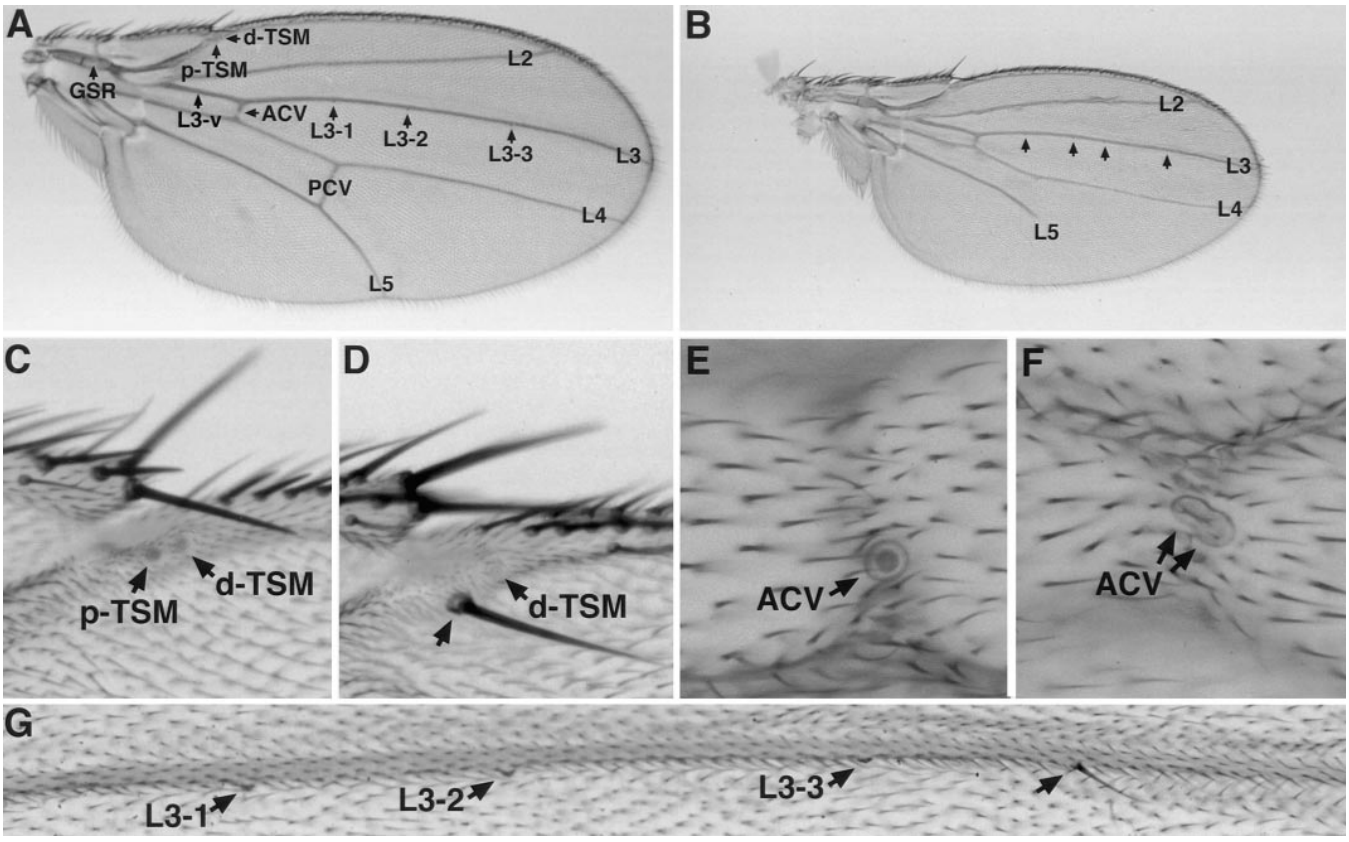

Figure 7.—Wing defects associated with expression of the BRM^{K804R} protein. Wings were dissected from control (*P[w$^+$, UAS$_{GAL}$hsp70:brm^{K804R}]2-2/P[UAS-lacZ 4-1-2]*; A, C, and E) or mutant (*P[w$^+$, UAS$_{GAL}$hsp70:brm^{K804R}]2-2/P[w$^+$, hsp70:GAL4]69B*; B, D, F, and G) individuals. (A) Normal wing. The positions of the campaniform sensilla are marked by arrows. (B) Mutant wing. Note the reduced size of the wing, the disruption of the L5 vein, and the absence of the posterior cross-vein (PCV). The positions of the campaniform sensilla along the L3 vein (including one ectopic sensillum) are marked by arrows. (C and D) Magnified views of the proximal and distal twin sensilla of the margin (p-TSM and d-TSM) of wings from normal (C) or mutant (D) individuals. Note the transformation of the d-TSM to a bristle in D. (E and F) Magnified views of the anterior cross-vein sensillum (ACV) in wings from normal (E) or mutant (F) individuals. Note the duplication of the ACV sensillum in F. (G) Magnification of the L3 vein of a wing from a mutant individual. An ectopic bristle distal to the L3-3 sensillum is marked by an arrow.

detected both the wild-type and BRM$^{\Delta 1446\text{-}1517}$ proteins in adults bearing the *P[w$^+$, brm$^{\Delta 1446\text{-}1517}$]22-1* transgene (Figure 8, lane 3). By contrast, these antibodies detect only the smaller BRM$^{\Delta 1446\text{-}1517}$ protein in *P[w$^+$, brm$^{\Delta 1446\text{-}1517}$]22-1*; *brm^2/Df(3L)th102* adults (Figure 8, lane 4). Similar results were obtained using the *P[w$^+$, brm$^{\Delta 1446\text{-}1517}$]13-1* transgene. These results demonstrate that the *brm^2* allele encodes either no protein, or a severely truncated protein lacking residues 505–1638 of BRM, including the entire ATPase domain, the nuclear localization signal and the bromodomain. We therefore conclude that *brm^2* is a null allele, and that the bromodomain is dispensable for both maternal and zygotic *brm* function.

Domain II is required for the assembly of the BRM complex: We next examined the function of domain II of the BRM protein. This 62–amino acid domain of BRM (residues 549–610) is located N-terminal to the ATPase domain, and is 48% identical to the corresponding region of SWI2/SNF2 (Tamkun *et al.* 1992). This domain is also conserved in the putative human homologs of BRM, BRG1, and hbrm (83% identity to BRM in both proteins; Khavari *et al.* 1993; Muchardt and Yaniv 1993). Using site-directed mutagenesis, we generated a transgene encoding an epitope-tagged BRM protein (BRM$^{\Delta 549\text{-}610}$) that lacks the 61 amino acids between residues 549 and 610, including all of domain II (Figure 1). A homozygous viable insertion of the *brm$^{\Delta 549\text{-}610}$* transgene on the *X* chromosome (*P[w$^+$, brm$^{\Delta 549\text{-}610}$]4-3*) was unable to rescue the hemizygous lethality of *brm^2*. Similar results were obtained with two independent transformants(*P[w$^+$, brm$^{\Delta 549\text{-}610}$]24-1* and *P[w$^+$, brm$^{\Delta 549\text{-}610}$]3-3*), suggesting that domain II is essential for the activity of the BRM protein.

Why does *brm$^{\Delta 549\text{-}610}$* fail to rescue *brm* mutations? Deletion of domain II has a reproducible effect on the level of BRM present in the Drosophila embryo. Using Western blotting, we found that the levels of epitope-tagged BRM$^{\Delta 549\text{-}610}$ protein in *P[w$^+$, brm$^{\Delta 549\text{-}610}$]4-3* embryos were fourfold lower than the level of epitope-tagged BRM protein in *P[w$^+$, brm$^+$]92C* embryos (data not shown). Similar results were obtained using two in-

TABLE 3

Defects in campaniform sensilla caused by expression of the BRM^{K804R} protein

Sensillum	Frequency of observed defects			
	Percent absent	Percent duplicated	Percent triplicated	Percent transformed
L3-1	—	—	—	1
L3-2	—	7	—	—
L3-3	—	—	—	2
L3-v	8	42	3	—
p-TSM	28	—	—	3
d-TSM	—	—	—	—
d-HCV	—	—	—	10
v-HCV	—	—	—	—
GSR	—	1	—	—
ACV	—	58	5	—

96 mutant wings from flies expressing BRM^{K804R} under the control of the 69B GAL4 insertion were mounted and scored. Thirty wings from individuals expressing β-galactosidase under the control of the 69B GAL insertion were mounted and scored; no defects were observed in any of the control wings. The dashes indicate that no defects were observed.

dependent transformants of *brm*$^{\Delta 549\text{-}610}$ (*P[w*$^+$, *brm*$^{\Delta 549\text{-}610}$*]24-1* and *P[w*$^+$, *brm*$^{\Delta 549\text{-}610}$*]3-3*). These results suggested that the failure of domain II to rescue *brm* mutations might be caused by decreased levels of BRM protein, as opposed to the elimination of a function specific to domain II. However, four copies of a *P[w*$^+$, *brm*$^{\Delta 549\text{-}610}$*]* transgene were unable to rescue the recessive lethality of *brm*^{1}, a hypomorphic *brm* allele, indicating that domain II is essential for *brm* function.

To determine if domain II is required for assembly into the BRM complex, we examined the native molecular weight of the epitope-tagged BRM$^{\Delta 549\text{-}610}$ protein in extracts prepared from *P[w*$^+$, *brm*$^{\Delta 549\text{-}610}$*]3-3* embryos. Although no monomeric BRM$^{\Delta 549\text{-}610}$ protein was present in these embryos, a significant proportion of the mutant BRM protein has a native molecular weight (~700 kD) smaller than that of the wild-type BRM protein (Figure 5). This size difference was also observed in extracts prepared from an independent transformant, *P[w*$^+$, *brm*$^{\Delta 549\text{-}610}$*]24-1*, and was confirmed by reprobing the Western blots with polyclonal antisera that recognize both the BRM$^{\Delta 549\text{-}610}$ and endogenous BRM proteins. Although we did not examine the subunit composition of the wild-type and mutant complexes, these results indicate that domain II contributes to the formation or stability of the BRM complex.

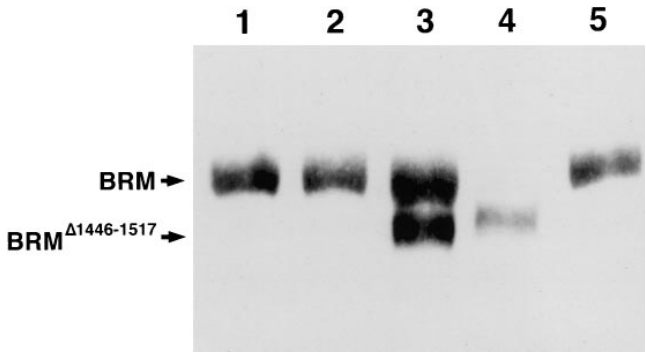

Figure 8.—Analysis of BRM and BRM$^{\Delta 1446\text{-}1517}$ proteins by Western blotting. Extracts from wild-type (lanes 1 and 5); *P[w*$^+$, *brm*$^+$*]21A* (lane 2); *P[w*$^+$, *brm*$^{\Delta 1446\text{-}1517}$*]22-1* (lane 3) and *P[w*$^+$, *brm*$^{\Delta 1446\text{-}1517}$*]22-1*; *brm*^{2}/*Df(3L)th102* females were fractionated on a 5% SDS-polyacrylamide gel and analyzed by Western blotting using a polyclonal antibody against the BRM protein. Antibodies against BRM recognize both the BRM protein and the smaller BRM$^{\Delta 1446\text{-}1517}$ proteins in transgenic flies bearing the *P[w*$^+$, *brm*$^{\Delta 1446\text{-}1517}$*]* transgene (lane 3). In *P[w*$^+$, *brm*$^{\Delta 1446\text{-}1517}$*]* adults hemizygous for the *brm*^{2} allele, only the BRM$^{\Delta 1446\text{-}1517}$ protein is detected (lane 4).

DISCUSSION

Our characterization of null and dominant-negative *brm* mutations confirms and extends previous studies which showed that *brm* plays an important role in the control of cell fate (Kennison and Tamkun 1988; Tamkun *et al.* 1992; Brizuela *et al.* 1994). Partial loss of *brm* function leads to a variety of homeotic transformations, including the transformations of haltere to wing and first leg to second leg. These transformations are identical to those associated with mutations in the *Ubx* and *Scr* genes, respectively. Complete loss of *brm* function was lethal, however, indicating that the function of *brm* is not limited to homeotic gene regulation. Although we are not certain how many genes require *brm* for their expression, the ubiquitous expression of high levels of the BRM protein (approximately one molecule of BRM per 20 nucleosomes in embryos) suggests that it may play a fairly general role in transcription or other processes.

The effect of *brm* mutations on the number, position, and identities of adult sensory organs indicates that *brm* plays an unanticipated role in the development of the adult peripheral nervous system. The mechanosensory structures of the peripheral nervous system consist of four unique cells derived from a common sensory organ precursor (SOP) cell (Jan and Jan 1993). Division of the SOP cell gives rise to two daughter cells, IIa and IIb. The IIa cell gives rise to the trichogen (shaft) cell and the tormogen (socket) cell; the IIb cell gives rise to the neuron and thecogen (sheath) cell. Some of the mechanosensory bristle defects observed in clones of mutant *brm* tissue, including abnormal shaft size and morphology and malformed or missing sockets, may be secondary consequences of decreased cell viability. Other defects associated with the *brm*^{2} and *brm*^{K804R} mutations, including the appearance of ectopic or duplicated sensory organs, may result from alterations in cell fate. For example, the transformation of the IIb cell into a IIa cell would lead to the twinning of both

bristle and socket at the expense of the neuron and sheath cells. Alternatively, the duplication of sensory organs may be caused by extra divisions of the SOP cell or its descendants resulting from loss of *brm* function.

How might *brm* control the fate or proliferation of the cells that form mechanosensory organs? The simplest explanation is that *brm* regulates the activity of one or more neurogenic genes. Mutations in several neurogenic genes cause sensory bristle abnormalities similar to those associated with *brm* mutations. For example, loss of *Notch* function early in pupal development causes a proliferation of SOP cells, resulting in the formation of supernumerary bristles and a low frequency of stunted, fused, and composite bristles (Hartenstein and Posakony 1990). Mutations in *Suppressor of Hairless* (*Su(H)*) cause transformations of socket to shaft, presumably caused by increased expression of *Hairless* (*H*; Schweisguth and Posakony 1994). Mutations in *twins* cause duplication of both sockets and bristles (Shiomi *et al.* 1994). Interactions between *brm* and one or more of these genes could account for the defects observed in *brm* clones.

Another possibility has been suggested by recent studies demonstrating that the human BRG1 and HBRM proteins cooperate with retinoblastoma tumor suppressor proteins to regulate cell cycle progression. Physical association between Rb family members and the BRG1 and HBRM proteins have been demonstrated using both the yeast two-hybrid system and coimmunoprecipitation assays (Dunaief *et al.* 1994; Singh *et al.* 1995). Rb, but not BRG1, is present in a human tumor cell line, SW13; transfection of BRG1 or HBRM into these cells causes a dramatic decrease in cell division and the restoration of normal cellular morphology (Dunaief *et al.* 1994; Strober 1996). These findings suggest that disruption of the interaction between BRG1 and Rb leads to increased rates of cell division and malignant transformation. Loss of *brm* function could similarly alter the activity of a Drosophila tumor suppressor protein, leading to extra divisions of SOP cells or their descendants. The recent identification of RBF, a Drosophila Rb family member (Du *et al.* 1996), should allow this possibility to be tested directly.

The peripheral nervous system defects associated with *brm* mutations are also similar to those associated with mutations in *ash2*, another trithorax group gene. *ash2* mutant hemizygotes display duplications of bristles and sockets, transformation of campaniform sensilla to bristles, and the appearance of ectopic bristles on wing veins (Adamson and Shearn 1996). Overexpression of the Polycomb group genes *Posterior sex combs* (*Psc*) and *Suppressor of zeste 2* (*Su(z)2*) in third instar larvae also results in a range of bristle abnormalities very similar to those observed in *brm*-deficient clones, including twinned, composite, and stunted bristles and partially duplicated or missing sockets (Brunk *et al.* 1991; Sharp *et al.* 1994). These findings suggest that *ash2* and *brm* function antagonistically to *Psc* and *Su(z)2* during the development of the peripheral nervous system.

Comparison of the sequences of BRM and its putative homologs in yeast and humans suggests that these proteins contain at least four functional domains: domain I, domain II, the ATPase domain, and the bromodomain. The ATPase domain is required for the catalytic activity of SWI2/SNF2 family members, while the regions flanking this domain are thought to contribute to their functional specificity by mediating interactions with other proteins. For example, domain I of the yeast SWI2/SNF2 protein interacts with the SNF11 subunit of the SWI/SNF complex (Treich *et al.* 1995). The significance of the interaction between SNF11 and SWI2/SNF2 is unknown, however, since domain I is not essential for SWI2/SNF2 function *in vivo*, and yeast cells lacking SNF11 are viable and phenotypically normal (Treich *et al.* 1995).

As previously shown for other SWI2/SNF2 family members (Laurent *et al.* 1993; Khavari *et al.* 1993; Peterson *et al.* 1994), we found that a mutation in the ATP-binding site of the BRM protein eliminates its function *in vivo* without disrupting its interactions with other proteins. By contrast, deletion of domain II causes a small but reproducible decrease in the size of the BRM complex, suggesting that it lacks one or more subunits. Although the effect of deleting domain II of the yeast SWI2/SNF2 protein has not been examined, the two-hybrid system has revealed an interaction between this domain and the SWI3 subunit of the SWI/SNF complex (Treich *et al.* 1995, 1997). Although a Drosophila homolog of SWI3 has not yet been identified, proteins related to SWI3 are present in the human BRG1 and hbrm complexes (Wang *et al.* 1996b). These observations strongly suggest that domain II of the BRM protein interacts with an as yet unidentified Drosophila relative of SWI3.

What is the function of the bromodomain? More than a dozen bromodomain proteins have been identified, including a subset of chromatin remodeling factors (SWI2/SNF2, STH1, BRM, BRG1, and HBRM), type A histone acetyltransferases (GCN5, PCAF, $TAF_{II}250$, and p300/CBP; Brownell *et al.* 1996; Yang *et al.* 1996; Mizzen *et al.* 1996; Ogryzko *et al.* 1996), and other proteins involved in transcriptional activation, including SPT7 (Gansheroff *et al.* 1995) and FSH/RING3 (Haynes *et al.* 1989; Beck *et al.* 1992). Most bromodomain proteins are components of multiprotein complexes, and they activate transcription by interacting with other proteins. For example, $TAF_{II}250$ interacts with the TATA-binding protein (Hisatake *et al.* 1993; Ruppert *et al.* 1993), and p300/CBP interacts with CREB, E1A, PCAF, c-jun, c-fos, c-Myb, MyoD, and TFIIB (Ogryzko *et al.* 1996; Yang *et al.* 1996). Type A histone acetyltransferases, which contain bromodomains, acetylate nucleosomal histones and are associated with transcriptional activation (Brownell and Allis 1996). The

bromodomain is thus an excellent candidate for a domain that mediates interactions with gene-specific regulatory proteins or general transcription factors. Such interactions might be important for targeting chromatin remodeling factors and histone acetyltransferases to specific regions of chromatin.

Although the bromodomain is required for full GCN5 function *in vivo* (Candau *et al.* 1997), it is dispensable for the function of two other yeast proteins, SWI2/SNF2 and SPT7 (Laurent *et al.* 1993; Gansheroff *et al.* 1995). The bromodomain is not required for the enzymatic activity of histone acetyltransferases *in vitro* (Mizzen *et al.* 1996; Ogryzko *et al.* 1996). We reasoned that Drosophila would provide a more stringent system for investigating the function of the bromodomain. To our surprise, we found that the bromodomain is dispensible for both maternal and zygotic *brm* function. The bromodomain is, therefore, either not essential for the function of the BRM protein or it is functionally redundant. Given the conservation of the bromodomain from yeast to humans, we favor the latter of these possibilities. The BRM complex may contain more than one bromodomain protein, or other proteins may have functions that overlap those of the BRM complex. Either of these possibilities would account for the ability of $brm^{\Delta 1446\text{-}1517}$ to rescue *brm* mutations.

It has recently become apparent that multiple complexes containing members of the SWI2/SNF2 family of ATPases are involved in chromatin remodeling in eukaryotic cells (Tsukiyama and Wu 1997; Pazin and Kadonaga 1997). For example, the Drosophila ISWI protein (Elfring *et al.* 1994), which is closely related to BRM within the ATPase domain, is a subunit of at least three chromatin remodeling complexes: NURF, CHRAC, and ACF (Tsukiyama *et al.* 1995; Varga-Weisz *et al.* 1997; Ito *et al.* 1997). Each of these complexes was identified using biochemical assays for chromatin remodeling, but their functions *in vivo* are unknown. The ability of these complexes to catalyze ATP-dependent alterations in chromatin structure *in vitro* suggests that their *in vivo* activities may overlap those of the BRM complex. Numerous other SWI2/SNF2 family members are also present in Drosophila, including CHD1 (Stokes and Perry 1995), 89B helicase (Goldman-Levi *et al.* 1994), RAD54 (Kooistra *et al.* 1997), and lodestar (Girdham and Glover 1991). Additional work will be necessary to clarify the distinct and common roles of these proteins in transcription, development, and other processes involving chromatin.

We thank Kathy Matthews and the Bloomington *Drosophila* Stock Center for providing numerous stocks. We are grateful to Matt Fish in Matthew Scott's laboratory at Stanford University for assistance with the generation of $P[w^+, brm^{\Delta 549\text{-}610}]$ transgenic strains. We gratefully acknowledge the contribution of Jesse Bentz and Kim Chansky to the analysis of dominant-negative *brm* mutations. We also thank Lisa Moore, Matthew Scott, and Bill Sullivan for many helpful discussions during the course of this work. This work was supported by a Basil O'Connor Starter Scholar Research Award from the March of Dimes Birth Defects Foundation, an American Cancer Society Junior Faculty Award, and a grant from the National Institutes of Health (GM49883) to J.W.T.

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Communicating editor: S. Henikoff