

# Genetic Screens for Enhancers of *brhma* Reveal Functional Interactions Between the BRM Chromatin-Remodeling Complex and the Delta-Notch Signal Transduction Pathway in *Drosophila*

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## ABSTRACT

The *Drosophila* trithorax group gene *brhma* (*brm*) encodes the ATPase subunit of a 2-MDa chromatin-remodeling complex. *brm* was identified in a screen for transcriptional activators of homeotic genes and subsequently shown to play a global role in transcription by RNA polymerase II. To gain insight into the targeting, function, and regulation of the BRM complex, we screened for mutations that genetically interact with a dominant-negative allele of *brm* (*brm*<sup>K804R</sup>). We first screened for dominant mutations that are lethal in combination with a *brm*<sup>K804R</sup> transgene under control of the *brm* promoter. In a distinct but related screen, we identified dominant mutations that modify eye defects resulting from expression of *brm*<sup>K804R</sup> in the eye-antennal imaginal disc. Mutations in three classes of genes were identified in our screens: genes encoding subunits of the BRM complex (*brm*, *moira*, and *osa*), other proteins directly involved in transcription (*zerknüllt* and *RpIII140*), and signaling molecules (*Delta* and *vein*). Expression of *brm*<sup>K804R</sup> in the adult sense organ precursor lineage causes phenotypes similar to those resulting from impaired Delta-Notch signaling. Our results suggest that signaling pathways may regulate the transcription of target genes by regulating the activity of the BRM complex.

**N**UCLEOSOMES and other components of chromatin can block the access of transcription factors and other regulatory proteins to DNA. Chromatin is not merely a passive barrier to transcription; eukaryotic cells exploit the repressive effects of chromatin to regulate gene expression. Chromatin repression is regulated via two general mechanisms: the covalent modification of nucleosomal histones and ATP-dependent chromatin remodeling (NARLIKAR *et al.* 2002). Histone-modifying enzymes alter the acetylation, methylation, phosphorylation, or ubiquitination of N-terminal histone tails and other regions on the surface of the nucleosome. These modifications modulate interactions between nucleosomes and a wide variety of structural and regulatory proteins (BERGER 2002; PETERSON and LANIEL 2004). By altering the structure or positioning of nucleosomes, chromatin-remodeling complexes can directly regulate

the access of transcription factors and other proteins to DNA in the context of chromatin (BECKER and HORZ 2002; MARTENS and WINSTON 2003; FLAUS and OWEN-HUGHES 2004). The coordinated actions of histone-modifying and chromatin-remodeling enzymes are critical for transcription in a chromatin environment.

Histone-modifying enzymes and ATP-dependent chromatin-remodeling complexes have been implicated in a broad range of biological processes, including transcription, DNA repair, recombination, viral integration, and malignant transformation (MARTENS and WINSTON 2003). Alterations in chromatin structure underlie many developmental processes, including the maintenance of cell fates and other epigenetic phenomena. In *Drosophila* and other metazoans, the identities of body segments are specified by transcription factors encoded by homeotic (Hox) genes (GELLON and MCGINNIS 1998). The initial patterns of Hox transcription are established in response to positional information in the early embryo. Once established, these patterns are maintained throughout development by two groups of regulatory proteins: the Polycomb group (PcG) of repressors and the trithorax group (trxG) of activators (SIMON 1995; GELLON and MCGINNIS 1998; FRANCIS and KINGSTON 2001). Counterparts of *Drosophila* PcG and trxG proteins play

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highly conserved roles in transcription and development in other metazoans, including humans (GOULD 1997; SCHUMACHER and MAGNUSON 1997).

A growing body of evidence suggests that PcG and trxG proteins regulate transcription via the covalent modification or remodeling of chromatin (SIMON and TAMKUN 2002). Two major complexes of PcG proteins have been identified: Polycomb repressor complex 1 (PRC1) and the enhancer of Zeste/extra sex combs [E(Z)/ESC] complex (CAO and ZHANG 2004; LEVINE *et al.* 2004). The E(Z)/ESC complex has histone methyltransferase activity that promotes the binding of PRC1 to its target genes and is required for PcG repression *in vivo*. The trxG proteins Drosophila Absent, small or homeotic 1 (ASH1) and Trithorax (TRX) also have histone methyltransferase activity that is required for their function *in vivo* (BEISEL *et al.* 2002; SMITH *et al.* 2004). Other trxG proteins appear to regulate transcription via ATP-dependent chromatin remodeling. For example, the trxG genes *brahma* (*brm*), *moira* (*mor*), *osa*, and *kismet* (*kis*) were identified in genetic screens for *Polycomb* antagonists and were subsequently found to encode subunits of ATP-dependent chromatin-remodeling complexes (SIMON and TAMKUN 2002).

Chromatin-remodeling complexes are large (up to 2 MDa), multisubunit protein machines with a catalytic subunit belonging to the SNF2 family of ATPases (LUSSEY and KADONAGA 2003). The trxG protein BRM is highly related to yeast SWI2/SNF2 and STH1, the ATPase subunits of the SWI/SNF and RSC chromatin-remodeling complexes, respectively. *mor* encodes a conserved SANT-domain protein related to yeast SWI3 and RSC8, while *osa* encodes a conserved ARID-domain protein related to SWI1 (PAPOULAS *et al.* 1998; COLLINS *et al.* 1999; CROSBY *et al.* 1999; VAZQUEZ *et al.* 1999).

How do SWI/SNF complexes regulate gene expression? *In vitro*, these complexes use the energy of ATP hydrolysis to influence many aspects of chromatin structure. Examples of *in vitro* activities associated with SWI/SNF complexes include the distortion of DNA on the nucleosomal surface, nucleosome sliding, H2A/H2B dimer exchange, nucleosome transfer or eviction, nucleosome assembly, and the disruption or creation of regularly spaced nucleosomal arrays (LUSSEY and KADONAGA 2003; EBERHARTER and BECKER 2004). The ATPase subunits of chromatin-remodeling complexes facilitate these reactions by functioning as ATP-dependent DNA translocases (SAHA *et al.* 2002; WHITEHOUSE *et al.* 2003). The ability of SWI/SNF complexes to remodel chromatin *in vitro* is inhibited by PRC1, suggesting a potential mechanism for PcG repression *in vivo* (FRANCIS *et al.* 2001).

In spite of the tremendous progress toward understanding the mechanism of action of SWI/SNF complexes *in vitro*, much remains to be learned about their mechanism of action and biological functions *in vivo*. In yeast, SWI/SNF has been shown to be involved in

both transcriptional activation and repression (MARTENS and WINSTON 2003). However, the genes encoding most of the SWI/SNF subunits are not essential and the SWI/SNF complex is required only for the expression of a small percentage of genes (HOLSTEGE *et al.* 1998; SUDARSANAM *et al.* 2000). By contrast, the RSC chromatin-remodeling complex, which contains the STH1 ATPase, is both abundant and essential. RSC is required for transcription of several groups of genes (ANGUS-HILL *et al.* 2001) as well as sister chromatin cohesion during mitosis (HUANG *et al.* 2004). Like their yeast and Drosophila counterparts, the human BAF and PBAF complexes regulate transcription by catalyzing ATP-dependent alterations in chromatin structure (NARLIKAR *et al.* 2002).

Although early work on the Drosophila BRM complex focused on its roles in Hox regulation, subsequent studies revealed that it plays a surprisingly general role in transcription. The BRM complex is essential for cell viability and extremely abundant; one copy of the BRM complex is present for every 20 nucleosomes in many cell types (ELFRING *et al.* 1998). Furthermore, the BRM complex is associated with virtually all transcriptionally active regions of chromatin in salivary gland nuclei and the loss of *brm* function leads to a dramatic reduction in RNA polymerase II transcription (ARMSTRONG *et al.* 2002). These findings raise many questions about the function of SWI/SNF-like complexes in higher eukaryotes. How are these complexes targeted to sites of active transcription? Which step(s) in the transcription cycle are dependent on their activity? Finally, how are the activities of these abundant and extremely stable complexes regulated?

The targeting of chromatin-remodeling complexes to specific chromosomal locations may involve interactions with both gene-specific transcriptional activators and components of the basal transcription machinery. The yeast SWI/SNF complex physically interacts with a variety of transcriptional activators (PETERSON and LOGIE 2000; PETERSON and WORKMAN 2000; NEELY *et al.* 2002), as do the human SWI/SNF-like complexes (KADAM *et al.* 2000; KADAM and EMERSON 2003). It is possible that the Drosophila BRM complex is targeted via analogous mechanisms. Indeed, the transcription factor Zeste recruits the BRM complex to chromatin *in vitro* (KAL *et al.* 2000) and *in vivo* (DEJARDIN and CAVALLI 2004). SWI/SNF also interacts with the general transcriptional machinery (SHARMA *et al.* 2003; YOON *et al.* 2003), and mutations in genes encoding components of RNA polymerase II impair the recruitment of SWI/SNF to the GAL1 promoter (LEMIEUX and GAUDREAU 2004).

Recent studies suggest that signal transduction pathways may also be important for the regulation and targeting of chromatin-remodeling complexes. The EBV latency C promoter binding factor (CBF-1) and the intracellular domain (ICD) of Notch both physically interact with human BRM and are perhaps responsible for

the targeting of BRM to the promoters of Notch target genes (KADAM and EMERSON 2003). Human SWI/SNF complexes are targeted to muscle-specific genes by the MAP kinase p38 (SIMONE *et al.* 2004). Interactions between chromatin-remodeling factors and signal transduction pathways are not limited to SWI/SNF complexes. *Drosophila* NURF, an ISWI-containing complex, is a negative regulator of the JAK/STAT signal transduction pathway (BADENHORST *et al.* 2002). Additional signal transduction pathways may also participate in the regulation of chromatin-remodeling factors since the activities of several chromatin-remodeling complexes, including *Drosophila* NURF and yeast SWI/SNF, are regulated by inositol polyphosphate second messengers (SHEN *et al.* 2003; STEGER *et al.* 2003). Several chromatin-remodeling complexes, including the BRM complex, play a general role in transcription; these complexes are therefore logical targets for regulation by signal transduction pathways. Targeting, inactivation, or activation of these complexes could have profound consequences for gene expression.

To gain insight into the function and regulation of the *Drosophila* BRM complex, we conducted genetic screens for modifiers of *brm*<sup>K804R</sup>. This conservative substitution in the ATP-binding site of the BRM protein renders it catalytically inactive without disrupting its ability to interact with other proteins (ELFRING *et al.* 1998). As a result, expression of *brm*<sup>K804R</sup> counteracts *brm* function *in vivo*. We therefore reasoned that mutations in genes that are important for *brm* function would strongly enhance phenotypes resulting from *brm*<sup>K804R</sup> expression, while mutations in *brm* antagonists would suppress them. In addition to mutations in subunits of the BRM complex and other proteins involved in transcription, our screens led to the recovery of mutations in genes involved in both the Notch and EGF receptor signal transduction pathways. These findings suggest that signal transduction pathways may regulate the activity of the BRM chromatin-remodeling complex to affect transcription of target genes.

## MATERIALS AND METHODS

***Drosophila* stocks and crosses:** Flies were raised on a cornmeal-molasses-yeast-agar medium containing Tegosept and propionic acid at 25° unless otherwise indicated. The autosomal deficiency kit and many other *Drosophila* stocks were obtained from the Bloomington *Drosophila* Stock Center (<http://flystocks.bio.indiana.edu>). Information concerning many of the preexisting mutations and chromosome aberrations used in this study can be found at FlyBase ([www.flybase.org](http://www.flybase.org)).

**Male-specific lethality screens:** To screen for dominant enhancers of *brm*<sup>K804R</sup> on the second chromosome, *al b cn sp* males were fed 20 mM ethylmethane sulfonate (EMS) in 1% sucrose for 12 hr and crossed to either *w; al b cn ISWI<sup>1</sup> sp/SM5, Cy sp* or *Df(2R)vg-C/SM5, Cy sp* females (Figure 1A). Individual *al b cn sp/SM5, Cy sp* males were mated to *Df(1)w67c2 y, P[w<sup>+</sup>, brm<sup>K804R</sup>]22D/Df(1)w67c2 y* females and their progeny were scored for the absence of males bearing both the muta-

genized chromosome and the *P[w<sup>+</sup>, brm<sup>K804R</sup>]22D* transgene (*Df(1)w67c2 y, P[w<sup>+</sup>, brm<sup>K804R</sup>]22D/Y; al b cn sp/+*). Potential enhancer mutations were recovered by mating *Df(1)w67c2 y/Y; al b cn sp/+* males to virgin females heterozygous for the second chromosome balancer. To identify dominant enhancers of *brm*<sup>K804R</sup> on the third chromosome, *ru h st ry e* males were fed EMS as described above and mated to *w; CxD/TM3, Sb* females (Figure 1B). Individual male progeny of the genotype *w/Y; ru h st ry e/CxD* or *w/Y; ru h st ry e/TM3, Sb* were mated to *Df(1)w67c2 y, P[w<sup>+</sup>, brm<sup>K804R</sup>]22D/Df(1)w67c2 y* females. Progeny of these crosses were scored for the absence of males carrying both the mutagenized chromosome and the *P[w<sup>+</sup>, brm<sup>K804R</sup>]22D* transgene (*Df(1)w67c2 y, P[w<sup>+</sup>, brm<sup>K804R</sup>]22D/Y; ru h st ry e/+*). Potential enhancer mutations were recovered by mating *Df(1)w67c2 y/Y; ru h st ry e/+* males to virgin females heterozygous for the third chromosome balancer.

To identify potential dominant suppressors of *brm*<sup>K804R</sup> on the second and third chromosomes *Df(3L)th102 ri Sb/TM6B, Hu e Tb ca* males were fed EMS as described above and mated to *Df(1)w67c2 y, P[w<sup>+</sup>, brm<sup>K804R</sup>]22D* virgins. The progeny of this cross were scored for *Df(1)w67c2 y, P[w<sup>+</sup>, brm<sup>K804R</sup>]22D/Y; +/+; Df(3L)th102 ri Sb/+* males.

**Eye-based modifier screens:** To screen for dominant modifiers of *brm*<sup>K804R</sup> on the third chromosome, *Df(1)w67c2 y* males were fed EMS as described above and mated to *w; CxD/TM3, Sb* females (Figure 3). Individual female progeny of the genotype *Df(1)w67c2 y/w; +/TM3, Sb* were mated to *w; P[w<sup>+</sup>, ey-GAL4], P[w<sup>+</sup>, UAS-*brm*<sup>K804R</sup>]/TM3, Sb* males. The resulting F<sub>2</sub> progeny were screened as described below for mutations that modify the rough eye phenotype resulting from expression of *brm*<sup>K804R</sup>. Candidate mutations were recovered by balancing the third chromosome of *w/Y; +/TM3, Sb* siblings. The *P[w<sup>+</sup>, ey-GAL4], P[w<sup>+</sup>, UAS-*brm*<sup>K804R</sup>]* chromosome was generated by recombination between *w; P[w<sup>+</sup>, ey-GAL4]* and *w; P[w<sup>+</sup>, UAS-*GAL*hsp70: *brm*<sup>K804R</sup>]2-2* as described (PAPOULAS *et al.* 2001).

To quantify the severity of eye defects, we assigned individual eyes a score from 1 to 6 as follows: (1) eye is wild type; (2) 50% or less of the eye is rough (as determined by disordered ommatidia under the light microscope); (3) >50% of the eye is rough; (4) the eye is rough and reduced in size by ≤50%; (5) the eye is rough and reduced in size by >50%; and (6) the eye is absent. To assay enhancement or suppression of the *brm*<sup>K804R</sup> rough eye phenotype, eye scores for individuals of the genotype mutation/*P[w<sup>+</sup>, ey-GAL4], P[w<sup>+</sup>, UAS-*brm*<sup>K804R</sup>]* were compared to eye scores for siblings of the genotype balancer/*P[w<sup>+</sup>, ey-GAL4], P[w<sup>+</sup>, UAS-*brm*<sup>K804R</sup>]*. A mutation was designated as an *Enhancer of *brm*<sup>K804R</sup>* [*E(brm<sup>K804R</sup>)*] if the cumulative frequency distributions of the eye scores of the two progeny classes were statistically different ( $P < 0.05$ ), using the Kolmogorov-Smirnov two-sample test. The lowest  $P$ -value given by this test is  $P < 0.001$  and the highest is  $P > 0.1$ . This GAL4-based assay is inherently temperature sensitive. The screens and subsequent crosses were done at 24°.

As a specificity control, potential *E(brm<sup>K804R</sup>)* mutations were assayed for their ability to modify eye defects caused by expression of *ISWI<sup>K159R</sup>* in the developing eye. Eye scores for individuals of the genotype mutation/*P[w<sup>+</sup>, ey-GAL4], P[w<sup>+</sup>, UAS-*ISWI*<sup>K159R</sup>]* were compared to eye scores for siblings of the genotype balancer/*P[w<sup>+</sup>, ey-GAL4], P[w<sup>+</sup>, UAS-*ISWI*<sup>K159R</sup>]*. The *P[w<sup>+</sup>, ey-GAL4], P[w<sup>+</sup>, UAS-*ISWI*<sup>K159R</sup>]* chromosome was generated by recombination between *w; P[w<sup>+</sup>, ey-GAL4]* and *w; P[w<sup>+</sup>, UAS-*GAL*hsp70: *ISWI*<sup>K159R</sup>]11-4* as described (PAPOULAS *et al.* 2001).

**Complementation analysis and genetic mapping:** Meiotic mapping was accomplished using either the *W Sb* or the *ru h th st cu sr e ca* chromosome, which do not themselves modify *brm*<sup>K804R</sup> phenotypes. Mapping by site-specific male recombination was carried out as previously described (CHEN *et al.* 1998). All *E(brm<sup>K804R</sup>)* alleles were tested for the ability to complement



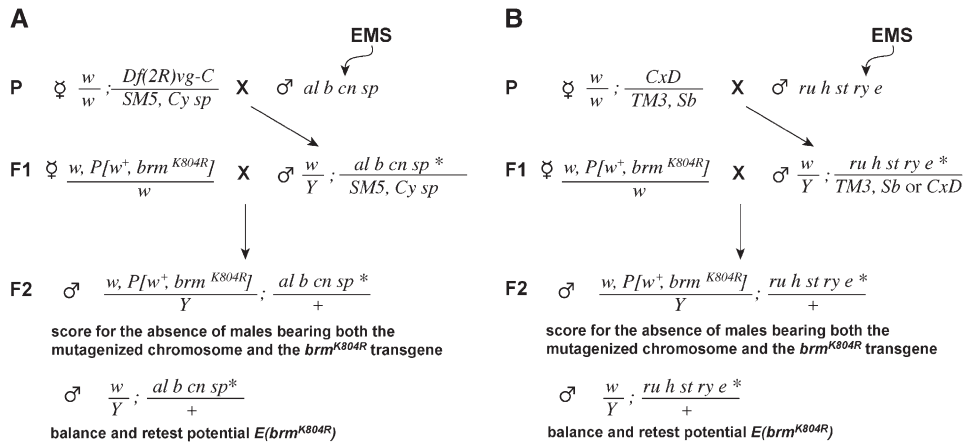


FIGURE 1.—Outline of F<sub>2</sub> screens for mutations in the (A) second and (B) third chromosomes that result in male-specific lethality in combination with the X-linked *brm*<sup>K804R</sup> transgene. Asterisks indicate mutagenized chromosomes.

each other as well as candidate genes and deficiencies. Alleles were placed into the same complementation group if *trans*-heterozygotes were not viable.

**Antibody staining and electron microscopy:** For immunofluorescent staining, animals of the genotypes *neuralized-GAL4/TM3* (control) or *neuralized-GAL4/UAS-brm*<sup>K804R</sup> (experimental) were allowed to develop at 18° until 22 or 30 hr after pupal formation (APF), hand dissected, fixed, and stained as described previously (MANNING and DOE 1999). We used the following primary antibodies: mouse anti-Pros ascites at 1:1000, rat anti-SuH 24E at 1:3000 (F. Schweisguth), and goat anti-HRP-FITC at 1:200 (Jackson ImmunoResearch, West Grove, PA). Fluorescently conjugated secondary antibodies were used at 1:200 (Jackson ImmunoResearch). Imaging was done on a Bio-Rad (Hercules, CA) Radiance confocal microscope and processed in Adobe Photoshop. For scanning electron microscopy, flies were air dried for several days in a fume hood, mounted, and sputter coated with gold/palladium. Imaging was done on an ISI WB-6 scanning electron microscope at 10 kV.

## RESULTS

**Genetic screens for dominant modifiers of an X-linked dominant-negative *brm* transgene:** To gain insight into regulation and function of the BRM chromatin-remodeling complex, we screened for modifiers of an engineered dominant-negative allele, *brm*<sup>K804R</sup>. This lysine-to-arginine substitution in the ATP-binding site of the BRM protein renders it catalytically inactive without disrupting its incorporation into the BRM complex. *brm*<sup>K804R</sup> therefore acts as a potent dominant-negative *brm* allele (ELFRING *et al.* 1998; ARMSTRONG *et al.* 2002; CORONA *et al.* 2004). The fly is extremely sensitive to changes in the relative ratio of the wild-type and BRM<sup>K804R</sup> proteins. Individuals expressing a 1:2 ratio of dominant-negative to wild-type BRM protein are phenotypically normal; individuals expressing a 1:1 ratio of the two proteins display a mild haltere-to-wing homeotic transformation due to decreased expression of *Ubx*; and a further doubling of the ratio of dominant-negative to wild-type BRM protein to 2:1 is lethal (ELFRING *et al.* 1998).

As reported previously, an X-linked transgene ex-

pressing *brm*<sup>K804R</sup> under control of the *brm* promoter (*P*[*w*<sup>+</sup>, *brm*<sup>K804R</sup>]*22D*) forms the basis of an unusual genetic screen (PAPOULAS *et al.* 1998). Females that carry the X-linked *brm*<sup>K804R</sup> transgene and are heterozygous for a *brm* null allele express a BRM<sup>K804R</sup> to BRM<sup>+</sup> ratio of 1:1 and are therefore viable. In males of the same genotype, dosage compensation of the X-linked transgene increases the ratio of BRM<sup>K804R</sup> to BRM<sup>+</sup> from 1:1 to 2:1. As a result, these individuals do not survive to adulthood. Thus, *brm* mutations or deficiencies cause male-specific lethality in individuals heterozygous for the X-linked *brm*<sup>K804R</sup> transgene. Alleles of *mor*, a *trxG* gene that encodes the BAP155 subunit of the BRM complex (CROSBY *et al.* 1999), also cause male-specific lethality in combination with the X-linked *brm*<sup>K804R</sup> transgene, suggesting that this genetic assay could be used to identify other genes that are critical for BRM function *in vivo* (PAPOULAS *et al.* 1998).

Previous studies showed that this genetic assay is highly selective; alleles of other *trxG* genes (including *ash1*, *ash2*, *dev*, *kis*, *kto*, *Trl*, *urd*, and *vtd*) and the majority of autosomal deficiencies present in deficiency kits provided by the Bloomington Stock Center failed to cause male-specific lethality in combination with the X-linked *brm*<sup>K804R</sup> transgene (PAPOULAS *et al.* 1998). Another advantage of this assay is that it is biased against recovery of mutations that merely reduce the expression of the BRM protein, since any mutation that decreases *brm* expression would similarly affect the expression of *brm*<sup>K804R</sup>. As a result, the relative levels of the two proteins would not change and no male-specific lethality would be observed.

To identify additional genes that functionally interact with *brm* *in vivo*, we screened for EMS-induced mutations that cause male-specific lethality in combination with the X-linked *brm*<sup>K804R</sup> transgene (Figure 1). We screened 6108 mutagenized second chromosomes and 3569 mutagenized third chromosomes and recovered five *E*(*brm*<sup>K804R</sup>) mutations that were placed into three lethal complementation groups. Complementation tests with existing alleles of candidate genes in addition to a com-

**TABLE 1**  
**Interactions of selected mutations with the**  
**X-linked *brm*<sup>K804R</sup> transgene**

|  | Mutant male | Control male | Mutant female | Control female | % survival |
|--|-------------|--------------|---------------|----------------|------------|
| <i>ru h st mor</i> <sup>1736</sup> <i>ry e</i> | 0           | 85           | 100           | 136            | 0          |
| <i>ru h st mor</i> <sup>2403</sup> <i>ry e</i> | 1           | 20           | 54            | 46             | 5          |
| <i>ru h st ry osa</i> <sup>2823</sup> <i>e</i> | 5           | 49           | 42            | 72             | 9          |
| <i>ru h st ry osa</i> <sup>3276</sup> <i>e</i> | 1           | 38           | 43            | 74             | 3          |
| <i>ru h st ry Dl</i> <sup>2321</sup> <i>e</i>  | 2           | 30           | 60            | 53             | 7          |
| <i>brm</i> <sup>2</sup> <i>e ca</i>            | 1           | 25           | 98            | 53             | 4          |
| <i>osa</i> <sup>2</sup>                        | 1           | 25           | 23            | 28             | 4          |
| <i>arl</i> <sup>1</sup>                        | 32          | 34           | 95            | 63             | 48         |
| <i>ru h st ry e</i>                            | 56          | NA           | 188           | NA             | NA         |

Virgin females homozygous for the X-linked *P[w<sup>+</sup>, brm<sup>K804R</sup>]* 22D transgene were mated to males bearing the mutant chromosome in *trans* to a balancer. The numbers of male and female progeny carrying either the mutant (mutant) or the balancer (control) chromosome are indicated. We determined percentage of survival by dividing the number of male mutant progeny by the total number of male progeny (male mutant + male control) and multiplying by 100. A percentage of survival of <10% was considered to be male-specific lethal in combination with the X-linked *brm*<sup>K804R</sup> transgene. *arl*<sup>1</sup> is an allele of the essential *arflike* gene that resides next to *brm* (TAMKUN *et al.* 1991) and is included as an example of a mutation that does not cause male-specific lethality in combination with the X-linked *brm*<sup>K804R</sup> transgene. *brm*<sup>2</sup> is included as a positive control. *ru h st ry e* is the parent chromosome that was mutagenized to screen for mutants on the third chromosome that interact with *brm*. NA, not applicable.

combination of meiotic and site-specific male recombination mapping (CHEN *et al.* 1998) allowed us to identify the modifiers of *brm*<sup>K804R</sup> as two *moira* alleles (*mor*<sup>1736</sup> and *mor*<sup>2403</sup>), two *osa* alleles (*osa*<sup>2823</sup> and *osa*<sup>3276</sup>), and one *Delta* allele (*Dl*<sup>2321</sup>) (Table 1). Thus, of the 9677 chromosomes screened, we recovered mutations in only three genes.

This level of selectivity was not completely unexpected since a deficiency screen of the second and third chromosomes revealed only three interacting regions, one of which spanned the *brm* gene (PAPOULAS *et al.* 1998). Our EMS screens did not identify interacting genes in the remaining two regions. We did not identify *Df(3R)Dl-BX12* (a deficiency that uncovers *Dl*) in our deficiency screen (PAPOULAS *et al.* 1998). This deficiency interacted weakly with *brm*<sup>K804R</sup>, but did not pass the stringent cutoff used in the screen (data not shown). It is possible that other genes uncovered by this deficiency obscured the genetic interaction between *brm* and *Dl*. Neither *mor* nor *osa* was uncovered by deficiencies tested in our deficiency screen (PAPOULAS *et al.* 1998).

The recovery of multiple alleles of *osa*, which encodes an ARID-domain protein found in a subset of BRM complexes (COLLINS *et al.* 1999; MOHRMANN *et al.* 2004), and of *mor*, which encodes a subunit common to all BRM complexes, confirmed the utility of our screen for identifying factors that are critical for BRM function *in*

*vivo*. The recovery of an allele of *Dl*, which encodes a ligand of the Notch receptor, may therefore reflect a close functional connection between Notch signaling and the BRM complex; this possibility is discussed at length below.

**Genetic screens for dominant suppressors of the X-linked *brm*<sup>K804R</sup> transgene:** The recovery of histone mutations in screens for suppressors of *snf2/swi2* mutations provided the first evidence that SWI/SNF counteracts chromatin repression in yeast (WINSTON and CARLSON 1992). The success of these screens motivated us to employ a similar approach in flies. To identify potential antagonists of BRM function *in vivo*, we performed an F<sub>1</sub> screen for mutations that allow *P[w<sup>+</sup>, brm<sup>K804R</sup>]* 22D/Y; *Df(3L)th102/+* individuals to survive to adulthood. The *th102* deficiency spans cytological region 72A2–72D10, including the *brm* gene. We used this deficiency for our suppressor screen instead of a *brm* null allele because it results in a lower background level of surviving males, which are invariably sterile (PAPOULAS *et al.* 1998). Mutagenized *Df(3L)th102 ri Sb/TM6B, Hu e Tb ca* males were mated to *Df(1)w67c2 y, P[w<sup>+</sup>, brm<sup>K804R</sup>]* 22D virgins and their progeny were scored for *Df(1)w67c2 y, P[w<sup>+</sup>, brm<sup>K804R</sup>]* 22D/Y; *Df(3L)th102 ri Sb/+* males. We recovered 73 males with potential suppressor mutations *vs.* 7001 sibling males of the genotype *Df(1)w67c2 y, P[w<sup>+</sup>, brm<sup>K804R</sup>]* 22D/Y; +/ *TM6B, Hu Tb*. We were unable to recover any potential suppressors, however, as each one of the 73 males was sterile. The recovery of 73 males (1%) is comparable to our background levels of male survival in a mock screen conducted without mutagen. The failure to recover mutations in genes encoding nucleosomal histones in this screen may be due to the presence of numerous copies of the histone gene cluster in flies. The failure to recover other dominant suppressors of *brm*<sup>K804R</sup> suggests that *brm* antagonists are either a relatively rare class of genes or not dosage sensitive.

**Development of an eye-based screen for dominant modifiers of *brm*<sup>K804R</sup>:** Due to the relatively small number of mutations recovered in the above screens, we developed a more sensitive, eye-based modifier screen to identify additional genes that interact with *brm*. We chose this approach because similar screens have been successfully used to study a wide variety of biological processes (THOMAS and WASSARMAN 1999). The expression of a *UAS-brm*<sup>K804R</sup> transgene in the eye-antennal disc using the *eyeless-GAL4 (ey-GAL4)* driver leads to the development of adults with eyes that are slightly rough and reduced in size. This phenotype is enhanced by mutations in genes encoding subunits of the BRM complex, including *brm*, *mor*, and *snr1* (Table 2 and Figure 4) and *BAP111* (PAPOULAS *et al.* 2001). We reasoned that additional factors that are critical for BRM function *in vivo* could be identified using this eye-based assay.

To further assess the feasibility of this approach, we screened the Bloomington Stock Center third chromosome deficiency kit for deficiencies that modify the

TABLE 2

Dominant interactions in the *brm*<sup>K804R</sup> eye-based assay

| Progeny expressing<br><i>brm</i> <sup>K804R</sup>    | Eye score |    |    |    |    |    | P-value |
|--|-----------|----|----|----|----|----|---------|
|  | 1         | 2  | 3  | 4  | 5  | 6  |         |
| <i>Df(1)w67c2</i>                                    | 0         | 82 | 10 | 14 | 3  | 1  | NA      |
| <i>brm</i> <sup>2</sup> <i>e ca</i>                  | 0         | 10 | 30 | 63 | 19 | 2  | <0.001  |
| <i>TM6B, Sb Hu Tb</i>                                | 0         | 93 | 12 | 9  | 0  | 0  |         |
| <i>snrI</i> <sup>3</sup>                             | 5         | 47 | 34 | 19 | 9  | 4  | <0.001  |
| <i>TM6B, Hu Tb</i>                                   | 21        | 26 | 6  | 2  | 1  | 0  |         |
| <i>brm</i> <sup>3369</sup>                           | 0         | 9  | 7  | 6  | 3  | 7  | <0.005  |
| <i>TM3, Sb</i>                                       | 4         | 27 | 6  | 7  | 0  | 0  |         |
| <i>mor</i> <sup>3090</sup>                           | 0         | 0  | 1  | 6  | 14 | 9  | <0.001  |
| <i>TM3, Sb</i>                                       | 0         | 16 | 16 | 2  | 0  | 0  |         |
| <i>zen</i> <sup>436</sup>                            | 0         | 11 | 17 | 16 | 6  | 3  | <0.001  |
| <i>TM3, Sb</i>                                       | 2         | 30 | 10 | 9  | 1  | 0  |         |
| <i>zen</i> <sup>714</sup>                            | 2         | 10 | 7  | 12 | 3  | 2  | <0.025  |
| <i>TM3, Sb</i>                                       | 4         | 19 | 7  | 4  | 0  | 0  |         |
| <i>zen</i> <sup>2</sup> <i>p</i> <sup>ϕ</sup>        | 0         | 18 | 6  | 1  | 4  | 15 | <0.001  |
| <i>TM3, Sb</i>                                       | 9         | 40 | 3  | 11 | 4  | 2  |         |
| <i>vn</i> <sup>643</sup>                             | 0         | 4  | 4  | 14 | 12 | 8  | <0.001  |
| <i>TM3, Sb</i>                                       | 0         | 10 | 13 | 3  | 0  | 0  |         |
| <i>P[ry<sup>+</sup>] vn</i> <sup>10567</sup>         | 0         | 1  | 10 | 17 | 7  | 9  | <0.001  |
| <i>TM3, Sb</i>                                       | 0         | 14 | 20 | 10 | 0  | 2  |         |
| <i>DI</i> <sup>2371</sup>                            | 0         | 0  | 7  | 14 | 9  | 8  | <0.001  |
| <i>TM3, Sb</i>                                       | 0         | 14 | 6  | 5  | 1  | 0  |         |
| <i>ru h th st cu sr DI</i> <sup>9P</sup> <i>e ca</i> | 0         | 1  | 11 | 19 | 8  | 5  | <0.001  |
| <i>TM3, Sb</i>                                       | 2         | 24 | 15 | 1  | 0  | 0  |         |
| <i>RpIII140</i> <sup>bric</sup>                      | 0         | 0  | 6  | 22 | 7  | 1  | <0.001  |
| <i>TM3, Sb</i>                                       | 1         | 11 | 10 | 1  | 1  | 0  |         |
| <i>RpIII140</i> <sup>A5</sup> <i>red e</i>           | 0         | 2  | 51 | 47 | 2  | 0  | <0.001  |
| <i>TM3, Sb</i>                                       | 6         | 78 | 36 | 2  | 0  | 0  |         |

With the exception of the *snrI*<sup>3</sup> stock, virgin females of the mutant stock of interest were mated to *ey-GAL4,UAS-brm*<sup>K804R</sup>/balancer males. Males of *snrI*<sup>3</sup>/*TM6B, Hu Tb* were mated to *ey-GAL4,UAS-brm*<sup>K804R</sup>/balancer virgin females. *Df(1)w67c2* was the parent stock mutagenized to screen for dominant enhancers of *brm*<sup>K804R</sup> on the third chromosome. Eyes of *ey-GAL4,UAS-brm*<sup>K804R</sup> progeny heterozygous for either a mutation of interest or the balancer chromosome were scored on a scale from 1 to 6, with 1 being wild type and 6 being complete absence of the eye as described in MATERIALS AND METHODS. P-values were determined using the Kolmogorov-Smirnov two-sample test.

*brm*<sup>K804R</sup> rough eye phenotype. We tested 62 deficiencies covering ~70% of the chromosome. Eyes were scored on a six-point scale for roughness and reduction in

TABLE 3

Representative deficiencies assayed for dominant interactions with *brm*<sup>K804R</sup>

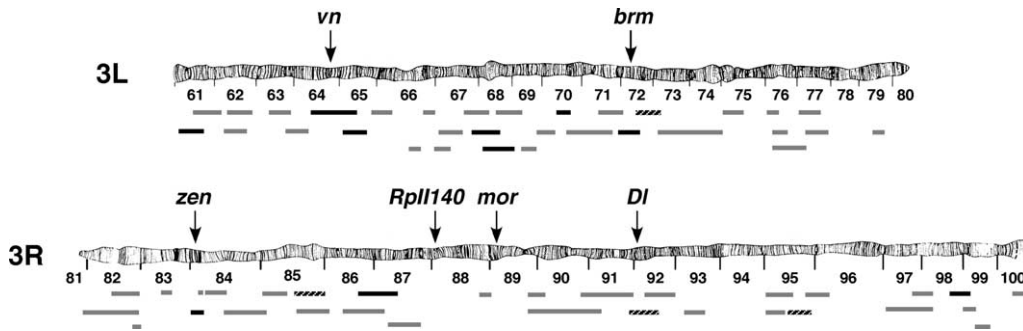
| Progeny expressing<br><i>brm</i> <sup>K804R</sup> | Eye score |    |    |    |    |   | P-value |
|---|-----------|----|----|----|----|---|---------|
|   | 1         | 2  | 3  | 4  | 5  | 6 |         |
| <i>Df(3L)vin2</i>                                 | 0         | 3  | 12 | 20 | 7  | 0 | <0.001  |
| <i>TM3, Sb</i>                                    | 0         | 20 | 7  | 3  | 0  | 0 |         |
| <i>Df(3R)3450</i>                                 | 0         | 5  | 5  | 14 | 12 | 0 | <0.05   |
| <i>TM3, Ser</i>                                   | 0         | 7  | 3  | 4  | 0  | 0 |         |
| <i>Df(3L)lxd6</i>                                 | 0         | 41 | 7  | 4  | 0  | 0 | >0.1    |
| <i>Tm3, Sb Ser</i>                                | 1         | 16 | 8  | 0  | 1  | 0 |         |
| <i>Df(3R)mbc-R1</i>                               | 16        | 50 | 1  | 3  | 2  | 0 | >0.1    |
| <i>TM3, Sb</i>                                    | 8         | 52 | 6  | 2  | 0  | 0 |         |

Virgin females heterozygous for a deficiency of interest were mated to balanced *ey-GAL4,UAS-brm*<sup>K804R</sup> males. Eyes of *ey-GAL4,UAS-brm*<sup>K804R</sup> progeny heterozygous for either the deficiency or balancer were scored as described in MATERIALS AND METHODS. P-values were determined using the Kolmogorov-Smirnov two-sample test. The representative interacting deficiencies are *Df(3L)vin2, ru<sup>1</sup> h<sup>1</sup> gl<sup>2</sup> e<sup>4</sup> ca<sup>1</sup>/TM3, Sb* (67F2–3;68D6) and *w<sup>1118</sup>; Df(3R)3450/TM6B, Tb* (98E3;99A6–8). Representative noninteracting deficiencies are *cn; Df(3R)mbc-R1, ry<sup>306</sup>/TM3, Sb ry* (95A5–7;95D6–11) and *y; Df(3L)lxd6/TM3, Sb Ser* (67E1–2;68C1–2).

size as described in MATERIALS AND METHODS. Data for representative interacting and noninteracting deficiencies are presented in Table 3. Of the 62 deficiencies tested, 14 enhanced the *brm*<sup>K804R</sup> rough eye phenotype [*Df(3L)emc-E12, Df(3L)ZN47, Df(3L)XD198, Df(3L)vin2, Df(L3)vin5, Df(3L)jz-Gfb, Df(3L)brm11, Df(3R)st-f13, Df(3R)Scr, Df(3R)by10, Df(3R)T-32, Df(3R)DI-BX12, Df(3R)crb-F89-4, and Df(3R)3450*] (Figure 2). By comparison, a screen of the third chromosome deficiency kit for regions that cause male-specific lethality in combination with the X-linked *brm*<sup>K804R</sup> transgene identified only two deficiencies: *Df(3L)ZN47* and *Df(3L)th102* (PAPOULAS *et al.* 1998). Both the *brm11* and *th102* deficiencies span the *brm* gene. Thus, the eye-based assay uncovers regions previously identified as important for *brm* function, as well as additional regions.

Rather than interacting with *brm*, it is possible that some of the deficiencies nonspecifically affect the GAL4 driver system. To control for this possibility, we performed a secondary screen in which the interacting deficiencies were tested for their ability to modify eye defects resulting from the expression of a dominant-negative form of ISWI (ISWI<sup>K159R</sup>), a chromatin-remodeling factor that is functionally distinct from BRM (DEURING *et al.* 2000). Four of the 14 deficiencies enhanced the ISWI<sup>K159R</sup> rough eye phenotype [*Df(3R)st-f13, Df(3R)by10, Df(3R)DI-BX12, and Df(3R)crb-F89-4*] (data not shown). The remaining 10 deficiencies specifically interact with *brm* and define at least eight interacting regions (Figure





gions indicate deficiencies that enhance eye defects resulting from expression of either *brm*<sup>K804R</sup> or *ISWI*<sup>K149R</sup>. Genes identified in subsequent eye-based screens as Enhancers of *brm*<sup>K804R</sup> are indicated.

FIGURE 2.—Deficiency screen of the third chromosome identified eight regions that dominantly enhance eye defects resulting from expression of *brm*<sup>K804R</sup>. Solid regions are deficiencies that specifically interact with *brm*<sup>K804R</sup> and not with *ISWI*<sup>K149R</sup> in this assay. Shaded regions indicate deficiencies that fail to enhance *brm*<sup>K804R</sup>. Hatched re-

2). By comparing interacting deficiencies to overlapping, but noninteracting deficiencies, the regions containing potential enhancers of *brm*<sup>K804R</sup> were determined to be 61A–C, 64C–65E, 68C1–11, 70C–D, 71F–72D, 84A1–5, 87B1–13, and 98E3–99A. From the results of this third chromosome deficiency kit screen, we concluded that our eye-based assay represents a sensitive but selective approach for identifying factors that functionally interact with the BRM complex.

**Most trithorax group genes do not enhance the *brm*<sup>K804R</sup> rough eye phenotype:** *brm* genetically interacts with several *trxG* genes, including *trx* and *ash1*. Flies doubly heterozygous for alleles of *brm* and *trx* display an increase in the incidence of homeotic transformations (including fifth abdominal segment to fourth and haltere to wing) (TAMKUN *et al.* 1992), while flies doubly heterozygous for *brm* and *ash1* display homeotic transformations including third to second leg (TRIPOULAS *et al.* 1994). Recent work suggests that methylation of histone tails by ASH1 may recruit the BRM complex to target promoters (BEISEL *et al.* 2002). We were therefore interested in whether mutations in *trxG* genes interacted with *brm* in our eye-based assay. Alleles of *breathless/devenir* (*bt<sup>l</sup>dev<sup>2</sup>*), *verthandi* (*vtd<sup>2</sup>*), *urdur* (*urd<sup>2</sup>*), *skuld* (*skd<sup>1</sup>* and *skd<sup>2</sup>*), *trithorax* (*trx<sup>E2</sup>*), and *kismet* (*kis<sup>1</sup>*) failed to modify the *brm*<sup>K804R</sup> rough eye phenotype (data not shown). Several *trxG* genes are uncovered by deficiencies that fail to interact with *brm* in the developing eye. These include *kohtalo* and *ash1* [*Df*(3L)*kto2*], *tonalli* [*Df*(3L)*lxd6*], and *osa* [*Df*(3R)*DG2*]. Thus, with the exception of *mor* it appears that the majority of *trxG* genes may not directly function with *brm* in the developing eye.

**Alleles of genes encoding subunits of RNA polymerase II interact with *brm*:** The BRM complex is required for global transcription by RNA polymerase II (pol II) on larval salivary gland polytene chromosomes (ARMSTRONG *et al.* 2002). To address whether mutations in genes encoding general regulators of transcription functionally interact with *brm*, we assayed alleles of genes that encode the two largest subunits of RNA polymerase II. *RpIII140<sup>A5</sup>*, *RpIII140<sup>Z45</sup>*, and *RpII215<sup>4</sup>* all enhanced the *brm*<sup>K804R</sup> rough eye phenotype (Table 2 and data not

shown). *RpII215<sup>4</sup>* enhanced eye defects resulting from expression of *ISWI*<sup>K159R</sup> (data not shown). By contrast, *RpIII140<sup>Z45</sup>* and *RpIII140<sup>A5</sup>* failed to enhance *ISWI*<sup>K159R</sup> eye defects (data not shown), suggesting that these interactions are specific to *brm*. Mutations in genes encoding components of the mediator complex (*Trap80<sup>S9256</sup>*, *Trap100<sup>BG01670</sup>*, *Trap150<sup>KG00948</sup>*, and *pap<sup>rK760</sup>*) failed to modify eye defects resulting from expression of *brm*<sup>K804R</sup>, as did alleles of genes encoding TBP-associated factors (*Taf4<sup>1</sup>* and *Taf10b<sup>KG01574</sup>*) (data not shown). Although negative results in the eye assay should be interpreted cautiously, these data suggest that *brm* functionally interacts with *RpIII140*, but not with subunits of TFIID or mediator.

#### Genetic screen for dominant modifiers of *brm*<sup>K804R</sup>:

As an unbiased approach to identify factors that functionally interact with the BRM complex, we screened for EMS-induced mutations on the third chromosome that enhance eye defects resulting from the expression of *brm*<sup>K804R</sup> (Figure 3). An F<sub>2</sub> screen was necessary for two reasons. First, the severity of the eye defects observed in *ey-GAL4*, *UAS-brm*<sup>K804R</sup> individuals was variable. Most of the eyes had disordered ommatidia covering less than half of the eye, but 5% (14 of 304 eyes) were severely

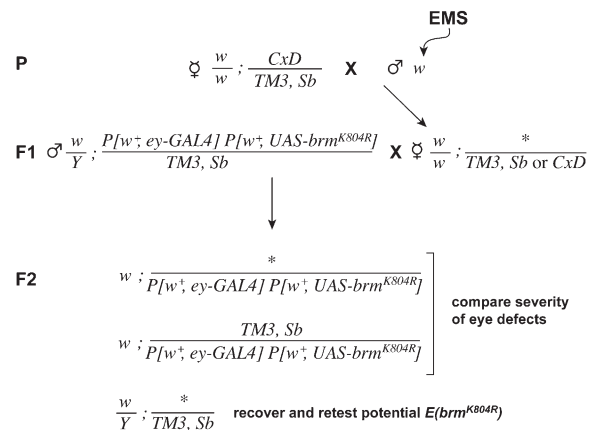


FIGURE 3.—Outline of eye-based screen for dominant modifiers of *brm*<sup>K804R</sup>. Asterisks indicate mutagenized chromosomes.

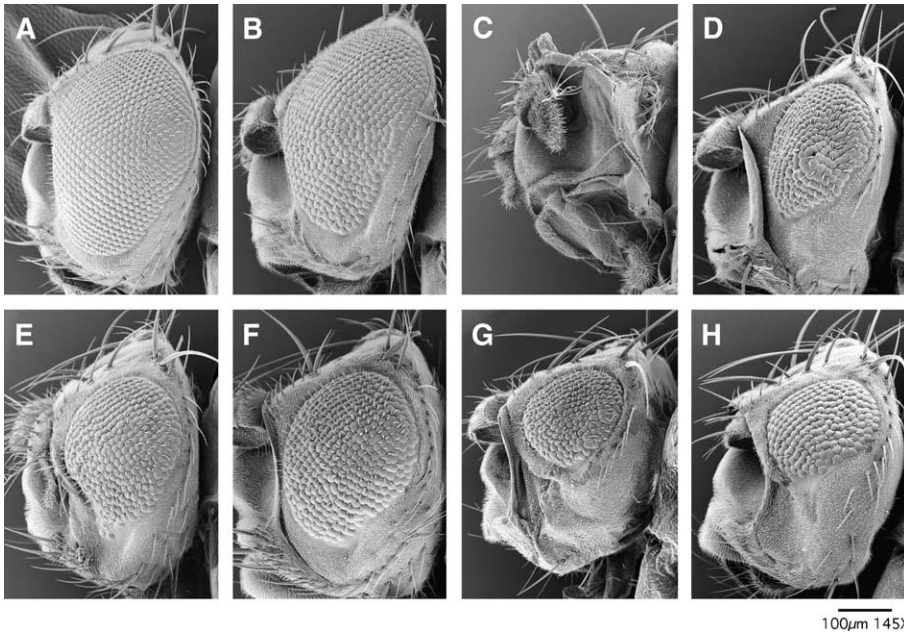


FIGURE 4.—Examples of enhancement of eye defects resulting from *brm*<sup>K804R</sup> expression by mutations in *mor*, *zen*, *Dl*, and *vn*. Scanning electron micrographs of eyes of (A) Oregon R (wild-type) and (B) *ey-GAL4, UAS-brm*<sup>K804R/+</sup> individuals are shown. Note the slight roughness resulting from the expression of *brm*<sup>K804R</sup>. This phenotype is enhanced in individuals heterozygous for *mor*<sup>3090</sup> (C), *zen*<sup>436</sup> (D), *Dl*<sup>2371</sup> (E), *Dl*<sup>9P</sup> (F), *vn*<sup>643</sup> (G), or *vn*<sup>10567</sup> (H). Representative examples are shown. Additional information on the strength of these interactions is presented in Table 2.

reduced in size or completely absent. In an F<sub>1</sub> screen, this background would result in a high number of false positives. Second, expression of the *ey-GAL4* driver is not limited to the developing eye. Mutations that strongly enhance *brm*<sup>K804R</sup> can lead to pupal lethality, as previously observed for the BAP111 subunit of the BRM complex (PAPOULAS *et al.* 2001); such mutations would be irretrievable in an F<sub>1</sub> screen.

We screened 7469 EMS-mutagenized third chromosomes for dominant modifiers of the eye defects observed in *ey-GAL4, UAS-brm*<sup>K804R</sup> adults. We simultaneously screened for mutations that were lethal in combination with *ey-GAL4, UAS-brm*<sup>K804R</sup> (Figure 3). Although the severity of the *ey-GAL4, UAS-brm*<sup>K804R</sup> eye phenotype would allow the identification of suppressor mutations, none were identified in our screen. We recovered 47 *E(brm*<sup>K804R</sup>) mutations, 13 of which were homozygous viable and were not pursued further. The remaining 34 mutations were placed into 20 lethal complementation groups. A combination of meiotic mapping and complementation tests with interacting deficiencies and alleles of candidate genes allowed us to identify six alleles of *brm* (*brm*<sup>795</sup>, *brm*<sup>963</sup>, *brm*<sup>2419</sup>, *brm*<sup>3244</sup>, *brm*<sup>3369</sup>, and *brm*<sup>1630</sup>), two alleles of *mor* (*mor*<sup>880</sup> and *mor*<sup>3090</sup>), two alleles of *zerknüllt* (*zen*<sup>436</sup> and *zen*<sup>714</sup>), eight alleles of *Dl* (*Dl*<sup>266</sup>, *Dl*<sup>470</sup>, *Dl*<sup>681</sup>, *Dl*<sup>2371</sup>, *Dl*<sup>1918</sup>, *Dl*<sup>1946</sup>, *Dl*<sup>4386</sup>, and *Dl*<sup>4585</sup>), one allele of *vein* (*vn*<sup>643</sup>), and one allele of *RpIII140* (*RpIII140*<sup>bric</sup>) (Figure 4 and Table 2). The mutations in *brm*, *mor*, *zen*, *RpIII140*, and *vn* failed to enhance the rough eye phenotype resulting from overexpression of *ISWI*<sup>K159R</sup>, suggesting that these genes specifically interact with *brm*. By contrast, the *Dl* alleles did enhance the *ISWI*<sup>K159R</sup> rough eye phenotype (data not shown). *Dl*, *brm*, *zen*, and *vn* all map to interacting defi-

ciencies (Figure 2), suggesting that these alleles behave as loss-of-function mutations. As discussed below, other loss-of-function alleles of these genes—including *brm*<sup>2</sup>, *mor*<sup>4</sup>, *zen*<sup>2</sup>, *vn*<sup>10567</sup>, *RpIII140*<sup>A5</sup>, and *Dl*<sup>9P</sup>—also dominantly enhance the *brm*<sup>K804R</sup> rough eye phenotype (Figure 4 and Table 2). The remaining 14 *E(brm)* alleles fall into single complementation groups and are currently under investigation.

**Genetic interactions between *brm* and mutations in genes involved in cell signaling:** One of the most surprising outcomes of our screens was the recovery of mutations in genes involved in signal transduction pathways. With eight alleles, *Delta* (*Dl*) was the largest complementation group recovered in the eye-based screen. Furthermore, *Dl* was the only gene recovered in the male-specific lethality screen that did not encode a subunit of the BRM complex. *Dl* encodes a ligand for the Notch receptor and is critical for development (LAI 2004). We also recovered one allele of *vein* (*vn*), which encodes a secreted ligand for the epidermal growth factor receptor (EGFR) (SCHNEPP *et al.* 1996). To determine whether genetic interactions between *brm* and these signaling pathways are limited to the developing eye, we examined *trans*-heterozygotes for adult phenotypes. This approach has proven useful for uncovering genes important for *brm* function. For example, individuals heterozygous for alleles of either *brm* or *mor* appear normal, while *trans*-heterozygous adults display loss of humeral bristles, duplicated or extra macrochaetae, ectopic wing veins, rough eyes, and held-out wings (Table 4) (BRIZUELA and KENNISON 1997). Likewise, individuals heterozygous for alleles of *brm* and *osa* display held-out wings (VAZQUEZ *et al.* 1999). These genetic interactions provided early evidence that the BRM protein



**TABLE 4**  
Genetic interactions between *brm* and *Dl*

| Genotype  | % with loss of humeral bristles | % with ectopic or duplicated macrochaetae | % with rough eyes | No. of flies |
|---|---------------------------------|---|-------------------|--------------|
| <i>brm</i> <sup>2</sup> /+                          | 0                               | 3.5                                       | 0                 | 57           |
| <i>brm</i> <sup>2</sup> / <i>mor</i> <sup>4</sup>   | 22                              | 35  | 10                | 49           |
| +/ <i>mor</i> <sup>4</sup>                          | 0                               | 12  | 0                 | 56           |
| <i>brm</i> <sup>2</sup> / <i>Dl</i> <sup>266</sup>  | 14                              | 42  | 58                | 48           |
| +/ <i>Dl</i> <sup>266</sup>                         | 0                               | 10  | 1.7               | 58           |
| <i>brm</i> <sup>2</sup> / <i>Dl</i> <sup>2371</sup> | 19                              | 52  | 69                | 42           |
| +/ <i>Dl</i> <sup>2371</sup>                        | 0                               | 27  | 2.4               | 41           |
| <i>brm</i> <sup>2</sup> / <i>Dl</i> <sup>9P</sup>   | 0                               | 28  | 76                | 49           |
| +/ <i>Dl</i> <sup>9P</sup>                          | 0                               | 20  | 0                 | 50           |

functionally interacts with MOR and OSA. We asked whether similar genetic interactions could be observed between *brm* and *Dl* or *vn*.

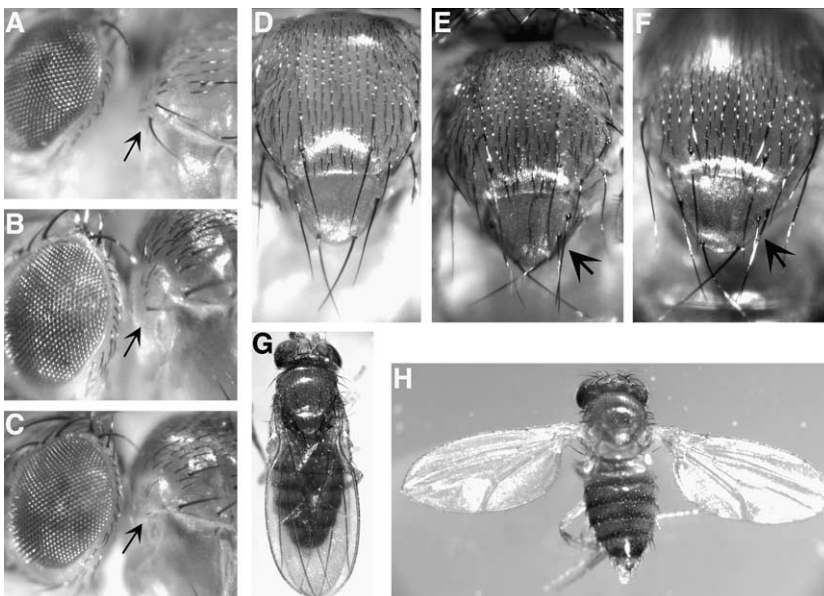
Individuals *trans*-heterozygous for *brm*<sup>2</sup> and *Dl*<sup>266</sup>, *Dl*<sup>2371</sup>, or *Dl*<sup>9P</sup> display a variety of phenotypes including loss of humeral bristles, duplicated or extra macrochaetae, and rough eyes (Table 4, Figure 5). Individuals heterozygous for only one of the alleles display some of these phenotypes at low penetrance, but the penetrance of phenotypes was greatly enhanced in the *trans*-heterozygotes (Table 4). Individuals *trans*-heterozygous for *brm* and *vn* also display a variety of adult phenotypes including held-out wings, loss of humeral bristles, duplicated or extra macrochaetae, and mildly rough eyes (Table 5, Figure 5). In the single heterozygotes, these phenotypes either are not observed or are present at low penetrance (Table 5). Thus, genetic interactions between *brm* and both *Dl* and *vn* are not limited to the developing eye and are not dependent on either the

**TABLE 5**  
Genetics interactions between *brm* and *vn*

| Genotype   | % with held-out wings | % with loss of humeral bristles | % with ectopic or duplicated macrochaetae | No. of flies |
|--|-----------------------|---------------------------------|---|--------------|
| <i>brm</i> <sup>2</sup> /+                               | 0                     | 0                               | 3.5                                       | 57           |
| <i>brm</i> <sup>2</sup> / <i>vn</i> <sup>643</sup>       | 96                    | 92                              | 21  | 53           |
| +/ <i>vn</i> <sup>643</sup>                              | 0                     | 0                               | 0   | 49           |
| <i>brm</i> <sup>2</sup> / <i>vn</i> <sup>221</sup>       | 13                    | 49                              | 5.7                                       | 53           |
| +/ <i>vn</i> <sup>221</sup>                              | 0                     | 2.1                             | 0   | 47           |
| <i>brm</i> <sup>2</sup> / <i>vn</i> <sup>10567</sup>     | 0                     | 93                              | 8.9                                       | 56           |
| +/ <i>vn</i> <sup>10567</sup>                            | 0                     | 0                               | 0   | 47           |
| <i>brm</i> <sup>2</sup> / <i>Egfr</i> <sup>12</sup>      | 0                     | 16                              | 2.0                                       | 49           |
| +/ <i>Egfr</i> <sup>12</sup>                             | 0                     | 0                               | 0   | 50           |
| <i>brm</i> <sup>2</sup> / <i>grk</i> <sup>3</sup>        | 0                     | 0                               | 0   | 36           |
| <i>brm</i> <sup>2</sup> / <i>spi</i> <sup>1</sup>        | 0                     | 0                               | 2.0                                       | 51           |
| <i>brm</i> <sup>2</sup> / <i>Df</i> (3L)Z <sup>N47</sup> | 0                     | 92                              | 62  | 50           |
| +/ <i>Df</i> (3L)Z <sup>N47</sup>                        | 0                     | 2.4                             | 7.3                                       | 41           |

GAL4 driver system or the dominant-negative *brm*<sup>K804R</sup> allele.

The signaling pathways involving *Dl* and *Vn* are complex since their respective receptors (Notch and EGF receptor) respond to more than one ligand. *Vn* signals via the EGF receptor, the *Drosophila* homolog of the epidermal growth factor receptor (SCHNEPP *et al.* 1996). We observed genetic interactions between *brm*<sup>2</sup> and *Egfr*<sup>12</sup>, a loss-of-function allele of *Egfr*. Of the *trans*-heterozygotes, 16% displayed loss of one or more humeral bristles (Table 5). This phenotype was not observed in either *brm*<sup>2</sup> or *Egfr*<sup>12</sup> heterozygotes. The EGF receptor responds to four different receptor ligands: *Vn*, *Gurken* (*Grk*), *Spitz* (*Spi*), and *Keren* (*Krn*) (SHILO 2003). We failed to detect similar types of genetic interactions be-



**FIGURE 5.**—Adult phenotypes illustrate genetic interactions between *brm* and *Dl* and *brm* and *vn*. (A) Arrows indicate wild-type humeral bristles in Oregon R flies. Bristles are lost in flies *trans*-heterozygous for (B) *brm*<sup>2</sup> and *Dl*<sup>2371</sup> or (C) *brm*<sup>2</sup> and *vn*<sup>643</sup>. (D) Wild-type Oregon R flies have four scutellar bristles. Extra scutellar bristles (indicated by arrows) are shown in flies that are *trans*-heterozygous for (E) *brm*<sup>2</sup> and *Dl*<sup>2371</sup> or (F) *brm*<sup>2</sup> and *vn*<sup>643</sup>. (G) Wild-type Oregon R flies do not hold out their wings while (H) *brm*<sup>2</sup>/*vn*<sup>643</sup> flies display held-out wings.

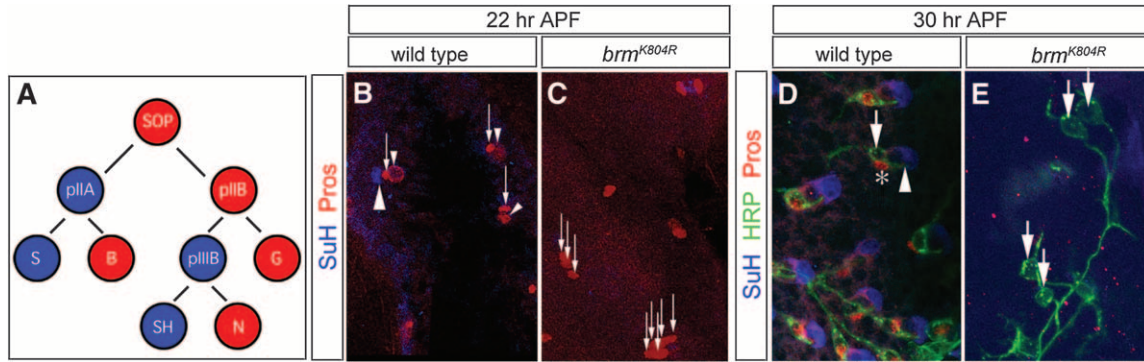


FIGURE 6.—Reduced BRM levels generate phenotypes similar to reduced Delta signaling in the adult SOP lineage. (A) Summary of the adult external sense organ lineage, with the cell fates requiring high DI-Notch signaling shown in blue and the cell fates requiring low DI-Notch signaling shown in red. S, socket; B, bristle; SH, sheath; N, neuron; and G, glial. (B and C) Pupal notum at 22 hr APF stained for socket cells (SuH, blue, large arrowhead), glia (strong Pros, red, thin arrows), and the pIIIB cell (weak Pros, red, small arrowheads). (B) Wild-type SOP lineage showing one socket, one pIIIB, and one glial cell visible. (C) The *neuralized-GAL4/UAS-brm*<sup>K804R</sup> SOP lineages contain fewer socket cells and extra glia (strong Pros, red, thin arrows). (D and E) Pupal notum at 30 hr APF stained for socket cells (SuH, blue, arrowhead), neurons (HRP, green, thick arrows) and sheath cells (Pros, red, asterisk). At this stage, the only Pros<sup>+</sup> cell present is the sheath cell, because the Pros<sup>+</sup> glial cell visible at 22 hr APF has migrated away (GHO *et al.* 1999; REDDY and RODRIGUES 1999). (D) Wild-type SOP lineages at this stage showing one socket, one neuron, and one sheath cell. (E) The *neuralized-GAL4/UAS-brm*<sup>K804R</sup> SOP lineages contain fewer socket cells, fewer sheath cells, and extra neurons.

tween *brm*<sup>2</sup> and alleles of *grk* or *spi* (Table 5). Furthermore, *grk*<sup>3</sup>, *spi*<sup>1</sup>, *spi*<sup>S3547</sup>, and *Df(3L)81k19* (a deficiency covering *km*) all failed to modify eye defects resulting from expression of *brm*<sup>K804R</sup> (data not shown). These data suggest that the BRM complex is important for signaling by the Vn ligand. The Notch receptor receives signals from one of two ligands, DI or Serrate (Ser) (LAI 2004). Ser is not expressed in the developing eye (VERHEYEN *et al.* 1996), and so we would not expect *Ser* alleles to modify eye defects resulting from loss of *brm* function. Mutations in *Notch* (*N*) would not have been identified in any of our screens since it is located on the X chromosome. However, a loss-of-function *N* allele, *N*<sup>264-39</sup>, dominantly enhances the rough eye phenotype resulting from expression of *brm*<sup>K804R</sup> (data not shown). These data suggest that BRM interacts with the Vn-Egfr and DI-Notch signaling pathways.

The identification of genetic interactions between *brm* and *Dl* was intriguing since previous studies had revealed a role for *brm* in the development of the peripheral nervous system (ELFRING *et al.* 1998). The external sense organs of the *Drosophila* peripheral nervous system are formed by the adult sense organ precursor (SOP) cells, which undergo asymmetric cell divisions to produce five different cell types: socket, bristle, sheath, neuron, and glia (Figure 6A). Cell fate in the SOP lineage is controlled by the level of DI-Notch signaling: high DI-Notch signaling promotes pIIA, pIIIB, socket, and sheath cell fates, whereas low DI-Notch signaling results in pIIB, bristle, neuron, and glial cell fates (HARTENSTEIN and POSAKONY 1990; PARKS and MUSKAVITCH 1993; GUO *et al.* 1996). If the BRM complex is required for DI-Notch signaling, the loss of *brm* function should cause lineage defects similar to those

resulting from reduced DI-Notch signaling, *i.e.*, an increase in bristles, glia, or neurons at the expense of other cell types.

To test the prediction, we expressed *brm*<sup>K804R</sup> in the SOP lineage and used cell-specific markers to observe the resulting cell types. We used the following markers to score sense organ cell fates: Suppressor of Hairless (SuH) for socket cells, Prospero (Pros) for glia in early lineages and sheath cell in late lineages, and anti-horse-radish peroxidase (HRP) for neuronal membranes (JAN and JAN 1982; MANNING and DOE 1999). Expression of *brm*<sup>K804R</sup> in the SOP lineage using the *neuralized-GAL4* driver at 20° resulted in high embryonic or larval lethality (78%; *n* = 803), early pupal lethality (17%; *n* = 803), and midpupal lethality (5%; *n* = 803); only the last class was used to score SOP phenotypes. Control genotypes containing the *UAS-brm*<sup>K804R</sup> transgene without a *neuralized-GAL4* showed excellent viability (97%; *n* = 800). We found that *neuralized-GAL4 UAS-brm*<sup>K804R</sup> pupae contained variable-sized patches of tissue containing wild-type or defective SOP lineages, and we confined our analysis to the defective tissue. Early SOP lineages, 22 hr APF, showed a loss of SuH<sup>+</sup> socket cells and an increase in Pros<sup>+</sup> glial cells (Figure 6, B and C; *n* = 371 lineages examined). Late SOP lineages, 30 hr APF, showed a loss of SuH<sup>+</sup> socket cells and Pros<sup>+</sup> sheath cells but an increase in HRP<sup>+</sup> neurons (Figure 6, D and E; *n* = 240). These two phenotypes were never observed in control 22-hr APF or 30-hr APF pupae containing the *UAS-brm*<sup>K804R</sup> transgene without the *neuralized-GAL4* transgene (22 hr APF, *n* = 272; 30 hr APF, *n* = 137). Both the early and late-lineage phenotypes are similar to those seen following loss of DI-Notch signaling, as summarized in Figure 6A (HARTENSTEIN and POSAKONY

1990; PARKS and MUSKAVITCH 1993; GUO *et al.* 1996). We conclude that BRM and D1 act together to specify cell fate within the adult SOP lineage.

## DISCUSSION

In this study we report the results of two different screens designed to identify factors that are critical for the function of the *Drosophila* BRM chromatin-remodeling complex. We screened a total of 17,146 mutant chromosomes and recovered 39 mutations that genetically interact with a dominant-negative allele of *brm* (*brm*<sup>K804R</sup>). Of the 25 mutations that we positively identified, nearly half (48%) are alleles of genes encoding subunits of the BRM complex (*brm*, *mor*, or *osa*), suggesting that the other genes identified in our screens are also critical for *brm* function. Similar screens could be used to study any *Drosophila* chromatin-remodeling factor that functions as the ATPase subunit of a protein complex (CORONA *et al.* 2004).

**Interactions between *brm* and other factors involved in transcription:** Our screens identified a single allele of *RpIII140*, which encodes the second largest subunit of RNA pol II. Other alleles of *RpIII140* also dominantly enhanced eye defects resulting from expression of *brm*<sup>K804R</sup>. This finding complements our observation that the BRM complex is required for global transcription by RNA pol II (ARMSTRONG *et al.* 2002) and suggests that the BRM complex may interact more closely than previously thought with the general transcriptional machinery. These findings are consistent with the observation that yeast TFIID and RNA pol II are required for the recruitment of SWI/SNF to the *RNR3* promoter (SHARMA *et al.* 2003). We have been unable to detect a physical interaction between RNA pol II and the BRM complex by co-immunoprecipitation (ARMSTRONG *et al.* 2002), however, and SWI/SNF recruitment does not depend upon RNA pol II at all yeast promoters (HIRSCHHORN *et al.* 1992; GAVIN and SIMPSON 1997). Why the basal transcription machinery targets chromatin-remodeling complexes to some, but not all, promoters remains to be determined.

Two distinct BRM complexes (called BAP and PBAP) were recently identified in *Drosophila* (MOHRMANN *et al.* 2004). Both complexes contain the BRM ATPase (related to the yeast SWI2/SNF2 and RSC ATPases), the SANT-domain protein Moira (MOR), the HMG-domain protein BAP111, the actin-related protein BAP55, actin, BAP60, and SNR1 (PAPOULAS *et al.* 1998; COLLINS *et al.* 1999; MOHRMANN *et al.* 2004). The BAP complex contains OSA, while the PBAP complex lacks OSA and instead contains Polybromo and the ARID-domain, zinc-finger protein BAP170 (COLLINS *et al.* 1999; MOHRMANN *et al.* 2004). BAP may represent the *Drosophila* counterpart of the yeast SWI/SNF and human BAF complexes, while PBAP appears more highly related to the yeast RSC and human PBAF complexes

(MOHRMANN and VERRIJZER 2005). Both BAP and PBAP are abundant and are widely associated with transcriptionally active chromatin in larval salivary glands (MOHRMANN *et al.* 2004). Both complexes use the BRM ATPase; the expression of BRM<sup>K804R</sup> should therefore interfere with the functions of both the BAP and PBAP complexes.

The presence or absence of the OSA subunit distinguishes the BAP complex from PBAP (MOHRMANN *et al.* 2004). We isolated two *osa* alleles from the male-specific lethality screens, suggesting that this screen has the potential to identify factors important for BAP function. Our *osa* alleles fail to modify the eye defects caused by expression of dominant-negative *brm* (as does a deficiency spanning *osa*), suggesting that our eye-based screen may select for genes important for PBAP function. In agreement with these observations, COLLINS *et al.* (1999) found that while *osa* interacted with *brm* in the wing, it acted in opposition to *brm* in the eye. The elucidation of the relative roles of BAP and PBAP *in vivo* will require the isolation of mutations in genes encoding unique subunits of this complex, including polybromo and BAP170 (MOHRMANN *et al.* 2004).

**Interactions between BRM and other proteins that regulate chromatin structure and function:** Numerous recent studies have revealed close functional relationships between chromatin-remodeling complexes and histone-modifying enzymes (HASSAN *et al.* 2002). For example, the MOF histone acetyltransferase functionally antagonizes the *Drosophila* ISWI chromatin-remodeling factor (CORONA *et al.* 2002); bromodomains within the yeast RSC chromatin-remodeling complex recognize acetylated histone H3 (KASTEN *et al.* 2004); and methylation of lysines 4 and 9 of H3 and lysine 20 of H4 by Ash1 may recruit the BRM complex (BEISEL *et al.* 2002). Histone modification, including methylation of lysine 4 of H3, is also required for expression of Notch target genes (BRAY *et al.* 2005).

However, to date we have not yet identified *E(brm)* mutations in genes encoding histone-modifying enzymes. We also failed to recover genes encoding structural components of chromatin or subunits of other chromatin-remodeling complexes. Why weren't mutations in these classes of genes recovered in our screens? We did not expect to recover mutations in histone genes in our screens since they are present in many copies in flies. Our eye-based screen was limited to the third chromosomes, and genes on the X chromosome would have escaped detection in both of our screens. Furthermore, we do not believe that either one of our genetic screens was taken to saturation. It is also possible that chromatin-remodeling and modifying enzymes that interact with *brm* are redundant or are not expressed in limiting quantities.

**The BRM complex and D1-Notch signaling:** *Dl* represented the largest *E(brm)* complementation group; over a third of the mutations (36%) were alleles of *Dl*. These



findings suggest that the functions of the BRM complex and the Notch signaling pathway are intimately related. Notch signaling is one of the most extensively studied signaling pathways (KADESCH 2004). It is essential for the development of most tissues and is likely present in all metazoans, although here we focus on the pathway in *Drosophila*. A transmembrane ligand (either Delta or Serrate) on the signaling cell binds the Notch receptor on the signal-receiving cell, resulting in two proteolytic cleavages of the Notch transmembrane protein. This proteolysis causes the release of the Notch ICD, which translocates to the nucleus to regulate gene expression. Once in the nucleus, the ICD forms a complex with the Suppressor of Hairless [Su(H)] transcription factor (a CSL protein) to activate Notch target genes. In the absence of signaling (and therefore the absence of ICD), Su(H) complexes with corepressors that deacetylate histones to repress transcription of target genes (LAI 2004; SCHWEISGUTH 2004). The role of Notch signaling is particularly well understood in regard to cell fate determinations within the adult SOP lineage. Loss of DI-Notch signaling can result in an increase of neurons or glia at the expense of other cell types (HARTENSTEIN and POSAKONY 1990; PARKS and MUSKAVITCH 1993).

Previous work suggested that the BRM complex was critical for the development of the peripheral nervous system; somatic clones of *brm* mutant tissue throughout the fly showed duplicated, stunted, or fused mechanosensory bristles (ELFRING *et al.* 1998). Expression of the dominant-negative allele of *brm* results in similar bristle defects, as well as alterations in the number and identities of campaniform sensilla, sensory organs used for flight (ELFRING *et al.* 1998). The identification of numerous alleles of *Dl* in our screens as well as the observation of increased penetrance of a variety of phenotypes in individuals heterozygous for alleles of both *brm* and *Dl* is consistent with these observations and points to a close functional connection between the Notch signaling pathway and the BRM complex.

To explore further the connection between the BRM complex and DI-Notch signaling, we investigated the role of the BRM complex in cell fate specification within the adult SOP lineage, where every stage of development is regulated by DI-Notch signaling. Reduced DI-Notch signaling within the imaginal disc proneural cluster that gives rise to the SOP leads to formation of ectopic SOPs that form perfectly normal sense organs, leading to bristle/socket duplications (HARTENSTEIN and POSAKONY 1990; PARKS and MUSKAVITCH 1993), a phenotype similar to the bristle defects seen in *brm* mutant clones (ELFRING *et al.* 1998). In contrast, reduced DI-Notch specifically within the SOP lineage results in loss of external cell types and production of ectopic internal cell types such as glia or neurons (HARTENSTEIN and POSAKONY 1990; PARKS and MUSKAVITCH 1993). This is precisely

the phenotype we observe following expression of *brm<sup>K804R</sup>* within the SOP lineage.

What is the role of the BRM complex in the Notch signaling pathway? Since the BRM complex plays a global role in transcription by RNA pol II (ARMSTRONG *et al.* 2002), it is possible that the genetic interactions and phenotypes that we have observed are the result of decreased DI expression. We believe this is unlikely due to the selectivity of our screens. Indeed, we failed to observe genetic interactions between DI and RpIII40 mutations (data not shown). It is also possible that the BRM complex and the DI-Notch pathway are independently regulating the same target genes. If both pathways are limiting, a reduction in DI-Notch signaling may enhance a *brm* phenotype. A more intriguing possibility is that DI-Notch signaling may regulate the activity or targeting of the BRM complex. As a ubiquitous complex that is critical for the transcription of most genes by RNA pol II genes, the BRM complex is a logical target for the signaling pathways. Once the ICD of Notch is in the nucleus, it may form complexes not only with Su(H), but also with the BRM complex, thus regulating its activity or its association with Notch target genes. Strong support for this model is provided by recent biochemical studies of the human BRM (hBRM) protein. hBRM physically interacts with the ICD of Notch and both hBRM and ICD are found to be associated with the promoters of Notch target genes (KADAM and EMERSON 2003). On the basis of these findings, further analyses of the interactions between DI-Notch signaling and the BRM chromatin-remodeling complex are clearly warranted.

Our data suggest that the BRM complex may play an important role in another signal transduction pathway. An allele of *vn*, which encodes a secreted protein related to the mammalian neuregulin family of ligands for the EGF receptor, was recovered as an enhancer of eye defects resulting from the expression of *brm<sup>K804R</sup>*. Many signal pathways intersect and complex interactions between EGF receptor signaling and the Notch pathway have been reported in *Drosophila*. EGF receptor signaling can work in concert with (FLORES *et al.* 2000; KUMAR and MOSES 2001; TSUDA *et al.* 2002) or antagonistically to Notch signaling (CULI *et al.* 2001; CARMENA *et al.* 2002; ROHRBAUGH *et al.* 2002). Our findings suggest that the BRM complex interacts with one or both of these pathways during eye development, but the precise nature of these interactions remains to be determined.

In conclusion, our unbiased genetic screens led us to an unexpected connection between the BRM chromatin-remodeling complex and DI-Notch signaling. Both the BRM complex and the DI-Notch signaling pathway are conserved in mammals; our results therefore suggest that similar interactions may be critical for mammalian development. In mice, loss of Notch activity leads to tumor formation (NICOLAS *et al.* 2003); similarly the genes encoding subunits of the mammalian BRM com-

plexes also act as tumor suppressors (DUNAIEF *et al.* 1994; VERSTEEGE *et al.* 1998). Further work is required to determine the precise nature and extent of interactions between the BRM chromatin-remodeling complex and signaling pathways.

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