The HMG-domain protein BAP111 is important for the function of the BRM chromatin-remodeling complex *in vivo*

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Edited by Roger D. Kornberg, Stanford University School of Medicine, Stanford, CA, and approved February 26, 2001 (received for review November 7, 2000)

The *Drosophila* trithorax group gene *brahma* (*brm*) encodes the ATPase subunit of a SWI/SNF-like chromatin-remodeling complex. A key question about chromatin-remodeling complexes is how they interact with DNA, particularly in the large genomes of higher eukaryotes. Here, we report the characterization of BAP111, a BRM-associated protein that contains a high mobility group (HMG) domain predicted to bind distorted or bent DNA. The presence of an HMG domain in BAP111 suggests that it may modulate interactions between the BRM complex and chromatin. BAP111 is an abundant nuclear protein that is present in all cells throughout development. By using gel filtration chromatography and immunoprecipitation assays, we found that the majority of BAP111 protein in embryos is associated with the BRM complex. Furthermore, heterozygosity for *BAP111* enhanced the phenotypes resulting from a partial loss of *brm* function. These data demonstrate that the BAP111 subunit is important for BRM complex function *in vivo*.

The packaging of eukaryotic DNA with histones into chromatin organizes and compacts the long, fragile DNA molecules and ensures their transmission during cell division. However, chromatin is a barrier to many processes that require access to DNA. Two common mechanisms used by eukaryotic cells to overcome this barrier are covalent modification of histones and ATP-dependent remodeling of chromatin structure (refs. 1 and 2 and references therein). One of the best characterized examples of histone modification is the regulated acetylation of specific lysines on histone tails by histone acetyltransferases and histone deacetylases (3–5). ATP-dependent remodeling of chromatin is carried out by large macromolecular protein complexes (reviewed in refs. 1 and 2). *In vitro* assays have demonstrated that these chromatin-remodeling complexes slide nucleosomes along DNA, assemble or displace nucleosomes, and alter the associations of nucleosomes with DNA. These activities are believed to create a more fluid chromatin structure that can increase the access of other regulatory proteins to nucleosomal DNA. Both histone-modifying enzymes and chromatin-remodeling complexes can be targeted to specific genes *in vivo*, resulting in local alterations of chromatin structure and transcription (6). Although our understanding of these complexes has grown immensely, much remains to be learned about how they interact with chromatin *in vivo*.

More than a dozen chromatin-remodeling complexes have been identified to date in yeast, flies, and vertebrates. These complexes contain related ATPases and have been divided into three groups based on whether their ATPase subunit is most similar to the SWI2/SNF2, ISWI, or Mi-2 protein (1, 2, 7). The first complex to be characterized biochemically was SWI/SNF, the SWI2/SNF2-containing complex in *Saccharomyces cerevisiae* (8, 9). Genetic and biochemical data indicate that the SWI/SNF complex can increase the access to DNA of transcription factors as well as histone-modifying complexes (reviewed in ref. 7). Genome-wide expression studies have shown that SWI/SNF is involved in both transcriptional activation and repression (10, 11). ATPases related to SWI2/SNF2—including *Drosophila* Brahma (BRM)(12), yeast STH1, and the human hBRM and BRG1 proteins (13)—function as the catalytic subunits of chromatin-remodeling complexes related to SWI/SNF (7).

Each SWI/SNF-like complex contains 4 core subunits homologous to the yeast SWI2/SNF2, SWI3, SNF5, and SWP73 proteins plus 3–12 additional subunits. Many of these additional subunits are unique to a given complex, tissue, or species and are dispensable for chromatin remodeling *in vitro* (14). These observations suggest that the additional subunits perform functions that are required only *in vivo*. One possible role of the additional subunits is the selective targeting of SWI/SNF-like complexes to genes that require their function. Recent work has demonstrated that SWI/SNF-like complexes are recruited to some sites through direct interactions with gene-specific regulators. For example, the yeast SWI/SNF complex is recruited to the HO locus by direct interactions with the DNA-binding transcription factor SWI5 (15, 16). Similarly, the glucocorticoid receptor (17), c/EBPβ (18), and the erythroid-specific transcription factor EKLF (19) all recruit human SWI/SNF-like complexes to specific genes. It is likely, therefore, that the function of some subunits is to mediate recruitment by specific transcription factors.

Much remains to be learned about how SWI/SNF-like complexes interact with chromatin *in vivo*. Although SWI/SNF-like complexes lack sequence-specific DNA-binding activity, several subunits of these complexes bind DNA nonspecifically. hBRM contains an A/T hook motif which mediates nonspecific DNA binding *in vitro* (20). The SWI1 subunit of the yeast SWI/SNF complex contains an ARID (AT-rich interaction domain) which seems to mediate nonspecific binding of this yeast complex to the minor groove of DNA (21, 22). Other ARID-containing proteins have been reported in human SWI/SNF-like complexes (p270; refs. 23 and 24) and in a subset of the *Drosophila* BRM complexes (OSA; refs. 25 and 26). In addition, a subunit of the human BRG-1 complex, BAF57, contains a high mobility group (HMG) domain, another motif predicted to bind nonspecifically to the minor groove of DNA (27). *In vitro* studies have confirmed that BAF57 binds DNA; however, human BRG1/hBRM complexes lacking the BAF57 HMG domain are nevertheless still able to bind DNA and remodel chromatin *in vitro* (27). Thus, nonspecific DNA binding is a conserved but poorly understood activity of SWI/SNF complexes.

Here, we report the characterization of the *Drosophila* BRM-associated protein BAP111. BAP111 is an HMG domain-containing protein related to the mammalian BAF57 protein.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: HMG, high mobility group; HA, hemagglutinin; BAP, BRM-associated protein; EST, expressed sequence tag.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession no. AF348329).

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Previous studies of BAF57 were unable to demonstrate an essential role for this subunit in chromatin remodeling *in vitro*. By using a genetic assay, we demonstrate that *BAP111* functionally interacts with *brm in vivo*. BAP111, and by extension BAF57, is likely, therefore, to play a critical role in the function of SWI/SNF-like complexes of multicellular eukaryotes.

Methods

Sequencing and Characterization of the *BAP111* Transcript. *BAP111* cDNAs were sequenced on both strands by using the Applied Biosystems BigDye reagents. Northern blots were probed with a 1-kb internal *Nsi* fragment of LD13023.

Production of Antibodies and Western Blotting. A *BAP111* cDNA fragment was generated by PCR using the primers 5′-CGTGGATCCGCATGAGACACCGTC-3′ and 5′-CATTGAATTCATCCAATCGCTGGC-3′, digested with *Bam*HI and *Eco*RI, and cloned in pGEX1. Rabbit polyclonal antibodies were raised against the glutathione *S*-transferase (GST)-fusion protein encoded by this plasmid as described (28). SDS/PAGE and Western blotting were performed as described (29), except that Tris-buffered saline/0.1% Tween-20/10% dry milk was used for blocking and antibody incubations. Other primary antibodies used were mouse monoclonal anti-hemagglutinin (HA) antibody 12CA5 (BabCo, Richmond, CA) and affinity-purified rabbit anti-BRM polyclonal antibodies (30). Immunoprecipitations were performed as described (29). Native embryo and pupal extracts (30), adult male or female proteins solubilized in electrophoresis sample buffer (30), or larval extracts prepared by homogenization in electrophoresis sample buffer with 1 mM EDTA and 8 M urea were analyzed by Western blotting. Chemiluminescent signals were quantified on a Bio-Rad GS-525 Molecular Imager.

Gel Filtration Chromatography. Superose 6 gel filtration chromatography (30) was performed with 1 mg of extract prepared from 0–12 h *P[w$^+$, brm-HA-6HIS]92C;brm^2/Df(3L)th102 h ri Sb ca^2* embryos.

Immunofluorescence. Embryos were dechorionated in 50% bleach, fixed in formaldehyde/heptane, and devitellinized by using methanol. Embryos were incubated with affinity-purified rabbit anti-BAP111 for 1 h at 4°C in phosphate-buffered saline/.05% Triton-X-100/1% BSA/.02% sodium azide. Secondary antibodies used were either goat anti-rabbit-FITC or -Cy5; embryos were mounted in Vectashield mounting medium (Vector Laboratories) containing 1.5 μg/ml propidium iodide. For propidium iodide staining, embryos were treated overnight at 37°C in PBS plus 5 mg/ml RNase A and were washed briefly in PBS before mounting. Images were captured at 250× magnification on a Leitz DMIRB inverted photoscope equipped with a Leica TCS NT laser confocal imaging system.

Generation of *BAP111* Transgenes. Sequences for *BAP111* transgenes were generated by PCR with the cDNA LD13023 (Berkeley Drosophila Genome Project). An *Xho*I–*Bgl*II HA-6His epitope tag fragment (31) was added at a C-terminal *Xho*I site created by PCR with the primers 5′-TTCCTCGAGGCATCCGCCTCG-3′ and 5′-TACTGCAGCGACCACAAC-3′. The epitope-tagged *BAP111* gene was cloned into pUAST (32) at the *Xba*I polylinker site by using both the LD13023 *Xba*I site located 90 nucleotides upstream of the initiating ATG and an *Xba*I site engineered 3′ of the epitope tag (final sequence 5′-CATTAGTAAGATCCTCTAGA-3′). The HMG domain of BAP111 was deleted in this construct by site-directed mutagenesis. Fragments were generated by PCR with the following

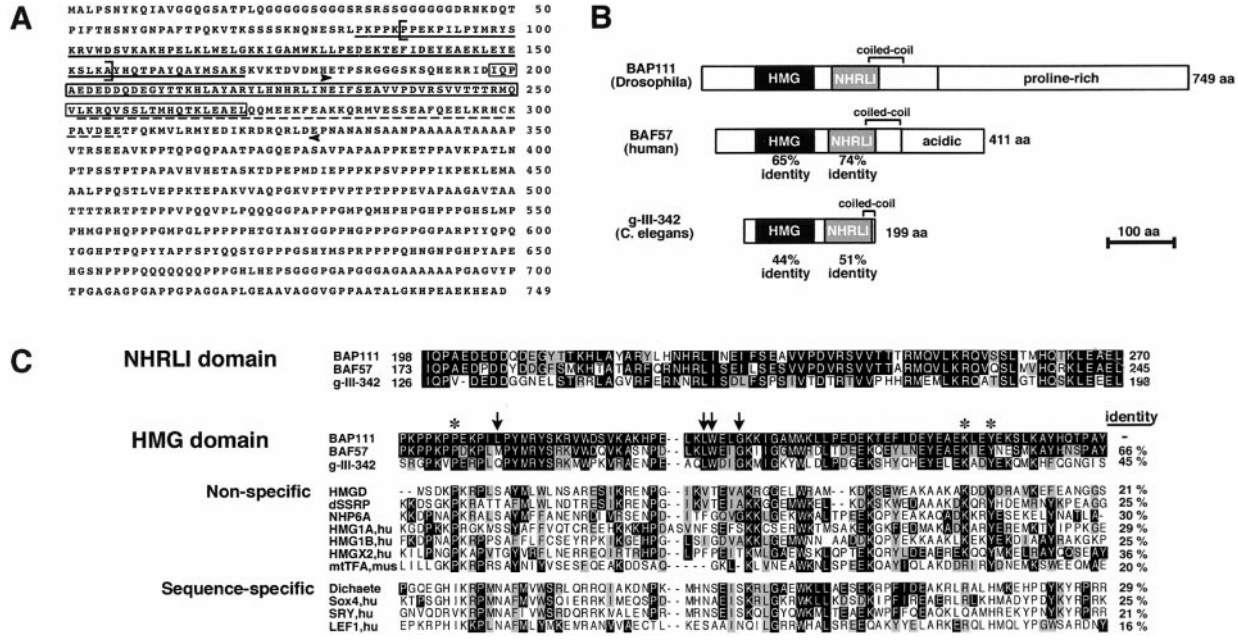

Fig. 1. Sequence and predicted domains of the BAP111 protein. (*A*) Amino acid sequence of the BAP111 protein. The HMG domain is indicated by a single underline, the NHRLI domain is boxed, and the coiled-coil region is indicated by a dashed line. Arrowheads mark the portion of BAP111 used to generate polyclonal antibodies. Brackets enclose the amino acids deleted in BAP111$^{\Delta HMG}$. (*B*) Domains of the BAP111 protein. Domains are drawn to scale. Percent amino acid identity to BAP111 is indicated below domains. The proline-rich region of BAP111 (amino acids 360–749) is 30.5% proline. BAF57 is a subunit of BRG1/hBRM complexes (27). g-III-342 is the translation of a *Caenorhabditis elegans* gene predicted by Genie (48–50) and annotated in Intronerator (43). This sequence is largely confirmed by the 5′ EST yk538e12.5. There is no 3′ EST currently available to confirm the C terminus. (*C*) Sequence alignment of conserved domains of BAP111. In the NHRLI domain alignment, black indicates identity and gray indicates conservation in two or more residues at a given position. Alignment of the HMG domains is similarly coded but homology is to BAP111. Percent identity to BAP111 is indicated at the right of each HMG-domain sequence. Residues cited in the text as marking distinctions between sequence-specific and nonspecific HMG domains are marked above the alignment. Asterisks specify residues conserved in nonspecific HMG domains; arrows specify residues conserved in sequence-specific HMG domains.

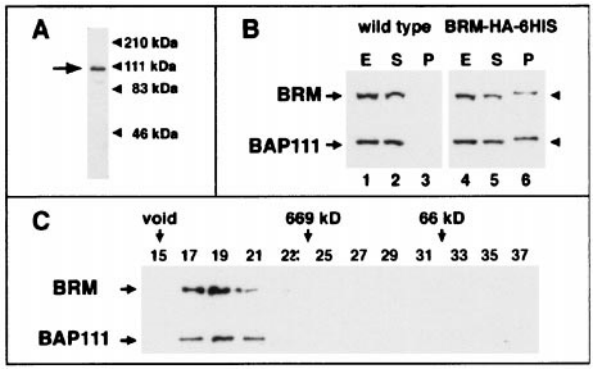

Fig. 2. BAP111 is associated with the BRM complex in embryo extracts. (*A*) Characterization of the BAP111 polyclonal antibody. Western blot of an SDS/8% polyacrylamide gel loaded with 30 μg of 0–12 h embryo extract and probed with affinity-purified anti-BAP111 antibodies. (*B*) BAP111 coimmunoprecipitates with BRM. Immunoprecipitations were performed by using anti-HA antibodies and either wild-type extract (lanes 1–3) or extract containing HA-tagged BRM (lanes 4–6). Western blotting was performed on 1/10 of the total starting extract (E) and supernatant (S) and 1/5 of the total pellet (P) by using anti-BRM and anti-BAP111 polyclonal antibodies. Arrowheads at right indicate molecular mass markers of 210 kDa and 111 kDa. Note that BAP111 is found only in the pellet when the BRM protein is immunoprecipitated. All proteins in pellet samples show slightly reduced migration relative to S and P samples because of differences in buffer conditions. (*C*) Western blot of fractions from a Superose 6 gel filtration column loaded with embryo extract and probed with anti-BRM and anti-BAP111 antibodies. Vertical arrows mark the void and elution volumes of native molecular mass markers. Note that all of the endogenous BAP111 coelutes with the 2-MDa BRM complex.

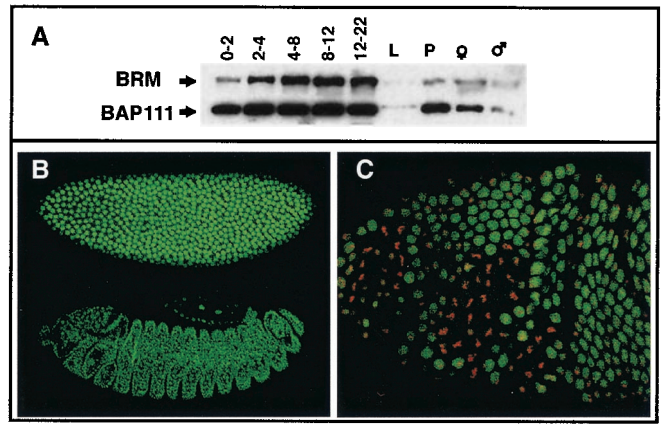

Fig. 3. Developmental expression of the BAP111 protein. (*A*) Ten micrograms of protein extracted from staged embryos (0–2, 2–4, 4–8, 8–12, and 12–22 h), larvae (L), pupae (P), and adult females (♀) or males (♂) was analyzed by Western blotting with anti-BRM and anti-BAP111 antibodies. Blots are overexposed to reveal the signal in larvae. (*B*) Affinity-purified anti-BAP111 antibodies detect ubiquitous nuclear staining of BAP111 in syncytial blastoderm embryos (*Upper*) and germ band retracted embryos (*Lower*). (*C*) BAP111 is not associated with mitotic chromosomes. Higher magnification (630×) view of a cephalic-furrow stage embryo stained with BAP111 antibodies (green) and propidium iodide to visualize DNA (red). BAP111 is nuclear except in domains of mitotic activity where BAP111 becomes dispersed throughout the cell.

primer pairs, and fused at the engineered *Bgl*II site: 5′-GCGAAGATCTTTGGTGGCTTGGGTAGC-3′ and T3 primer; 5′-CGCAGATCTACCACCAGACGCCCGCC-3′ and 5′-CTCGCTCCTCGTCACTG-3′. The resulting sequence replaced the 68 amino acids P88 through A155 of the BAP111 coding sequence with an isoleucine. All fragments generated by PCR were sequenced before use. Transgenic strains were generated by *P*-element-mediated transformation (33). *P[w$^+$, UAS-BAP111] 22–1* and *P[w$^+$, UAS-BAP111$^{\Delta HMG}$] 42–1* are strains bearing a second chromosome insertion of the indicated construct.

***Drosophila* Stocks and Genetic Crosses.** Flies were raised on cornmeal/molasses/yeast/agar medium containing Tegosept and propionic acid. *Df(1)18.1.15* is described in FlyBase (http://flybase.bio.indiana.edu). The *eyGAL4 UAS-brm^{K804R}* chromosome was generated by recombination between *w; P[w$^+$, eyGAL4]* (34) and *w; P[w$^+$, UAS$_{GAL}$hsp70:brm^{K804R}]2–2* (30). The presence of both transgenes was detected by roughened eyes. The *eyGAL4 UAS-ISWI^{K159R}* chromosome was generated by recombination between *w; P[w$^+$, eyGAL4]* (34) and *w; P[w$^+$, UAS$_{GAL}$hsp70:ISWI^{K159R}]11–4* (35). The presence of both transgenes was detected by roughened eyes. To detect modification of the rough-eye phenotype caused by the expression of *brm^{K804R}* in the eye, the following crosses were performed and female progeny were scored: (*i*) *Df(1)18.1.15/FM7c* virgins were mated to *w/Y*; *P[w$^+$, UAS-BAP111] 22–1/Pin^{88k}; ey-GAL4 UAS-brm^{K804R}/TM6B, Hu Tb* males, and (*ii*) *Df(1)18.1.15/FM7c* virgins were mated to *w/Y*; *P[w$^+$, UAS-BAP111$^{\Delta HMG}$] 42–1/Pin^{88k}; ey-GAL4 UAS-brm^{K804R}/TM6B, Hu Tb* males. Pharate adults were dissected and females scored for eye defects where indicated. The following control crosses were performed: (*i*) *Df(1)18.1.15/FM7c* virgins were mated to *w/Y; P[w$^+$, UAS-lacZ4–1-2]/Pin^{88k}; ey-GAL4 UAS-brm^{K804R}/TM6B, Hu Tb* males, and (*ii*) *Df(1)18.1.15/FM7c* virgins were mated to *w/Y; ey-GAL4 UAS-ISWI^{K159R}/TM3, Sb* males. Individual eyes of female progeny from the above crosses were scored for the severity of eye defects on a scale of 1–6 (see legend to Table 1).

Results and Discussion

BAP111 Is an HMG-Domain Protein Unique to Multicellular Eukaryotes. In another study, we purified BRM and associated proteins to near homogeneity from *Drosophila* embryos. BAP111 was one of seven prominent copurifying proteins designated BAPs (BRM-associated proteins; ref. 29). The two peptides derived from BAP111 perfectly matched translations of *Drosophila* expressed sequence tag (EST) sequences, including LD13023. *In situ* hybridization of cDNA probes to salivary gland polytene chromosomes mapped the BAP111 gene to cytological interval 8C10–12 of the X chromosome. Northern blotting with DNA fragments derived from the BAP111 EST clone LD13023 identified a 3-kb transcript in *Drosophila* embryos (data not shown). Sequencing of this clone and the overlapping EST clones (LD02725, LD15079, and LD03794) generated a 2,649-nt cDNA sequence that matched the predicted transcript (CT21811) of the *Drosophila* gene CG7055.

The *BAP111* RNA contains a single long ORF with an in-frame termination codon 21 nucleotides upstream of the initiating methionine. This ORF encodes a 749-aa polypeptide (Fig. 1*A*) with a predicted pI of 7.32 and a predicted molecular mass of 79 kDa. To facilitate characterization of the BAP111 protein, we raised antisera to a glutathione *S*-transferase (GST) fusion containing amino acids 179–328 of BAP111 (Fig. 1*A*). On Western blots of *Drosophila* embryo extracts, immune sera (but not preimmune sera; data not shown) detected a single polypeptide with a molecular mass of 111 kDa, identical to the mass of the BAP111 subunit of the BRM complex (Fig. 2*A*). The difference between the apparent and predicted molecular masses of BAP111 may be caused by protein modifications or unusual structural features, such as its high proline content (see below).

BLAST searches (36) revealed a strong homology between amino acids 83–170 of BAP111 and the HMG domains of a large number of proteins (Fig. 1*C*). HMG domains form a three-helix DNA-binding domain that binds the minor groove with relatively

low affinity but is capable of recognizing or inducing bends in DNA (reviewed in refs. 37–39). HMG domains fall into two groups: those that bind DNA nonspecifically and those that bind to specific sequences. HMG domains of the sequence-specific class are usually found in transcriptional regulators, including LEF-1, SRY, and the Sox family of proteins. These proteins contain a single HMG domain as well as a transcriptional activation or repression domain (37, 38, 40). By contrast, the non-sequence-specific HMG domains are highly abundant nonhistone chromosomal proteins considered to have a more architectural role; they shape DNA to facilitate the function of other factors. Such structural roles frequently require multiple HMG domains within a single polypeptide (37, 38).

BAP111 shows equal overall sequence similarity to both the sequence-specific and nonspecific classes of HMG domains (Fig. 1*C*). This degree of similarity is likely to reflect requirements for DNA binding and bending that are common to both sequence-specific and nonspecific HMG domains. Like the sequence-specific class, BAP111 contains a single HMG domain. However, the critical residues used by HMG domains to recognize specific DNA sequences are generally hydrophilic (37, 39), but in BAP111, the residues are hydrophobic (arrows in Fig. 1*C*). Furthermore, the BAP111 HMG domain shares three extremely conserved residues (Pro-89, Lys-146, and Tyr-149) with HMG domains of the nonspecific class (residues marked by * in Fig. 1*C*). Thus, based on its sequence, BAP111 is likely to bind distorted or bent DNA without sequence specificity.

Another member of the nonspecific class of HMG-domain proteins, SSRP1, has been implicated recently in chromatin-based regulation of transcription. SSRP1 is a subunit of the human histone chaperone complex FACT (facilitates chromatin transcription), which is required for efficient elongation on chromatin templates (41). Although this functional similarity is intriguing, BAP111 is no more related to SSRP1 than to other HMG-domain proteins. Furthermore, BAP111 does not show particular homology to any of the recognized subgroups of nonspecific HMG domains, as defined by Baxevanis and Landsman (42), indicating that it may define a new subgroup of nonspecific HMG domains.

A small number of HMG-domain proteins are strikingly related to BAP111 outside the HMG domain, suggesting that they may be functional homologs. These HMG-domain proteins include the human and mouse BAF57 proteins (27), the zebrafish protein identified by the EST fe48d03.y1, and the predicted *Caenorhabditis elegans* protein g-III-342 (43). Comparison of these proteins revealed a segment (amino acids 198–270) with an even greater degree of conservation than the HMG domain (Fig. 1 *B* and *C*). We have designated this segment the NHRLI domain based on a conserved block of amino acids within the heart of this domain (Fig. 1*C*). This domain is 74% identical between *Drosophila* BAP111 and human BAF57 over 73 aa. The last 19 residues of the NHRLI domain overlap a region previously predicted to form a coiled-coil structure in BAF57 (Fig. 1*B*; ref. 27). Computer predictions of the BAP111 structure using COILS (44) confirmed the presence of this putative coiled-coil region in the BAP111 protein. By using the PHDSEC program (45), the HMG domain and the coiled-coil region were predicted to be helical. There was no strong prediction of structure for the initial 54 aa of the NHRLI domain. The remainder of BAP111, including the proline-rich C terminus (30.5% proline over 390 aa), has no significant similarity to any known sequences. The evolutionary conservation of the HMG and NHRLI domains suggests that they are critical for the function of the BAP111 protein, with the rest of the molecule having either dispensable or species-specific functions. No potential homologs of BAP111 are present in *S. cerevisiae*, suggesting that this subfamily of HMG-domain proteins is unique to higher eukaryotes. Thus, it is possible that BAP111 is involved in an aspect of chromatin remodeling that is unique to metazoa.

BAP111 Is Quantitatively Associated with the BRM Complex. There are ≈100,000 copies of the BRM complex per cell, or roughly 1 molecule per 20 nucleosomes (30). Because BAP111 is a stoichiometric subunit of purified BRM complexes (29), it is at least equally abundant. To verify that BAP111 is a subunit of the BRM complex, as opposed to a copurifying contaminant, we examined the association between BAP111 and BRM by using a coimmunoprecipitation assay. Anti-HA antibodies immunoprecipitated both BRM and BAP111 from extracts prepared from *Drosophila* embryos expressing HA-tagged BRM protein (Fig. 2*B*, lane 6). By contrast, neither BAP111 nor BRM was immunoprecipitated from wild-type embryo extracts (Fig. 2*B*, lane 3). These data confirm that BAP111 is a *bona fide* subunit of the BRM complex.

Because many HMG-domain proteins interact with multiple partners to mediate cell-type-specific functions (40), we considered the possibility that BAP111 might also function independently of the BRM complex. To address this issue, we examined the native molecular mass of BAP111 in *Drosophila* embryo extracts by gel filtration chromatography. BAP111 and BRM precisely coeluted from a Superose 6 gel filtration column with apparent native molecular masses of ≈2 MDa (Fig. 2*C*). No monomeric BAP111 could be detected in these experiments, even after longer exposures (Fig. 2*C* and data not shown). These results suggest that all of the BAP111 is associated with the BRM complex, but do not exclude the possibility that BAP111 is also present in additional complexes of the same size. If all of the BAP111 in the cell is associated with BRM, the ratio of BRM to BAP111 should be identical in whole embryo extracts and purified BRM complex. To address this issue, we used quantitative Western blotting to compare the ratios of BRM to BAP111 in whole embryo extracts and immunoprecipitated BRM complex. The ratios of BRM to BAP111 were similar in both samples in multiple experiments (Fig. 2*B* and data not shown), indicating that most, if not all, of the BAP111 is associated with the BRM complex.

BAP111 Is a Ubiquitous Nuclear Protein. Vertebrate hBRM/BRG1 complexes contain a number of tissue-specific subunits (46, 47). To determine whether BAP111 might be a stage- or tissue-specific subunit of the BRM complex, we examined its expression throughout development. Western blotting of staged extracts revealed that the expression of BAP111 parallels that of BRM throughout embryonic, larval, pupal, and adult life (Fig. 3*A*). BAP111, like BRM, is a nuclear protein (Fig. 3 *B* and *C*). Closer examination of dividing cells in early embryos revealed that BAP111 (Fig. 3*C*) and BRM (data not shown) diffuse throughout the cell as the nuclei break down for mitosis and are not associated with the condensed metaphase chromosomes. We also failed to detect an association of BAP111 with larval salivary-gland polytene chromosomes (data not shown). BAP111, like BRM, is expressed ubiquitously throughout embryogenesis (Fig. 3*B*) and appears to be enriched during later stages of embryogenesis in rapidly dividing tissues such as the central nervous system (data not shown), as has been reported for BRM (30). Thus, BAP111 does not appear to be a stage- or tissue-specific subunit of the BRM complex.

***BAP111* Functionally Interacts with *brm in Vivo*.** To determine whether BAP111 is required for the function of the BRM complex *in vivo*, we examined whether reduction of *BAP111* function would modify the phenotypes that result from a partial loss of *brm* function. We previously demonstrated that the replacement of a conserved lysine by an arginine in the ATP-binding site of the BRM protein eliminates the activity of the BRM protein without disrupting its

Table 1. *BAP111* specifically interacts with *brm in vivo*

Progeny	Eye score 1	2	3	4	5	6
Expressing brm^{K804R}						
FM7 live	0	12	19	11	0	0
FM7 pharate	0	0	6	2	0	0
FM7 total	0	12	25	13	0	0
Df(1)18.1.15 live	0	3	1	5	2	3
Df(1)18.1.15 pharate	0	0	0	9	3	18
Df(1)18.1.15 total	0	3	1	14	5	21
Df(1)18.1.15, [w^+, UAS-BAP111] live	4	74	62	9	3	0
Df(1)18.1.15, [w^+, UAS-BAP111] pharate	0	2	2	0	0	0
Df(1)18.1.15, [w^+, UAS-BAP111] total	4	76	64	9	3	0
Expressing brm^{K804R}						
FM7 live	0	4	13	23	0	0
FM7 pharate	0	0	6	4	0	0
FM7 total	0	4	19	27	0	0
Df(1)18.1.15 live	0	0	0	5	5	4
Df(1)18.1.15 pharate	0	0	0	8	11	17
Df(1)18.1.15 total	0	0	0	13	16	21
Df(1)18.1.15, [w^+, UAS-BAP111$^{\Delta HMG}$] live	0	7	8	24	11	1
Df(1)18.1.15, [w^+, UAS-BAP111$^{\Delta HMG}$] pharate	0	0	3	3	0	1
Df(1)18.1.15, [w^+, UAS-BAP111$^{\Delta HMG}$] total	0	7	11	27	11	2
Expressing $ISWI^{K159R}$						
FM7 live	0	22	14	0	0	0
FM7 pharate	0	3	3	0	0	0
FM7 total	0	25	17	0	0	0
Df(1)18.1.15 live	13	19	20	0	0	0
Df(1)18.1.15 pharate	2	2	0	0	0	0
Df(1)18.1.15 total	15	21	20	0	0	0

Eyes of female progeny from crosses described in *Methods* were scored for defects. Numerical scores are defined as follows: 1 = wild-type eye, 2 = roughness comprising ≤50% of normal eye area, 3 = roughness comprising >50% of normal eye area, 4 = roughness of eye and eye slightly reduced in size, 5 = roughness of eye and eye size reduced by >50%, and 6 = eye absent (no recognizable omatidia). FM7 (the *FM7c* balancer chromosome bearing the dominant eye marker *Bar*) was scored as a 2. The difference in *FM7c* vs. *Df(1)18.1.15* data is significant for the flies expressing brm^{K804R} ($P < 0.001$), as is the rescue by the wild-type BAP111 transgene ($P < 0.001$ for *Df(1)18.1.15* vs. *Df(1)18.1.15, P[w+, UAS-BAP111]22-1*). No significant shift was caused by the *Df(1)18.1.15* in the flies expressing $ISWI^{K159R}$ ($P < 0.10$).

assembly into the BRM complex (30). This brm^{K804R} mutation behaves, therefore, as a strong dominant-negative allele. Expression of brm^{K804R} in a variety of tissues antagonizes the function of endogenous BRM protein (30). We found that expression of a GAL4-responsive brm^{K804R} transgene (*UAS-*brm^{K804R}) under control of the *eyeless* driver (*ey-GAL4*) results in adults with slightly smaller or rough eyes (Fig. 4*A*; Table 1). Furthermore, a small percentage of *ey-GAL4 UAS-*brm^{K804R} individuals die late in pupal development (Table 1, pharate), presumably because of leaky expression of GAL4 in non-eye tissues.

We reasoned that mutations in genes that functionally interact with *brm in vivo* should enhance or suppress the pupal lethality and eye phenotypes resulting from brm^{K804R} expression. Because no *BAP111* mutations had been identified, we tested a deficiency [*Df(1)18.1.15*] that spans *BAP111* and more than 50 other predicted genes in the cytological region 8C10;8E1–2. Females heterozygous for this deficiency were crossed to flies bearing insertions of both *ey-GAL4* and *UAS-*brm^{K804R} transgenes. Female progeny heterozygous for the *BAP111* deficiency are indistinguishable from wild type (Fig. 4, compare *B* and *C*). However, consistent with a strong interaction between *brm* and *BAP111*, the majority of the progeny heterozygous for the *BAP111* deficiency and *ey-GAL4 UAS-*brm^{K804R} failed to eclose (Table 1). When these individuals were dissected from the pupal case, they were found to have extremely rough, reduced, or missing eyes (Fig. 4*D* and Table 1). By contrast, siblings het-

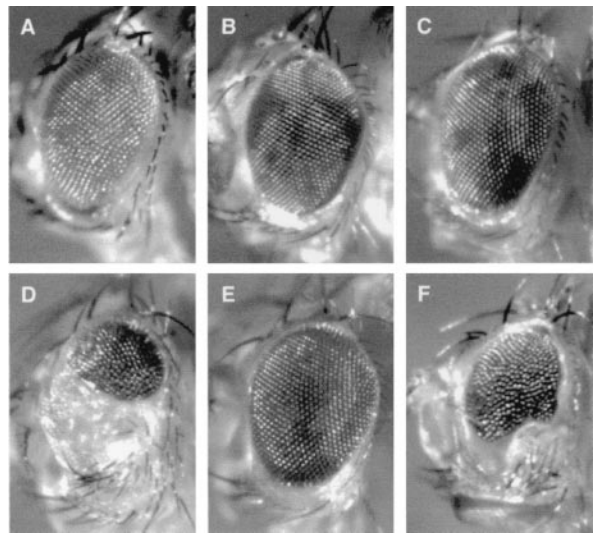

Fig. 4. Loss of *BAP111* enhances the phenotype caused by expression of brm^{K804R} in the developing eye. Representative eyes of the following genotypes are presented: (*A*) *ey-GAL4 UAS-*brm^{K804R}/+. (*B*) Wild type (Oregon-R). (*C*) *Df(1)18.1.15*/+, Pin^{88k}/+, *TM6B, Hu Tb*/+. (*D*) *Df(1)18.1.15*/+; *ey-GAL4 UAS-*brm^{K804R}/+. (*E*) *Df(1)18.1.15*/+; *P[w+, UAS-BAP111] 22–1*/+; *ey-GAL4 UAS-*brm^{K804R}/+. (*F*) *Df(1)18.1.15*/+, *P[w+, UAS-BAP111*$^{\Delta HMG}$*] 42–1*/+, *ey-GAL4 UAS-*brm^{K804R}/+.

erozygous for *ey-GAL4 UAS-brm^{K804R}* but wild-type for *BAP111* usually survived into adulthood and displayed much less severe eye defects (Table 1). Thus the BAP111 deficiency enhanced both *brm^{K804R}*–dependent lethality and eye defects.

If the strong genetic interaction between the *BAP111* deficiency and *brm^{K804R}* is caused by the loss of *BAP111*, as opposed to one of the other genes in the deficiency, the expression of wild-type *BAP111* should block the enhancement of *brm^{K804R}*-dependent phenotypes. We generated a GAL4-responsive transgene (*P[w^{+}, UAS-BAP111] 22–1*) to express the full-length BAP111 protein. This transgene blocked the ability of the *BAP111* deficiency to enhance *brm^{K804R}* phenotypes (Table 1 and Fig. 4*E*), whereas a control transgene expressing β-galactosidase had no effect (data not shown). These data confirm that loss of *BAP111*, and not some other gene within *Df(1)18.1.15*, is responsible for the enhancement of *brm^{K804R}*.

The enhancement of *brm^{K804R}* phenotypes by the *BAP111* deficiency could be caused by an independent effect on eye development or cell viability. To control for these possibilities, we tested the deficiency for the ability to interact with *Drosophila* Imitation SWI (*ISWI*) by using a similar assay. *ISWI* encodes an ATPase related to BRM, but is a subunit of chromatin-remodeling complexes that are distinct from the BRM complex. Expression of a mutant form of the ISWI protein (ISWI^{K159R}) that is unable to hydrolyze ATP in the developing eye causes a rough or reduced eye phenotype virtually indistinguishable from that caused by BRM^{K804R} (ref. 35, and data not shown). However, the *BAP111* deficiency had no effect on the survival or phenotype of these individuals (Table 1). Thus, the deficiency specifically enhances phenotypes caused by disruption of *brm* function. We conclude, therefore, that BAP111 is a component of the BRM complex required for its normal function *in vivo*.

The HMG Domain of BAP111 Is Important for the Function of the BRM Complex *in Vivo*. To investigate whether the HMG domain is essential for BAP111 function *in vivo*, we generated a strain bearing a GAL4-responsive transgene encoding a mutant protein that lacked 68 aa of the HMG domain (Fig. 1*A*). An *Actin5C-GAL4* driver was used to ubiquitously express epitope-tagged wild-type or BAP111$^{\Delta HMG}$ protein in *Drosophila* embryos. Quantitative Western blotting revealed that the BAP111$^{\Delta HMG}$ and wild-type BAP111 proteins are expressed at comparable levels (data not shown). Furthermore, when embryo extracts were fractionated by gel filtration chromatography, BAP111$^{\Delta HMG}$ precisely coeluted with BRM with an apparent molecular mass of ≈2 MDa (data not shown). Thus, the HMG domain is not required for either the stability of the BAP111 protein or its incorporation into the BRM complex.

We next examined the function of the HMG domain by using the genetic assay described above. Unlike wild-type BAP111, BAP111$^{\Delta HMG}$ did not fully rescue the eye defects caused by the expression of *brm^{K804R}* in *BAP111* hemizygotes (Fig. 4*F* and Table 1). The HMG domain is important, therefore, for BAP111 function *in vivo*. However, BAP111$^{\Delta HMG}$ was able to rescue the pupal lethality caused by expression of *brm^{K804R}* in *BAP111* hemizygotes, indicating that deletion of the HMG domain does not completely eliminate BAP111 function. Therefore, other conserved domains of BAP111, including the NHRLI domain, warrant further investigation.

How might BAP111 contribute to the function of the BRM complex? Because the HMG domain of BAP111 is likely to bind DNA, it is possible that it mediates interactions between the BRM complex and a subset of its target genes, a function similar to that proposed for the ARID protein OSA (25, 26). For example, BAP111 might recognize an unusual chromatin structure present at particular target loci. Alternatively, a promoter-specific transcription factor might recruit the BRM complex, but efficient chromatin remodeling might require stabilization of the recruitment by the nonspecific DNA-binding affinity of BAP111. A third possibility is that BAP111 is involved not in gene-specific recruitment of the BRM complex, but rather in the catalytic event itself. For example, BAP111 might bind to transiently distorted DNA to stabilize a chromatin-remodeling intermediate. Further studies will be necessary to uncover the roles of this somewhat unconventional HMG-domain protein.

We thank Matt Fish for technical assistance and D. Corona, G. Hartzog, and J. Sisson for critical reading of the manuscript. This work was supported by National Institutes of Health Grant GM49883 (to J.W.T.) and Grant 5 PO1 CA70404 (to M.P.S.). J.A.A. was supported by Grant DRG-1556 from the Cancer Research Fund of the Damon Runyon–Walter Winchell Foundation Fellowship.

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