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Two Subunits Specific to the PBAP Chromatin Remodeling Complex Have Distinct and Redundant Functions during *Drosophila* Development▽†

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Chromatin remodeling complexes control the availability of DNA binding sites to transcriptional regulators. Two distinct conserved forms of the SWI/SNF class of complexes are characterized by the presence of specific accessory subunits. In *Drosophila*, the core Brahma complex associates either with Osa to form the BAP complex or with Bap170 and Bap180 to form the PBAP complex. *osa* mutations reproduce only a subset of the developmental phenotypes caused by mutations in subunits of the core complex. To test whether the PBAP complex performs the remaining functions, we generated mutations in *bap170* and *bap180*. Surprisingly, we found that Bap180 is not essential for viability, although it is required in ovarian follicle cells for normal eggshell development. Bap170 is necessary to stabilize the Bap180 protein, but a mutant form that retains this function is sufficient for both survival and fertility. The two subunits act redundantly to allow metamorphosis; using gene expression profiling of *bap170 bap180* double mutants, we found that the PBAP complex regulates genes involved in tissue remodeling and immune system function. Finally, we generated mutants lacking Bap170, Bap180, and Osa in the germ line to demonstrate that the core Brahma complex can function in oogenesis without any of these accessory subunits.

Morphogenesis and differentiation require the integration of multiple developmental signals to produce different patterns of gene expression. The packaging of eukaryotic DNA into chromatin presents a challenge for the transcriptional regulatory proteins that establish these patterns (50). Chromatin remodeling complexes use the energy generated by ATP hydrolysis to alter histone-DNA contacts, controlling the availability of DNA binding sites to sequence-specific activators or repressors and to the general transcriptional machinery (10). This may occur by nucleosome sliding, distortion of DNA on the surface of the nucleosome, nucleosome removal, or histone exchange (56). Several classes of ATP-dependent chromatin remodeling complexes can be distinguished by the identity of their core ATPase subunits (56, 58). Complexes related to *Saccharomyces cerevisiae* SWI/SNF, which include the *Drosophila* Brahma (Brm) complex and the human BRG-1 and human BRM-containing complexes, have been implicated in numerous developmental functions (9, 20, 34). Chromatin remodeling in vitro requires only a minimal complex that contains the ATPase subunit SWI2/SNF2, STH1, BRG-1, or Brm; the SANT domain protein(s) SWI3, BAF170 and BAF155, or Moira; and SNF5, INI1, or Snr1 (54), suggesting that additional subunits may control target gene specificity. For instance, neuronal differentiation requires the incorporation of distinct isoforms of the BAF45 and BAF53 subunits (70), and BAF60 isoforms can mediate interaction of the complex with nuclear receptors (19).

Interestingly, SWI/SNF-related complexes in multiple species exist in two forms defined by the presence of distinctive accessory subunits that are not isoforms of the same protein. One class, consisting of SWI/SNF in yeast, BAF in mammals, and BAP in *Drosophila*, contains the AT-rich interaction domain (ARID) protein SWI1, BAF250, or Osa (17, 52, 67), while the other class, consisting of the RSC (for remodels the structure of chromatin) complex in yeast, PBAF in mammals, and PBAP in *Drosophila*, instead contains two characteristic subunit types, the bromodomain protein(s) RSC1, RSC2 and RSC4, BAF180, or Bap180/Polybromo and the ARID protein RSC9, BAF200, or Bap170 (44, 45, 72, 73). While the yeast SWI/SNF complex regulates the transcription of only about 5% of yeast genes and is not required for cell viability, yeast RSC is essential and more abundant (11, 26, 60). Both complexes are required for DNA repair, but they have distinct roles in this process (13). The human PBAF complex has been found to localize to kinetochores of mitotic chromosomes (72), suggesting that it may be involved in cell division. An in vitro study showed that both complexes had equivalent chromatin remodeling activity on promoters responsive to nuclear receptors, but only PBAF complexes could promote transcriptional activation by the vitamin D receptor, retinoid X receptor, peroxisome proliferator-activated receptor, Sp1, or SREBP (38).

The relative lack of activity of the BAF and SWI/SNF complexes in vitro and in yeast (26, 38) contrasts with the early lethality and dramatic developmental defects caused by mutations in the *Drosophila osa* gene or the mouse *baf250a* gene (23, 62, 65), demonstrating the importance of examining the functions of these complexes in vivo. Supporting a role for the PBAF complex in transcriptional activation by nuclear recep-

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tors, a mouse *baf180* knockout shows defects in the activation of retinoic acid receptor target genes during heart development (68). However, functional comparisons between the two classes of complexes in multicellular organisms have primarily been carried out using RNA interference in cell culture (47, 73). A microarray analysis done in *Drosophila* S2 cells revealed largely antagonistic functions for the BAP- and PBAP-specific subunits and suggested that the core complex is inactive in the absence of these subunits (47). In human HeLa cells, the BAF and PBAF complexes regulate different sets of interferon-induced target genes (73). It is not yet clear whether these results can be generalized beyond the specific cell types studied.

We have previously shown that *Drosophila* Osa is required for normal embryonic segmentation and photoreceptor differentiation and contributes to the repression of genes regulated by the Wnt protein Wingless (18, 29, 62). Osa also controls the expression of homeotic genes and genes regulated by the transcription factors Pannier and Apterous (25, 42, 65). However, unlike the core subunits Brm, Moira, and Snr1, Osa is not required for oogenesis and must therefore mediate only a subset of Brm complex functions (6, 7, 62, 76). To test whether the PBAP complex was responsible for the remaining functions, we generated mutations in the genes encoding its two specific subunits, Bap180 and Bap170. Surprisingly, we found that both *bap180* and *bap170* mutants were able to survive to adulthood although *bap180* was required in ovarian follicle cells for eggshell formation and female fertility, and *bap170* had a role in wing vein patterning. Double mutants died early in metamorphosis, revealing an unexpected redundancy between these two unrelated proteins. Microarray analysis of gene expression levels in these double mutants at puparium formation showed altered expression of genes involved in morphogenesis and the immune response but no correlation with genes regulated by the ecdysone receptor. Genes regulated by the PBAP complex were not affected in the same way by mutations in *osa*, supporting the hypothesis that Bap180/Bap170 and Osa direct the Brm complex to distinct regulatory regions. Unlike core Brm complex subunits, none of these three accessory subunits was required for germ line differentiation in oogenesis, suggesting that the core complex may be sufficient for this process.

MATERIALS AND METHODS

Fly stocks and transgenic lines. Stocks used were osa^{4H}, osa^{308}, brm^{2}, *da*-GAL4, *ap*-GAL4, *T155*-GAL4 (Flybase), *drosomycin*-green fluorescent protein (GFP) (21), and *7XEcRE-lacZ* (61). Clones in imaginal discs were generated by crossing FRT82, $bap180^{\Delta 86}$ males to *hs*FLP122; FRT82, *Ubi*-GFP/TM6B females and heat shocking the progeny for 1 h at 38.5°C at 2 and 3 days after egg laying (AEL). brm^{2} germ line clones were generated by heat shocking *hs*FLP122/+; FRT2A, brm^{2}/FRT2A, ovo^{D} larvae at 2 and 3 days AEL. Clones lacking both *bap170* and *bap180* in the germ line were generated by crossing *hs*FLP122/Y; $bap170^{\Delta 65}$/*Sp*; FRT82, ovo^{D}/TM6B males with $bap170^{\Delta 65}$; FRT82, $bap180^{\Delta 86}$/SM6-TM6B females. Clones lacking *osa*, *bap170*, and *bap180* in the germ line were generated by crossing the same males with $bap170^{\Delta 65}$; FRT82, osa^{308}, $bap180^{\Delta 86}$/SM6-TM6B females. In both cases, progeny were heat shocked at 5 to 6 days AEL, and the resulting *hs*FLP122/+; $bap170^{\Delta 65}$; FRT82, osa^{308} (some experiments), $bap180^{\Delta 86}$/FRT82, ovo^{D} females were crossed to $bap170^{\Delta 65}$; FRT82, osa^{308} (some experiments), $bap180^{\Delta 86}$/SM6-TM6B males. Full-length *bap170* (RE33012) and *bap180* (LD41562) cDNAs obtained from the *Drosophila* Genomics Resource Center were cloned into the pUAST vector, and transgenic flies were generated by standard methods. Nucleotides 481 to 1020 in *bap170* were amplified with primers that introduced XbaI sites, and the product was cloned into the pWIZ vector (37) twice in a tail-to-tail orientation to generate UAS-*bap170RNAi*.

Generation and characterization of mutations. The EY10238 and EY14080 insertions were obtained from the Gene Disruption Project of the laboratories of H. Bellen, R. Hoskins, and A. Spradling. These P elements were mobilized by crossing them to the transposase stock Δ*2-3*, *CyO*; *TM3*/*T*(*2;3*)ap^{Xa}. Ninety independent excision lines were generated for *bap180*, and 150 independent lines were generated for *bap170*. The alleles described here were identified by PCR using primers flanking the deleted regions, and the breakpoints were determined by sequence analysis of the PCR products. $bap170^{\Delta 135}$ retains a 1.6-kb fragment of the P element.

Antibody production, immunohistochemistry, and Western blotting. A glutathione *S*-transferase fusion protein containing amino acids 1157 to 1527 of Bap180 was expressed in *Escherichia coli* and purified using glutathione-agarose (Pharmacia). Purified protein was used to immunize guinea pigs (Covance). A peptide corresponding to amino acids 211 to 227 of Bap170 was synthesized and used to immunize rabbits (Biosource). Eye and wing discs were stained as described previously (36). Antibodies used were rat anti-Elav (1:100), mouse anti-Wg (1:10; Developmental Studies Hybridoma Bank), guinea pig anti-Bap180 (1:500), rabbit anti-Bap170 (1:500), rat anti-Ci (1:1) (48), and rabbit anti-GFP (1:1,000; Molecular Probes).

Total protein was extracted from adult flies or dechorionated embryos by homogenization in extraction buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 mM NaF, 1 mM Na_3VO_4, 1 mM EDTA, 1% NP-40, 0.5% Na deoxycholate). The same buffer with 4% NP-40 was used to extract protein from larvae. Polyacrylamide gel electrophoresis and Western blotting were performed as described previously (43). Antibodies used were guinea pig anti-Bap180 (1:5,000), rabbit anti-Bap170 (1:5,000), mouse anti-beta-tubulin (1:5,000; Covance MMS410P), and mouse anti-Armadillo (1:20; Developmental Studies Hybridoma Bank).

Analysis of follicle cells, vitelline membrane, embryonic cuticle, and crystal cells. Analysis of bromodeoxyuridine (BrdU) incorporation in follicle cells was performed as described previously (4). The neutral red uptake assay was done as described previously (39). Embryos were fixed in glycerol-acetic acid (4:1) and cleared in Hoyer's-lactic acid (2:1) overnight at 65°C. To detect crystal cells, larvae were heated at 65°C for 10 min.

Microarray analysis of gene expression. Total RNA from single white prepupae (0 h after puparium formation [APF]) was isolated using TRIzol reagent (Invitrogen). Each sample was homogenized in 200 μl of TRIzol and spun briefly, and 50 μg of glycogen was added. After 5 min at room temperature the samples were mixed vigorously with 40 μl of chloroform and spun for 5 min. RNA was precipitated overnight with 100 μl of isopropanol, washed with 70% ethanol, and resuspended in water. Four replicas (two females and two males) from wild-type (w^{1118}) and $bap170^{\Delta 65}$ $bap180^{\Delta 86}$ double mutant white prepupae were performed. The RNA quality and quantity were assessed using an Agilent 2100 Bioanalyzer and Nanodrop ND-1000. One microgram of total RNA was converted to cRNA following the Affymetrix one-cycle protocol and hybridized to Affymetrix *Drosophila* Genome 2.0 arrays according to the manufacturer's recommendations for hybridization, fluidics processing, and scanning. The raw data were processed in GeneSpring, version 7.2 (Agilent). First, the Affymetrix CEL files were normalized using a robust multiarray average expression measure. Next, to identify the differentially abundant mRNAs between the two genotype groups, the preprocessed data were statistically filtered by a *t* test ($P < 0.05$, alpha correction) or by significance analysis of microarray at a false discovery rate set to 5% (see Table S1 in the supplemental material) (64). Functional annotations of the resulting gene lists were performed using Gene Ontology and information available on Flybase (http://flybase.bio.indiana.edu/).

RT-PCR. To measure *bap170*, *bap180*, and *Trap1* mRNA levels, total RNA was extracted from pools of six late-third-instar larvae as above. To validate microarray targets in $bap170^{\Delta 65}$ $bap180^{\Delta 86}$ double mutants and test expression levels of these genes in osa^{308}/osa^{4H} mutants, RNA was extracted from pools of six white prepupae of the corresponding genotypes. Reverse transcription (RT) reactions were performed using Superscript II (Invitrogen) following the manufacturer's protocol. Quantitative PCR analysis of the resulting cDNA was performed using Power Sybr green and a real-time PCR ABI 7900HT sequence detection systems machine (Applied Biosystems). The relative abundances of transcripts were calculated using the comparative threshold cycle method based on the $2^{-\Delta\Delta CT}$ formula (40) with genes *ostStt-3* and *rpl39* used as normalization controls for array validation and *rpl39* and *rpl32* used as controls for RNA quantifications shown in Fig. 3 and 4. Primer sequences are available on request.

Microarray data accession number. Primary data from this study has been deposited in the NCBI Gene Expression Omnibus database under accession number GSE 11825.

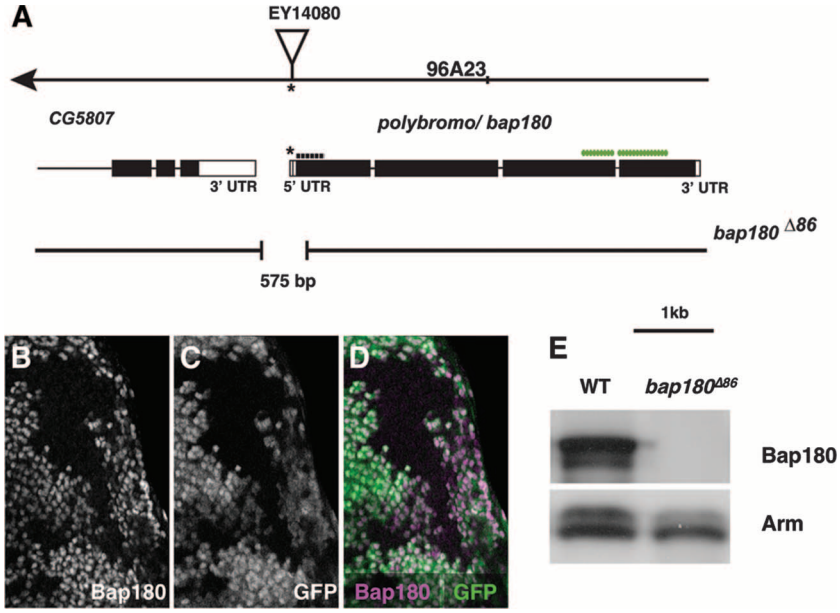

FIG. 1. Generating a mutation in *bap180*. (A) Diagram of the *bap180* genomic region, the P element EY14080 used to generate imprecise excisions, and the extent of the deletion in $bap180^{\Delta 86}$. An asterisk indicates the P element insertion site in the 5′ UTR, dashed lines indicate the region amplified by quantitative RT-PCR, and green plus signs indicate the region used to make a fusion protein for antibody generation. (B to D) Third-instar eye imaginal discs containing $bap180^{\Delta 86}$ homozygous clones marked by the absence of GFP (C; green in D) and stained with Bap180 antibody (B; magenta in D). (E) Western blot of extracts from wild-type (WT) and $bap180^{\Delta 86}$ homozygous adults, blotted with anti-Bap180 and the loading control anti-Armadillo (Arm) as indicated. No Bap180 protein can be detected in $bap180^{\Delta 86}$ mutant cells or individuals.

RESULTS

***bap180* is required for eggshell formation but not for viability.** In order to study the functions of the PBAP complex in vivo, we generated mutations in the genes encoding its distinctive subunits. We mobilized a P element inserted in the 5′ untranslated region (UTR) of *bap180*, EY14080, to produce deletions by imprecise excision. We isolated a 575-bp deletion, $bap180^{\Delta 86}$, that removes the *bap180* 5′ UTR and extends 120 bp into the coding sequence (Fig. 1A). Using an antiserum that we raised against a region near the C terminus of Bap180, we detected no protein in clones of cells homozygous for $bap180^{\Delta 86}$ in eye imaginal discs (Fig. 1B to D) or in extracts from homozygous mutant adults (Fig. 1E), indicating that this deletion is a protein null.

Homozygous $bap180^{\Delta 86}$ mutant flies could survive to the adult stage with no obvious morphological defects. However, $bap180^{\Delta 86}$ females were sterile. The eggs they laid failed to hatch (hatching rate of 0.3%; $n = 1{,}467$) and had eggshell defects. The eggshell is a multilayered extracellular matrix consisting of an inner vitelline membrane, a wax layer, and an outer chorion (69). Eggs laid by $bap180^{\Delta 86}$ mutant females had very thin and irregularly deposited chorions (Fig. 2B). In addition, dechorionated embryos were permeable to the dye neutral red, indicating defects in the barrier function of the vitelline membrane (Fig. 2E) (39).

Both the chorion and vitelline membrane are made up of proteins secreted during oogenesis by the somatic follicle cells surrounding the oocyte. To determine whether *bap180* function was required in these cells, we expressed an upstream activation sequence (UAS)-*bap180* transgene specifically in the follicle cells of $bap180^{\Delta 86}$ mutants using the driver *T155*-GAL4 (55). This expression rescued the chorion and vitelline membrane defects (Fig. 2C and F) and allowed 23.5% of the embryos to hatch ($n = 1{,}211$). A similar percentage of embryos (24.3%; $n = 1{,}027$) hatched from eggs produced by mothers containing $bap180^{\Delta 86}$ germ line clones, confirming that *bap180*

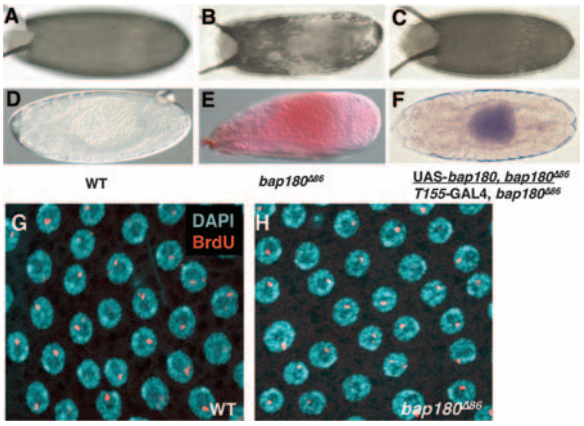

FIG. 2. *bap180* is required for eggshell development. Eggs laid by wild-type (WT) mothers (A and D), $bap180^{\Delta 86}$ mutant mothers (B and E), or $bap180^{\Delta 86}$ mutant mothers rescued by UAS-*bap180* expression with *T155*-GAL4 (C and F). (A to C) External views of the chorion. (D to F) Dechorionated embryos following incubation with neutral red. *bap180* mutant mothers lay eggs with irregular and thin chorions and vitelline membranes that are permeable to neutral red; both defects are rescued by expressing *bap180* in the follicle cells. (G and H) Stage 12 egg chambers after BrdU labeling (red). Nuclei are stained with DAPI (4′,6′-diamidino-2-phenylindole; blue). Incorporation of BrdU at sites of chorion gene amplification appears normal in *bap180* mutants.

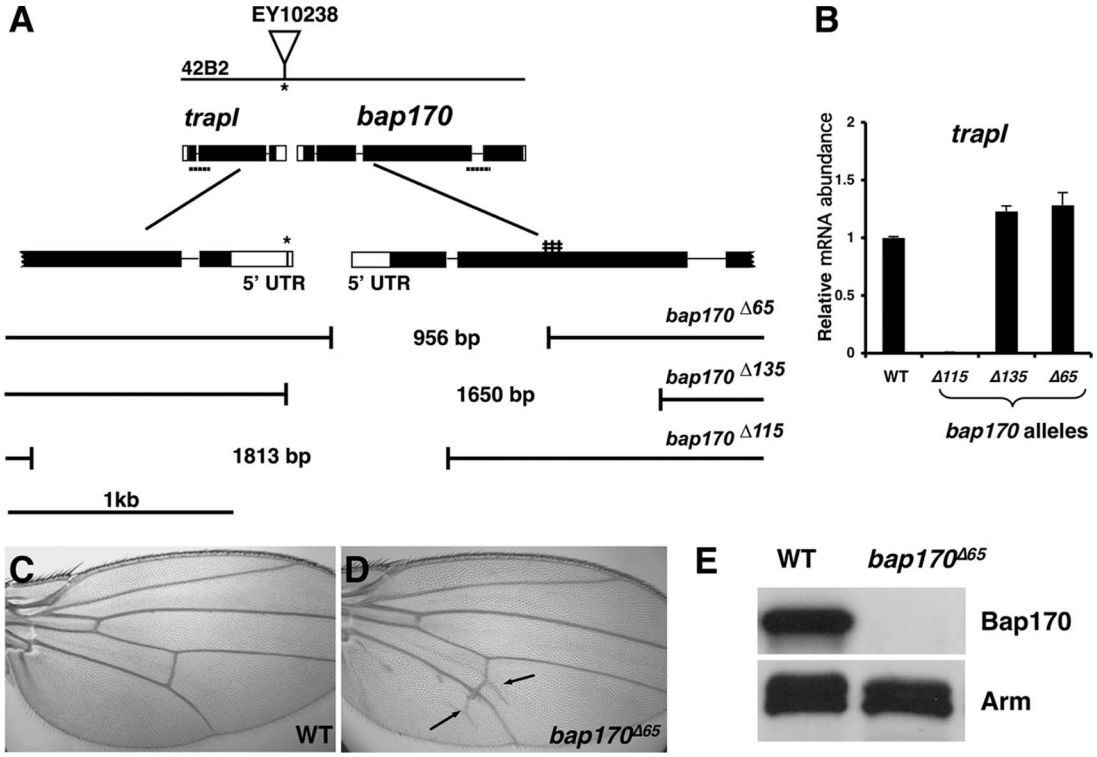

FIG. 3. Generating a mutation in *bap170*. (A) Diagram of the *bap170* genomic region, the P element EY10238 used to generate imprecise excisions, and the extent of the deletions in $bap170^{\Delta 65}$, $bap170^{\Delta 135}$, and $bap170^{\Delta 115}$. An asterisk indicates the P element insertion site in the 5′ UTR of *trap1*, and plus signs indicate the peptide used as an immunogen for antibody generation. Dashed lines on the upper diagram indicate the regions amplified by quantitative RT-PCR. (B) Quantification of *trap1* mRNA by quantitative RT-PCR in larvae that are either wild type or homozygous for each of the indicated *bap170* alleles. The $bap170^{\Delta 65}$ and $bap170^{\Delta 135}$ deletions do not affect *trap1* expression. (C and D) Adult wings. Loss of *bap170* results in extra wing vein material (arrows) in the $bap170^{\Delta 65}$ mutant. (E) Western blot of extracts from embryos laid by wild-type or $bap170^{\Delta 65}$ mutant parents, blotted with anti-Bap170 and the loading control anti-Arm as indicated. This antibody detects no Bap170 protein in $bap170^{\Delta 65}$ mutants. WT, wild type.

function in somatic cells is sufficient for hatching but indicating an additional role for *bap180* in the germ line.

Genes encoding many chorion and vitelline membrane components undergo genomic amplification during follicle cell development; this process is essential to allow levels of gene expression adequate for fertility (16). Sites of gene amplification can be visualized as foci of BrdU incorporation within follicle cell nuclei (Fig. 2G) (12). These foci remained visible in $bap180^{\Delta 86}$ mutant follicle cells (Fig. 2H). *bap180* is thus not required for the amplification of chorion and vitelline membrane genes, and its phenotype may instead be due to an effect on their transcription. Embryos derived from germ line clones that lacked both the maternal and zygotic contribution of *bap180* died at a range of developmental stages, but some were able to develop into normal adults. Unlike core subunits of the Brm complex, *bap180* is thus not essential for either viability or germ line differentiation.

***bap170* is not essential for viability.** To generate mutations in *bap170*, we made imprecise excisions of the EY10238 P element, which is located in the 5′ UTR of the neighboring gene, *trap1* (Fig. 3A). In addition to two deletions that remove the intergenic sequence and extend into both the *trap1* and *bap170* genes, we isolated a 956-bp deletion that extends from upstream of the *bap170* transcriptional start site into the second exon and removes the first 213 amino acids, including the entire ARID. This deletion, $bap170^{\Delta 65}$, leaves *trap1* and the intergenic region intact and presumably resulted from a transposition into the 5′ UTR of *bap170*, followed by an imprecise excision (Fig. 3A). Expression of *trap1* is not affected, as shown by quantitative RT-PCR (Fig. 3B). Using an anti-Bap170 antibody that we produced against a peptide representing amino acids 211 to 227, we detected no protein in extracts from embryos homozygous for $bap170^{\Delta 65}$ (Fig. 3E). This allele reduced adult viability to a similar extent (30 to 33% at 25°C) when homozygous or heterozygous with a large deficiency for the region, but adult survivors could be obtained in both cases and showed no morphological defects except for variable ectopic wing vein material (Fig. 3D). These adults were fertile, and their progeny, which lacked maternal and zygotic *bap170*, could again develop into fertile adults. Thus, neither *bap180* nor *bap170* is essential for adult survival.

Bap170 is required to stabilize Bap180 protein in vivo. Two larger deletions that extend into the *trap1* transcription unit, $bap170^{\Delta 135}$ and $bap170^{\Delta 115}$ (Fig. 3A), caused pupal lethality when homozygous. This is unlikely to be due to the loss of *trap1*, because other deletions we generated that removed only *trap1* were homozygous viable and because the lethality of $bap170^{\Delta 135}$ and $bap170^{\Delta 115}$ could be rescued by ubiquitous expression of UAS-*bap170* with a *tubulin*-GAL4 driver. Another possibility is that the lethality is a synthetic phenotype

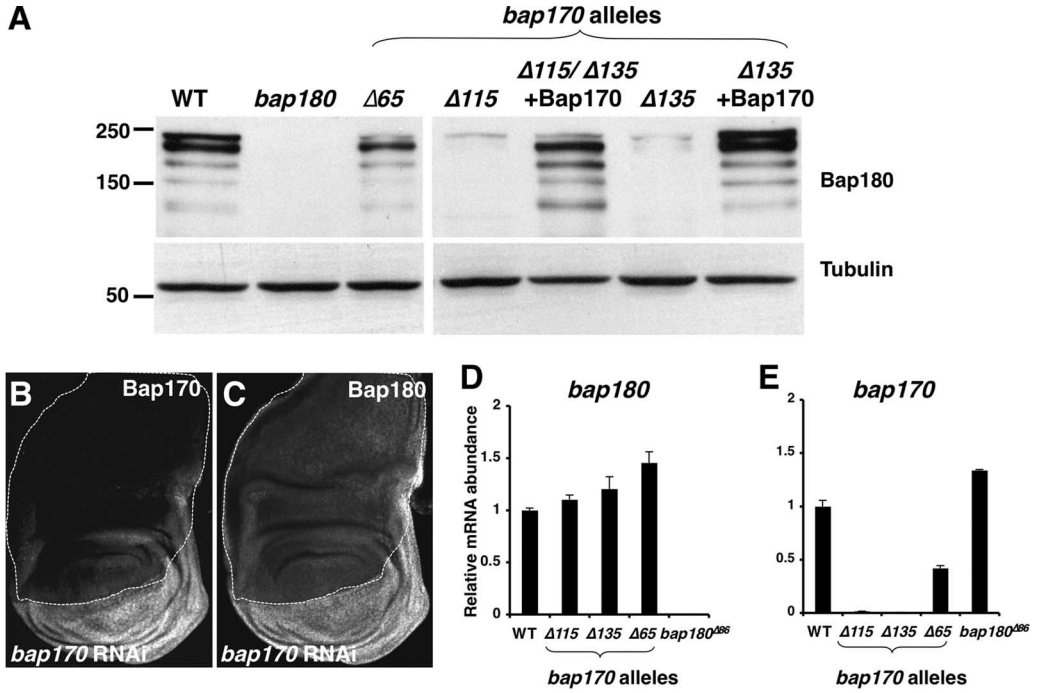

FIG. 4. Bap170 stabilizes Bap180 protein in vivo. (A) Western blotting with anti-Bap180 and the loading control antitubulin of extracts from larvae with the indicated genotypes. (+Bap170: *tub*-GAL4 UAS-*bap170*). Bap180 protein is almost completely absent from $bap170^{\Delta 115}$ and $bap170^{\Delta 135}$ mutants but only reduced in $bap170^{\Delta 65}$ mutants. Bap180 levels are restored to normal by expression of *bap170* from a transgene, indicating that the loss is specifically due to the absence of Bap170. (B and C) Wing discs from flies expressing UAS-*bap170RNAi* in the dorsal compartment (outlined) with *ap*-GAL4. Panel B is stained with anti-Bap170 and panel C is stained with anti-Bap180. Both proteins show strongly reduced levels in the dorsal compartment. (D and E) Quantification of *bap180* mRNA and *bap170* mRNA by quantitative RT-PCR in wild-type, *bap170*, and *bap180* mutant larvae as indicated. *bap180* mRNA is unaffected in *bap170* mutants, suggesting that the effect is on protein stability. Using primers to the 3′ end of the gene, some *bap170* mRNA is detectable in $bap170^{\Delta 65}$ mutants (E). WT, wild type.

due to loss of both *bap170* and *trap1*. However, this is unlikely because $bap170^{\Delta 135}$, which removes the *trap1* 5′ UTR but leaves the coding sequence intact, did not reduce *trap1* mRNA levels (Fig. 3A and B).

The stability of subunits in a multiprotein complex may depend on the presence of other subunits in the same complex. For example, the core subunit Moira/SRG3 is required for the stability of several other subunits of the Brm/BRG1 complex (47, 59). We found that Bap180 protein levels were strongly reduced in larvae homozygous for $bap170^{\Delta 115}$ or $bap170^{\Delta 135}$ (Fig. 4A). Normal Bap180 levels were restored by ubiquitous expression of a *bap170* transgene in these mutants (Fig. 4A), indicating that loss of Bap180 was specifically due to the absence of *bap170*. This effect on Bap180 protein was not transcriptional since quantitative RT-PCR showed that *bap180* mRNA was not decreased in $bap170^{\Delta 115}$ or $bap170^{\Delta 135}$ mutants (Fig. 4D). In addition, we generated a transgene expressing a *bap170* hairpin to reduce Bap170 protein levels in vivo by RNA interference (32). Expression of this transgene in the dorsal compartment of the wing disc using *apterous*-GAL4 reduced the levels of both Bap170 and Bap180 proteins (Fig. 4B and C), confirming a requirement for Bap170 to stabilize Bap180. A similar reduction of Bap180 protein has been reported when Bap170 is knocked down by RNA interference in S2 cells (47), and BAF200 is likewise required for the stability of BAF180 in vertebrate cells (73).

To investigate the reason for the phenotypic differences between our *bap170* mutant alleles, we analyzed *bap170* mRNA expression levels by quantitative RT-PCR using primers spanning the last intron (Fig. 4E). While $bap170^{\Delta 115}$ and $bap170^{\Delta 135}$ larvae showed no detectable *bap170* transcription, $bap170^{\Delta 65}$ larvae produced approximately half the normal quantity of *bap170* transcripts in this 3′ region of the gene. These mutants showed a partial reduction in Bap180 protein (Fig. 4A), suggesting that they may express a truncated Bap170 protein lacking the ARID domain that retains some ability to stabilize Bap180. To test whether the viability of $bap170^{\Delta 65}$ mutants was due to the presence of Bap180, we generated $bap170^{\Delta 65}$ $bap180^{\Delta 86}$ double mutants. These showed fully penetrant pupal lethality that could be rescued by ubiquitous expression of either UAS-*bap170* or UAS-*bap180*, confirming that *bap170* and *bap180* are redundantly required for viability. The increased severity of the $bap170^{\Delta 65}$ $bap180^{\Delta 86}$ phenotype compared to $bap180^{\Delta 86}$ single mutants indicates that the truncated Bap170 protein produced from the $bap170^{\Delta 65}$ allele must be defective for Bap170 functions other than stabilizing Bap180. This allele thus allows a specific assessment of the function of Bap170, while previous RNA interference (RNAi) studies have depleted both proteins simultaneously. Since neither Bap180 nor Bap170 is essential for the stability or assembly of the core Brm complex (47), it is likely that the proteins have redundant functions in recruiting the PBAP complex to specific target genes.

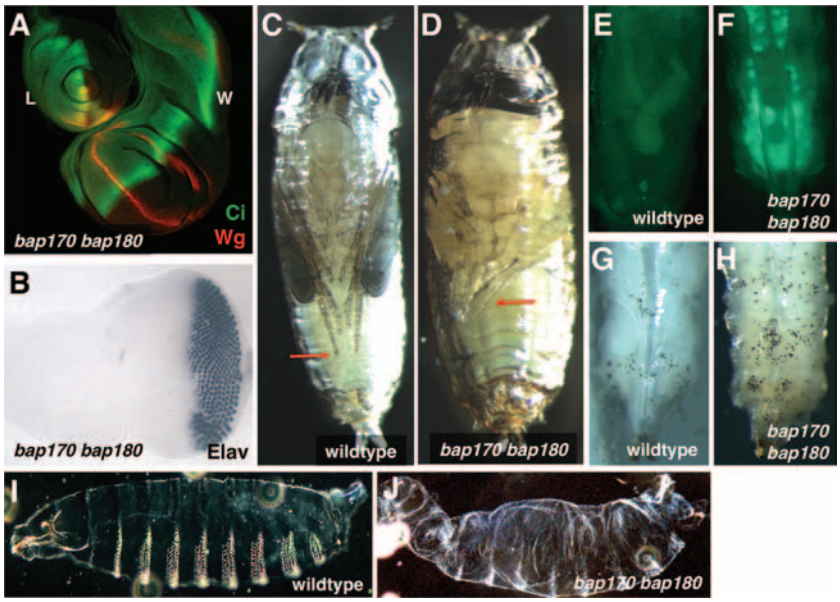

FIG. 5. Phenotype of *bap170 bap180* double mutants. (A and B) Imaginal discs from $bap170^{\Delta 65}$ $bap180^{\Delta 86}$ mutant larvae. In panel A wing (W) and leg (L) discs are stained with anti-Wg (red) and anti-Cubitus interruptus (green); in panel B an eye disc is stained with anti-Elav. All markers are expressed normally, indicating no apparent defects in anterior-posterior or dorsal-ventral patterning of the wing or leg discs or in photoreceptor differentiation in the eye disc. (C and D) Pupae at 88 h APF in wild type and the $bap170^{\Delta 65}$ $bap180^{\Delta 86}$ mutant. The legs have not everted completely (arrows). (E to H) Posterior region of wild-type and $bap170^{\Delta 65}$ $bap180^{\Delta 86}$ mutant third-instar larvae. Panels E and F show expression of the *drosomycin*-GFP reporter in uninfected larvae, and panels G and H show crystal cells identified by melanization after heating to 65°C. Both reporter expression and crystal cell number are increased in the double mutant larvae. (I and J) Cuticle preparations of a wild-type embryo and an embryo laid by a $bap170^{\Delta 65}$ mother with a $bap180^{\Delta 86}$ germ line clone. The ventral denticle belts are reduced, especially in the anterior region (left), suggestive of dorsalization.

The PBAP complex regulates genes required for metamorphosis and the immune response. To reveal the role of the PBAP complex in vivo, we further examined the $bap170^{\Delta 65}$ $bap180^{\Delta 86}$ double mutant phenotype. These mutants showed normal imaginal disc patterning in the third larval instar (Fig. 5A and B), but abnormalities appeared at the pupal stage, beginning at approximately 12 h APF. The mutants failed to fully evert their legs and sometimes exhibited melanotic patches (Fig. 5D). To uncover the genetic basis for these defects, we carried out a microarray analysis comparing these double mutants to wild-type at the white prepupal stage (0 h APF), prior to the appearance of any morphological defects. RNA from two individual males and two individual females of each genotype was hybridized to the Affymetrix *Drosophila* Genome 2.0 Array, and the resulting data were subjected to normalization and statistical analysis as described in Materials and Methods. We identified 261 genes that were significantly downregulated in $bap170^{\Delta 65}$ $bap180^{\Delta 86}$ double mutants compared to wild type and 264 genes that were significantly upregulated (see Table S1 in the supplemental material). We validated the results for 12 of these genes by quantitative RT-PCR on pools of wild-type and mutant white prepupae and observed similar effects on expression levels (data not shown). Many of the genes with altered expression in mutant prepupae encode enzymes likely to be directly involved in tissue remodeling during metamorphosis, while few encode transcription factors (Fig. 6A), suggesting that the PBAP complex acts near the bottom of the transcriptional hierarchy controlling metamorphosis.

In addition to genes involved in metabolism, transport, proteolysis, and chitin synthesis, we noted effects of the PBAP complex on genes implicated in morphogenesis and signal transduction (Table 1). Components of the Notch signaling pathway were particularly prominent among the downregulated genes (Table 1), suggesting that the PBAP complex may regulate cellular sensitivity to Notch signaling. Another interesting category of genes included those involved in the immune response, which were primarily upregulated (Table 1). To further test the role of the PBAP complex in suppressing inappropriate immune responses, we examined the expression of a GFP reporter driven by the regulatory sequences of drosomycin, an antifungal peptide induced by the Toll pathway in response to infection in the fat body (21). $bap170^{\Delta 65}$ $bap180^{\Delta 86}$ double mutant third-instar larvae expressed this reporter in the fat body in the absence of infection (55%; $n = 49$) while larvae heterozygous for $bap170^{\Delta 65}$ did not (2.3%; $n = 129$) (Fig. 5E and F). These double mutant larvae also showed an overproduction of crystal cells (Fig. 5H), which produce prophenoloxidases required for melanization during the immune response, and a corresponding increase in melanin production (data not shown). One function of the PBAP complex may thus be to downregulate the immune response.

Metamorphosis is initiated by ecdysone signaling through the nuclear ecdysone receptor (EcR); a role for the PBAP complex in EcR-mediated transcription would be consistent with the effect of the core subunits Brm and Snr1 on ecdysone-inducible gene expression (77). However, when we compared our data (using identical statistical approaches as described in

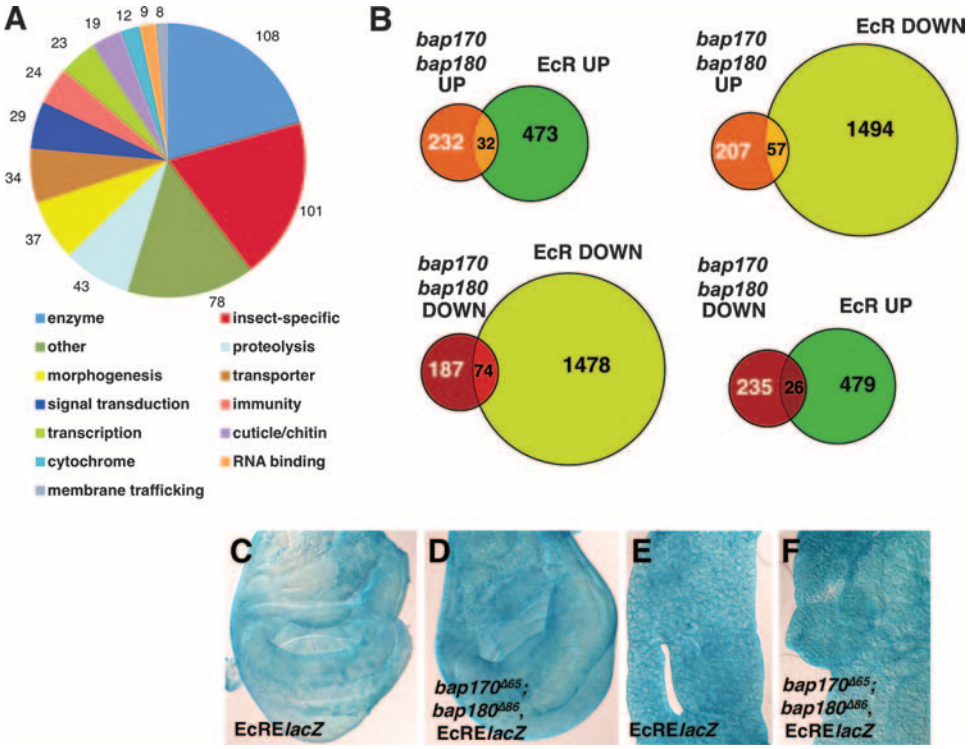

FIG. 6. The PBAP complex regulates genes required for metamorphosis independently of EcR. (A) Pie chart indicates the functional classes of genes with altered expression levels in *bap170*$^{\Delta 65}$ *bap180*$^{\Delta 86}$ mutant white prepupae compared to wild type. (B) Venn diagrams indicate the overlap between genes upregulated or downregulated in *bap170*$^{\Delta 65}$ *bap180*$^{\Delta 86}$ white prepupae and in white prepupae expressing an RNAi construct directed against the EcR (5). The extent of overlap is not significant and is not restricted to one direction of change. (C to F) Expression of an EcRE-*lacZ* reporter detected by 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside staining in wild-type (C and E) and *bap170*$^{\Delta 65}$ *bap180*$^{\Delta 86}$ (D and F) white prepupal wing discs (C and D) and fat body (E and F). No significant differences are observed.

Materials and Methods) to the results of a microarray study that examined the effects of EcR knockdown by RNAi at the same developmental stage (5), we found that genes that required EcR for their expression were almost equally likely to be upregulated as downregulated in *bap170 bap180* double mutants and that a large number of EcR-regulated genes were independent of *bap180* and *bap170* (Fig. 6B). In particular, expression of *Eip74EF* and *Eip75B*, two primary ecdysone target genes with critical roles in metamorphosis (1), was not significantly altered. We also examined expression of the Broad-Complex proteins, early EcR targets (31), in mutant third-instar larvae and saw no significant difference from wild type (data not shown). Finally, expression of a reporter reflecting the activity of EcR and other nuclear receptors (7XEcRE-lacZ) (61) was unaffected in the *bap170 bap180* mutants (Fig. 6C to F). A much closer correlation has been seen between genes regulated by EcR and by the NURF chromatin remodeling complex, which contains a distinct ATPase subunit, ISWI (3). It is thus unlikely that the PBAP complex is dedicated to EcR-mediated transcription.

The BAP, PBAP, and core Brm complexes perform different functions in vivo. The viability of *bap180* and *bap170* single mutants and the late lethality of the double mutant were surprising in view of the fully penetrant embryonic lethality of mutants lacking Osa, the subunit specific to the BAP complex (17, 52, 62). To test whether embryonic and larval development of *bap170 bap180* double mutants was rescued by maternally contributed gene products, we generated *bap180*$^{\Delta 86}$ germ line clones in homozygous *bap170*$^{\Delta 65}$ mutant mothers. The resulting embryos, which lacked the maternal and zygotic products of both genes, died before the third larval instar, supporting functions for the PBAP complex prior to metamorphosis. Cuticles secreted by the embryos that failed to hatch showed dorsalization, especially in the anterior region (Fig. 5J), in contrast to the segmentation defects seen in embryos lacking maternal and zygotic *osa* (62).

Although null *osa* mutants die during embryogenesis, the weaker allelic combination *osa*^{4H}/*osa*^{308} can survive until early pupal stages (18). We used quantitative RT-PCR to test whether a sample of genes with clear changes in expression in *bap170 bap180* mutants was affected in *osa*^{4H}/*osa*^{308} white prepupae. In most cases there was little change in expression in *osa* mutants, and several genes showed opposite expression changes in *osa* mutants and in *bap170 bap180* mutants (Fig. 7A to F; also data not shown). These results support the model derived from cell culture (47) that the BAP and PBAP complexes direct largely distinct processes and can act antagonistically.

The core subunits of the Brm complex, Brm, Mor, and Snr1, are required in the germ line for oogenesis (6, 7, 76), while mothers lacking either the BAP-specific subunit Osa or the PBAP-specific subunits Bap180 and Bap170 in their germ lines can lay eggs (62) (Fig. 5J). To test the possibility that the BAP and PBAP complexes have redundant functions in oogenesis,

TABLE 1. Classes of genes showing altered expression in *bap170*$^{\Delta 65}$ *bap180*$^{\Delta 86}$ white prepupae relative to wild type[a]

Regulated gene grouped by functional category	Product or function	Gene identifier	Fold change	*P* value	Affymetrix ID
Immune response					
Upregulated genes					
MtnC	Metal ion binding and detoxification	CG5097	31.64	0.002	1628446_at
He	Modulates the activation or recruitment of hemocytes	CG31770	5.54	0.146	1640654_at
AttA	Antibacterial peptide	CG10146	4.62	0.115	1625124_at
CG7227	Scavenger receptor	CG7227	3.38	0.003	1634702_at
eater	Phagocytosis of bacterial pathogens	CG6124	3.32	0.000	1627746_at
dl	Transcription factor downstream of Toll	CG6667	2.76	0.074	1623415_at
nec	Serine protease inhibitor that may modulate Toll activation	CG1857	2.46	0.082	1636653_at
AttB	Antibacterial peptide	CG18372	2.39	0.116	1627551_s_at
pes	Scavenger receptor	CG7228	2.29	0.007	1634913_s_at
BG4	Death receptor binding protein downstream of Imd	CG12297	2.25	0.002	1630987_at
Tab2	Component of the Imd signaling pathway	CG7417	1.84	0.002	1627702_at
GNBP3	Pattern recognition receptor	CG5008	1.76	0.004	1635470_at
srp	Transcription factor required for hemocyte differentiation	CG3992	1.71	0.013	1629229_a_at
CG12207	Bacterial cell wall catabolism	CG12207	1.52	0.002	1628761_s_at
Downregulated genes					
LysX	Lysozyme; bacterial cell wall catabolism	CG9120	−74.85	0.005	1632720_at
Tsf3	Ferric iron transporter	CG3666	−2.63	0.006	1633017_at
Zip3	Metal ion transporter	CG6898	−2.38	0.047	1637577_at
CG6429	Lysozyme; bacterial cell wall catabolism	CG6429	−2.37	0.041	1641698_at
PGRP-SD	Pattern recognition receptor	CG7496	−2.26	0.009	1633545_at
dro5	Antifungal peptide	CG10812	−1.74	0.000	1627327_at
Signal transduction					
Upregulated genes					
Cbl	Ubiquitin ligase for receptor tyrosine kinases	CG7037	3.40	0.000	1626708_at
InR	Insulin-like receptor	CG18402	2.46	0.015	1629141_at
Pka-C3	Cyclic AMP-dependent protein kinase 3	CG6117	1.73	0.015	1635928_a_at
puc	Phosphatase that inhibits the JNK pathway	CG7850	1.66	0.002	1631765_at
msn	Activates JNK signaling pathway	CG16973	1.50	0.003	1632475_at
Jra	Transcription factor in the JNK pathway	CG2275	1.49	0.023	1635224_s_at
Downregulated genes					
Dl	Notch ligand	CG3619	−5.78	0.004	1634398_a_at
fz	Wingless receptor	CG17697	−3.20	0.015	1639883_at
Brd	Inhibitor of Neuralized	CG3096	−2.44	0.003	1636672_at
ac	Proneural transcription factor	CG3796	−1.86	0.040	1638596_at
Tom	Inhibitor of Neuralized	CG5185	−1.75	0.033	1624476_at
m4	Transcription factor in the Notch pathway	CG6099	−1.69	0.037	1628334_at
Traf1	Activates JNK signaling pathway	CG3048	−1.63	0.008	1627704_a_at
Morphogenesis					
Upregulated genes					
Sop2	Actin branching	CG8978	3.02	0.000	1626492_s_at
Mbs	Myosin light chain phosphatase	CG32156	2.77	0.004	1630456_at
Rhp	GTP-Rho binding	CG8497	2.17	0.003	1634557_at
zormin	Myosin light chain kinase	CG32311	1.99	0.018	1640817_at
Myo61F	Myosin	CG9155	1.99	0.029	1638278_s_at
rhea	Focal adhesion component talin	CG6831	1.65	0.017	1628141_at
Mmp1	Matrix metalloproteinase 1	CG4859	1.51	0.018	1632204_at
Khc-73	Kinesin	CG8183	1.51	0.010	1639305_a_at
Myo95E	Myosin	CG31134	1.39	0.003	1631844_at
Downregulated genes					
rpr	Apoptosis inducer	CG4319	−4.36	0.010	1624859_at
m	Matrix protein required for wing morphogenesis	CG9369	−3.51	0.027	1626235_at
PhKγ	Phosphorylase kinase required for leg development	CG1830	−3.05	0.059	1632955_at
RhoBTB	Atypical Rho GTPase	CG5701	−1.94	0.001	1624530_at
ImpE2	Secreted protein required for imaginal disc eversion	CG1934	−1.82	0.002	1634928_at
debcl	Proapoptotic	CG33134	−1.80	0.003	1629916_at
grh	Transcription factor required for tracheal morphogenesis	CG5058	−1.59	0.001	1634573_a_at
tok	Transforming growth factor beta processing	CG6863	−1.46	0.068	1641053_s_at

Continued on following page

TABLE 1—*Continued*

Regulated gene grouped by functional category	Product or function	Gene identifier	Fold change	*P* value	Affymetrix ID
Proteolysis					
Upregulated genes					
CG8871	Jonah serine protease	CG8871	10.70	0.042	1624506_at
β*Try*	Trypsin serine protease	CG18211	8.18	0.032	1630320_at
ϵ*Try*	Trypsin serine protease	CG18681	5.70	0.077	1637492_at
CG33013	Metalloprotease	CG33013	3.01	0.008	1637916_at
CG11034	Dipeptidyl-peptidase IV	CG11034	2.43	0.059	1636409_at
CG18563	Serine protease	CG18563	2.31	0.028	1623871_at
η*Try*	Trypsin serine protease	CG12386	2.22	0.008	1639322_at
Downregulated genes					
CG11865	Astacin metalloprotease	CG11865	−9.75	0.005	1636201_at
CG6580	Jonah serine protease	CG6580	−7.77	0.000	1641190_at
CG11319	Dipeptidyl-peptidase IV	CG11319	−6.04	0.015	1634246_at
CG31205	Serine protease	CG31205	−4.97	0.008	1640666_at
Acer	Angiotensin-converting enzyme	CG10593	−2.39	0.001	1640031_at
CG10477	Serine protease	CG10477	−2.10	0.102	1629050_at
CG11529	Chymotrypsin serine protease	CG11529	−2.07	0.080	1634279_at
Cuticle metabolism					
Upregulated genes					
CG32302	Chitin binding	CG32302	2.77	0.019	1639143_at
CG7290	Chitin binding	CG7290	2.50	0.022	1640275_at
obst-J	Chitin binding	CG7348	2.12	0.050	1638298_at
CG15313	Chitin binding	CG15313	1.66	0.006	1639149_at
Downregulated genes					
CG18349	Structural constituent of chitin-based cuticle	CG18349	−9.94	0.016	1632080_s_at
Lcp65Ab2	Larval cuticle protein	CG18773	−7.53	0.023	1623637_s_at
Cht4	Chitinase	CG3986	−5.18	0.002	1631472_at
Cpr65Ec	Structural constituent of cuticle	CG8634	−1.75	0.005	1636659_at
Cpr67B	Structural constituent of cuticle	CG3672	−1.59	0.024	1635998_at
CG8192	Chitin binding	CG8192	−1.41	0.008	1632091_at

[a] The table shows regulated genes in several broad functional categories that are of interest for metamorphosis; within each category genes are listed in groups that are upregulated or downregulated in the double mutant background. Each gene is defined by its symbol and/or CG number, a brief functional description derived from data available on Flybase, and Affymetrix identifier (ID). The relative change in mRNA abundance in *bap170 bap180* mutants normalized to wild type as well as the *P* value from the microarray analysis are indicated.

we generated *osa bap180* double mutant germ line clones in homozygous *bap170* mutant mothers. Although the resulting females lacked all the BAP- and PBAP-specific subunits in their germ lines, they were able to differentiate oocytes (Fig. 7H) and to lay eggs. In contrast, germ line clones homozygous for the null allele brm^2 arrested their development early in oogenesis (Fig. 7G). Since removing *osa*, *bap170*, and *bap180* is not sufficient to phenocopy *brm*, a core complex lacking any of these specificity-determining subunits must retain the functions necessary to mediate normal oogenesis.

DISCUSSION

We have taken a genetic approach to compare the roles of the BAP and PBAP chromatin remodeling complexes in vivo. Surprisingly, we found that two PBAP-specific subunits, Bap170 and Bap180, are individually dispensable for adult survival in *Drosophila*. The requirement for Bap170 to stabilize Bap180 protein has previously prevented an independent assessment of the role of Bap170 (47, 73). Our isolation of the $bap170^{\Delta 65}$ allele allowed us to show that the two proteins have independent functions in vivo; only Bap180 is required to promote normal eggshell formation, while the removal of Bap170 functions other than Bap180 stabilization reduces viability and leads to ectopic wing vein differentiation. Despite the lack of homology between the two proteins, double mutant analysis showed that there is strong functional redundancy between the proteins, which act together to regulate genes required for metamorphosis and to suppress immune response genes. The basis for this redundancy is unknown but might reflect binding of Bap170 and Bap180 to different factors associated with the same target genes, providing redundant mechanisms for recruitment of the PBAP complex. Finally, our analysis of triple mutants lacking Bap180, Bap170, and the BAP-specific subunit Osa has revealed a function for the core Brm complex in oogenesis.

The yeast SWI/SNF complex, which is most similar to the BAF complex, is not required for viability, while the RSC complex related to PBAF is abundant and essential (11, 26). In addition, a previous study that examined a small number of mammalian promoters in vitro had suggested a more important role in transcriptional activation for the PBAF than the BAF complex (38), although the BAF complex has been shown to play a role in transcriptional activation by the glucocorticoid, estrogen, and androgen receptors (27, 52, 63). In contrast, we found that the Osa subunit of the BAP complex is critical for survival early in development (62), and our current results show that early developmental lethality is observed only when the maternal and zygotic contributions of both PBAP-specific subunits are removed. This suggests that the BAP complex has

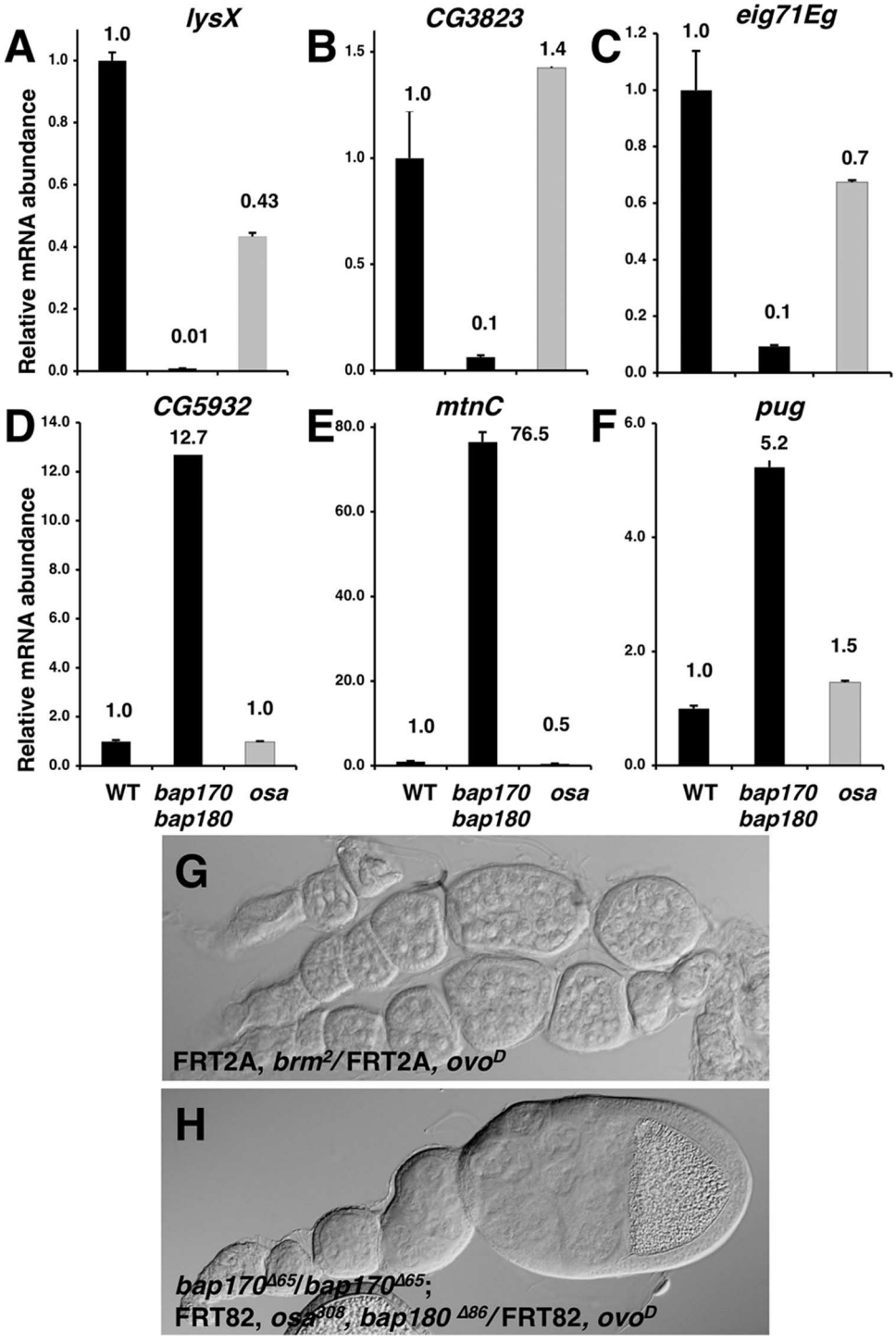

FIG. 7. Target genes of the PBAP complex are not regulated in the same way by *osa*. (A to F) RT-PCR mRNA quantifications in wild type (WT), $bap170^{\Delta 65}$ $bap180^{\Delta 86}$ double mutants (*bap170 bap180*), and osa^{308}/osa^{4H} mutants (*osa*) (bars are in respective order in all panels). Panels A to C show genes with decreased expression in *bap170 bap180* mutants, and panels D to F show genes with increased expression in *bap170 bap180* mutants. Error bars represent the coefficient of variance expressed as a percentage of the base. Genes affected in *bap170 bap180* double mutants can be unaffected in *osa* mutants (D), affected to a lesser extent (A, C, and F), or affected in the opposite way (B and E). (G and H) Nomarski images of ovaries from females with brm^2 germ line clones (G) or from $bap170^{\Delta 65}$ females with osa^{308} $bap180^{\Delta 86}$ germ line clones (H). No brm^2 egg chambers develop beyond stage 6, the latest stage reached by ovo^D egg chambers (G); however, egg chambers lacking *osa*, *bap170*, and *bap180* function can develop to stage 9 (H), and some of the resulting eggs are laid.

taken on crucial developmental functions during the evolution of higher eukaryotes. Recent in vivo studies in mouse support this conclusion. While the mouse BAF180 protein is required for normal cardiac development, other organs appear normal in the mutant embryos (68). Consistently, the Bap170 homologue BAF200 has been shown to activate cardiac genes in cell culture (75). However, a knockout of the *osa* homologue *baf250a* causes early embryonic arrest and failure to differentiate mesoderm (23). Both BAF250a and BAF250b are also required for the self-renewal and pluripotency of embryonic stem cells (23, 74).

Since chromatin remodeling in vitro requires only four subunits common to both complexes (54), the remaining subunits are likely to control target gene selection. They might do this by binding to DNA, modified histones, or sequence-specific transcription factors. Although both the Osa and Bap170/BAF200 proteins contain DNA-binding ARIDs, these have no apparent sequence specificity (17, 53) and are therefore unlikely to recruit the BAP and PBAP complexes to specific target promoters through interactions with DNA. However, Bap170 and Bap180 contain additional potential DNA-binding domains, the specificity of which has not been determined. Bap180 also contains multiple bromodomains that could recruit the complex by binding to histones acetylated at specific lysine residues (15). Several subunits of SWI/SNF family complexes have been shown to bind to specific transcription factors, leading to either activation or repression of their target genes (19, 22, 24, 28, 51, 66). Osa homologues have been shown to interact directly with the glucocorticoid receptor, Zeste, the GATA transcription factor Pannier, the coactivator Chip, and acidic activators (25, 30, 51, 52). Human BAF200 can bind to serum response factor (75) while Bap180 homologues have no identified transcription factor binding partners. A preliminary search for overrepresented sequence motifs in the flanking regions of genes with altered expression in our microarray (J. Wang, personal communication) did not enable us to identify any candidate transcription factor partners for the PBAP complex through their binding sites.

The requirement of BAF180 for the expression of retinoic acid receptor target genes (68) suggested a possible interaction of this protein with nuclear receptors. However, the results of our microarray analysis of *bap170 bap180* mutants did not show any significant correlation with genes regulated by the EcR at puparium formation (Fig. 6B). A previous study showed that Brm and Snr1 regulate the expression of a cluster of nine ecdysone-induced genes (*eig*) at position 71E in early pupae (77); only three of these, *Eig71Eb*, *Eig71Eg*, and *Eig71Ek*, were significantly altered in our microarray. The BAP complex may thus be the primary mediator of ecdysone responses; Osa contains an LXXLL motif that might interact with EcR. The NURF chromatin remodeling complex is another good candidate to mediate EcR signaling as it has a strong effect on the expression of ecdysone-regulated genes at the larval stage (3). It is still possible that the PBAP complex interacts with one or more of the other 17 *Drosophila* nuclear receptors (33). Human BAF180 has been shown to induce p21 expression in breast cancer cells (71); however, the *Drosophila* p21 homologue *dacapo* was not significantly affected in our microarray or in a previous study in S2 cells (47), suggesting that p21 regulation may be specific to tumor cells. Interestingly, we see an effect of the PBAP complex on the expression of genes involved in the immune response, a function likely to be conserved in mammals (73). The effects on the immune system we observed in *bap180 bap170* mutants may be due to upregulation of the transcription factors Dorsal and Serpent (Table 1), which regulate, respectively, *drosomycin* expression and crystal cell production (35, 41). The dorsalization of double mutant embryos might also be due to an effect on Dorsal (46).

Since the core complex but not *osa* is required for oogenesis (6, 7, 62, 76), we hypothesized that this function might be performed by the PBAP complex. However, we found that loss of PBAP-specific subunits from the germ line did not prevent oogenesis. A previous microarray study in cell culture suggested that knocking down Osa, Bap170, and Bap180 was equivalent to removing one of the core subunits Brm, Mor, or Snr1 (47). In contrast, we found that the absence of all three accessory subunits in the germ line did not block oogenesis, while the loss of Brm arrested oogenesis at an early stage. This indicates that the core complex, alone or with additional accessory subunits, is sufficient to regulate some target genes. The BAP and PBAP complexes may be specialized for subsets of regulatory functions, such as control of the cell cycle and Wingless target genes by BAP (8, 18, 47, 49) and control of metamorphosis and the immune response by PBAP.

While the manuscript was in preparation, Chalkley et al. (14) reported that the PBAP complex contains a third specific subunit, Supporter of activation of *Yellow* protein (SAYP), that is required for the stability of Bap180 and Bap170. Although homologues of this subunit have not been reported in purified vertebrate PBAF complexes, genome-wide gene expression changes in S2 cells after RNAi knockdown of SAYP, Bap170, and Bap180 were strongly correlated, suggesting that the three proteins act as a functional unit. These results are surprising given that SAYP is encoded by the *enhancer of yellow 3* [*e(y)3*] gene, which is essential for early embryogenesis (57). It is possible that zygotic SAYP is required for embryonic functions performed by maternally provided Bap170 and Bap180 (Fig. 5J). An alternative possibility is that SAYP has additional functions outside the PBAP complex. Although immunodepletion experiments suggested that the majority of cellular SAYP protein is associated with the Brm complex (14), previous studies have shown that SAYP is localized to and functionally required in heterochromatin (57), while Brm and other PBAP complex subunits are restricted to euchromatin (2, 44). A hypomorphic *e(y)3* allele is female sterile (57), suggesting that SAYP is required for oogenesis, but it has not been determined whether it functions in the soma, like Bap180, in the germ line, like the core Brm complex, or in both. The different phenotypes of mutations in the three PBAP complex-specific subunits demonstrate the importance of complementing in vitro cell-based assays with in vivo studies in whole organisms.

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