Hemocytes are essential for wing maturation in *Drosophila melanogaster*

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Newly eclosed flies have wings that are highly folded and compact. Within an hour, each wing has expanded, the dorsal and ventral cuticular surfaces bonding to one another to form the mature wing. To initiate a dissection of this process, we present studies of two mutant phenotypes. First, the *batone* mutant blocks wing expansion, a behavior that is shown to have a mutant focus anterior to the wing in the embryonic fate map. Second, ectopic expression of protein kinase A catalytic subunit (PKAc) using certain *GAL4* enhancer detector strains mimics the *batone* wing phenotype and also induces melanotic ''tumors.'' Surprisingly, these *GAL4* strains express GAL4 in cells, which seem to be hemocytes, found between the dorsal and ventral surfaces of newly opened wings. Ectopic expression of Ricin A in these cells reduces their number and prevents bonding of the wing surfaces without preventing wing expansion. We propose that hemocytes are present in the wing to phagocytose apoptotic epithelial cells and to synthesize an extracellular matrix that bonds the two wing surfaces together. Hemocytes are known to form melanotic tumors either as part of an innate immune response or under other abnormal conditions, including evidently ectopic PKAc expression. Ectopic expression of PKAc in the presence of the *batone* mutant causes dominant lethality, suggesting a functional relationship. We propose that *batone* is required for the release of a hormone necessary for wing expansion and tissue remodeling by hemocytes in the wing.

The final step in morphogenesis of the adult fly is wing maturation. Newly eclosed flies have wings that are highly folded and compact. Their cuticle is pale and pliable. Soon after eclosion, an increase in blood pressure begins to expand the wings. Within about 1 h, the wings have unfolded, and the dorsal and ventral cuticular panels of each wing have bonded to one another. Subsequent tanning of the cuticle over a period of several hours forms a strong but flexible flight organ.

The development of the wing before eclosion is well documented (1–3). The events accompanying wing maturation subsequent to eclosion of the adult fly are less clearly understood. At eclosion, the epithelial cells that secreted the wing cuticle are in a state of dissolution (4). A recent review has described the death of these epithelial cells as a process distinct from apoptosis because of the absence of accompanying phagocytosis (5). How the dorsal and ventral surfaces of the wing come to bond together after expansion does not seem to have been addressed as a process separate from the tanning process.

Further insight might be gained from the study of mutants that impede or completely block wing expansion and/or joining of dorsal and ventral surfaces. Here we present studies of two such mutant phenotypes. One is caused by the X-linked mutant *batone* (*bae*, ''club'' in Italian). The other is a phenotype, produced by *UAS*-controlled expression of protein kinase A catalytic subunit (PKAc) using certain *GAL4* enhancer detector strains, that mimics the wing phenotype of *bae*. In addition to the wing phenotype, PKAc expression induces melanotic ''tumors'' in the fly, melanized aggregations of a form of hemocyte called a lamellocyte.

The expression patterns of these *GAL4* strains have been monitored by use of a *UAS-GFP* transgene expressing green fluorescent protein (GFP). We report here that GFP is expressed in previously undescribed cells present in the wing at eclosion of normal and *bae* flies. Simultaneous GAL4-driven expression of GFP and PKAc visibly alters the behavior of these cells and causes abnormal wing maturation in otherwise normal flies. Simultaneous expression of GFP and Ricin A (the catalytic subunit of ricin toxin) reduces the number of these cells and prevents bonding of dorsal and ventral wing surfaces. We conclude that these cells are hemocytes that are essential for normal wing maturation. We present evidence that ectopic PKAc expression interferes with normal hemocyte function in the wing by blocking signaling through the *wg/Wnt* pathway.

Ectopic expression of PKAc in heterozygous *bae* females or hemizygous *bae* males causes dominant lethality. Therefore, we propose that the *bae* mutation may alter normal hemocyte behavior in producing its effect on wing maturation. This proposal is supported by gynandromorph fate-mapping of *bae*, which places its focus of activity in the anterior dorsal neuroectoderm of the embryo, demonstrating the nonautonomy of the *bae* phenotype in the wing.

Materials and Methods

Most *GAL4*-enhancer detector strains and the *UAS-PKAc* and *UAS-GFP* transgenes used have been described along with genetic and microscopic techniques (6, 7). The studies presented here use the strongly expressing transgenes *UAS-PKAc 5.2* and *15.3*. To view GFP fluorescence, wings were pulled from the thorax, mounted dry under a small coverslip, viewed with a Zeiss Axioplan microscope fitted for epifluorescence, and photographed using a Zeiss camera with automatic exposure control. The *UAS-Ricin A* transgene was obtained from Alicia Hidalgo (Univ. of Cambridge, Cambridge, U.K.). The *UAS-dCbz* transgene was obtained from Mariann Bienz (Medical Research Council-Laboratory of Molecular Biology, Cambridge, U.K.). *GAL4–684* and the *UAS-*α_{PS2} *integrin* transgene were obtained from Danny Brower (University of Arizona, Tucson, AZ). *Stat92E* alleles were obtained from Norbert Perrimon (Harvard Medical School, Boston, MA). Alleles or *UAS* transgenes of other genes studied were obtained from the Bloomington Stock Center (Bloomington, IN).

The *bae* mutant was discovered by M.M.G. in a laboratory stock on April, 22, 1968. Mutant flies never expand their wings after eclosion (Fig. 1*B*). The mutant maps to the X chromosome at 14F6-15A1 based on its inclusion in $Df(1)r^{D17}$ (14F6-15A6) and that it maps genetically to the left of *rudimentary*, which is located at 15A1 (8). *bae* is semidominant with ≈5% of heterozygous females exhibiting the phenotype. When soaked in buffer, wings dissected from *bae* flies expand and look identical to unopened wings dissected from newly eclosed wild-type flies (or

Abbreviations: ECM, extracellular matrix; GFP, green fluorescent protein; PKAc, protein kinase A catalytic subunit.

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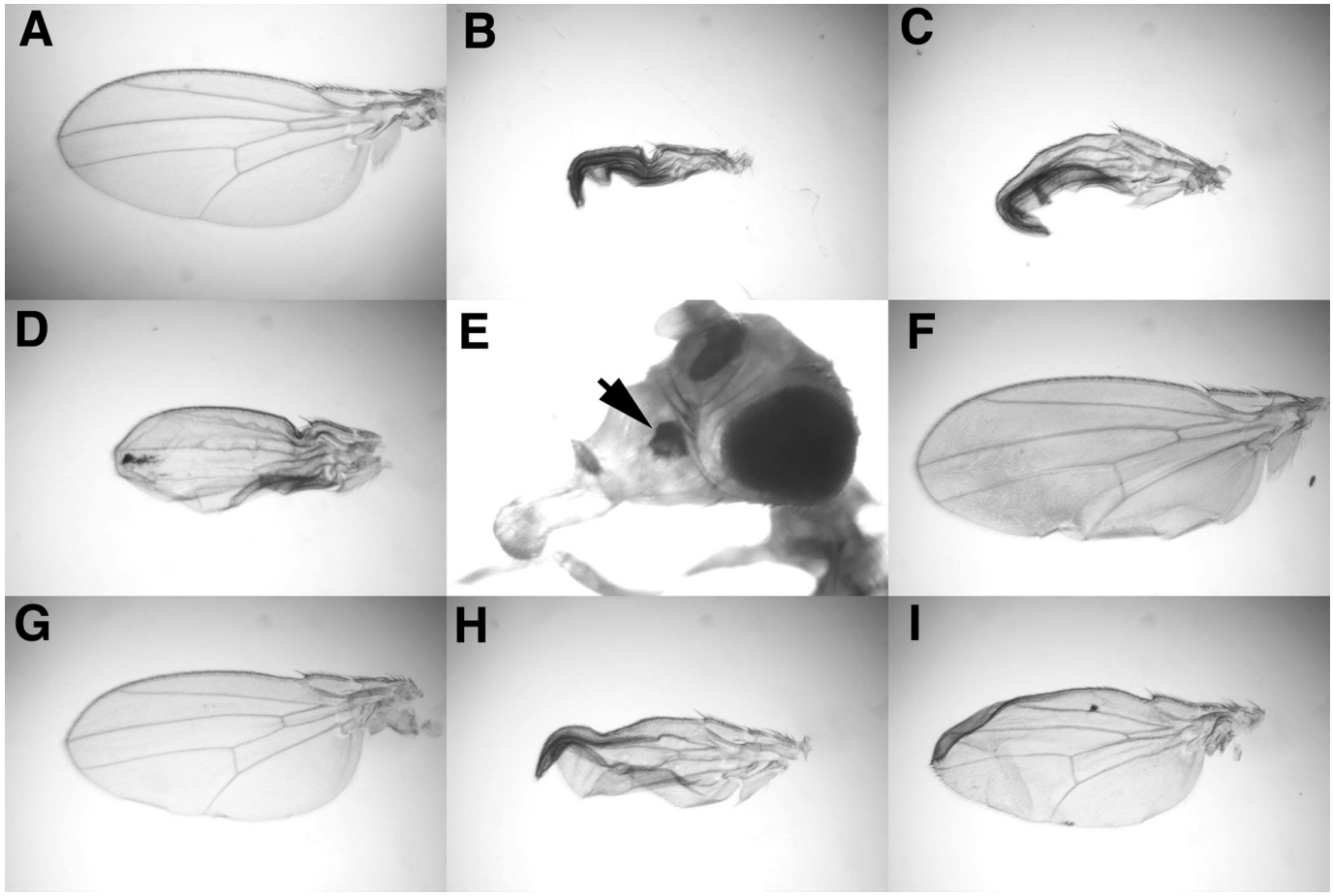

Fig. 1. Morphological observations. (*A*) Wild-type wing of a *GAL4–30A* fly. (*B*) Wing of a *bae* mutant fly. (*C* and *D*) Wings of *GAL4–30A/+ ; UAS-PKAcF 5.2/+* flies. (*E*) Head of a *GAL4–30A/+ ; UAS-PKAcF 5.2/+* fly; the arrow points to a small melanotic tumor. (*F*) Wing of a *GAL4–30A, UAS-GFP/UAS-Ricin A* fly. Failure of dorsal and ventral surfaces to bond is apparent along the posterior edge of the wing and from the precipitated debris in the distal portion of the wing blade. (*G*) Wing of a *GAL4–30A, UAS-PKAc 15.3/UAS-pan 24* fly, sibling to that shown in *H*. (*H*) Wing of a *GAL4–30A, UAS-PKAc 15.3/Sp* fly, sibling to that shown in *G*. (*I*) Wing of a *GAL4–30A/+ ; UAS-panΔN5/+* fly.

pharate adults). There are no apparent structural defects in *bae* wings that might account for their failure to open.

Gynandromorph analysis of the *bae* phenotype was carried out as described by Hotta and Benzer (9) by using a ring-X chromosome of genotype *R(1), $w^{vc}p[w^{+}$ ftz:lacZ]* provided by Susan Younger and Yuh Nung Jan (Univ. of California, San Francisco). Pertinent structures scored were the head (eye), wing (blade), and anterior leg. A structure was scored as hemizygous *y w bae* if the majority of the tissue showed the recessive phenotype (*y* or *w*) rather then the dominant phenotype (y^{+} or w^{+}). In analyzing the gynandromorph data, the figures were adjusted to account for the fact that 5% of genetically *bae/+* gynandromorphs will show a mutant wing phenotype.

Results

Fate Mapping the *bae* Phenotype. A gynandromorph analysis was undertaken to determine the blastoderm fate map location of the *bae* phenotype (9, 10). The results of this analysis are summarized in Table 1. The wing phenotype and genotype are not coincident, indicating that *bae* acts nonautonomously. A feature of the data not evident in Table 1 is that gynandromorphs either have both wings fully normal or fully mutant. The data show that the *bae* mutant focus is located in the dorsal anterior region of the embryonic fate map as shown in Fig. 2.

***UAS-PKAc* Transgenes Mimic the *bae* Phenotype.** In the course of studying phenotypic effects caused by ectopic expression of PKAc (6), a number of *GAL4*-enhancer detector strains were found that mimic the *bae* phenotype. We focus here on the effects of one such strain, *GAL4–30A* (11), driving expression of one or another *UAS-PKAc* transgene. The resulting wing phenotypes are similar to but more variable than that of *bae*, as illustrated by wings in Fig. 1 *C* and *D*. The wings are variably unfolded or unfolded and blistered (dorsal and ventral surfaces of the wing blade separated) or collapsed (the wing blade not fully expanded). All pattern elements of the wing (veins, bristles, and hairs) are present and normal. In addition, and unlike *bae* flies, flies ectopically expressing PKAc frequently have mela-

Table 1. Gynandromorph mapping of *bae* mutant focus

Wing phenotype		All *bae/+*	All *bae/0*	Bilateral mosaic
			Head genotype	
Normal	108	79	2	27
Mutant	25	10	11	4
			Wing genotype	
Normal	108	46	6	56
Mutant	25	14	4	7
			1st Leg genotype	
Normal	108	59	6	43
Mutant	25	13	6	6

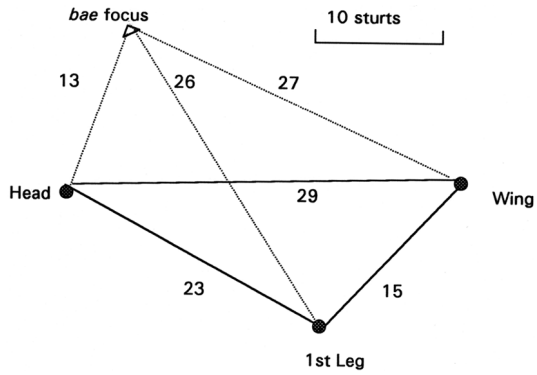

Fig. 2. Blastoderm fate map of the *bae* mutant focus. The *bae* mutant focus is positioned on the blastoderm fate map relative to markers for the head, wing, and anterior leg. Sturt distances between cuticular markers are shown with solid lines, and the distances between the *bae* focus and cuticular markers are shown with dotted lines. Distances were generated from the data in Table 1 by using the Hotta and Benzer (9) procedure.

notic tumors (12) of variable sizes within the head capsule (Fig. 1*E*). Melanotic tumors may also be found in the wing (Fig. 1*D*) or other parts of the body. Occasionally, dead or moribund flies are found that have become entirely melanized after partially opening their wings. These phenotypic effects are caused by PKAc phosphorylation of target protein(s) because *GAL4–30A*-driven expression of *UAS-PKAcA*^{75} transgenes, mutant at the active site lysine^{75}, produces flies that do not exhibit these effects (7).

Expression Pattern of the *GAL4–30A* Transgene. We investigated the expression pattern of *GAL4–30A* by using a *UAS-GFP* transgene. In third-instar larvae, GFP fluorescence is strong in the salivary glands and in the wing discs, in a ring of cells that give rise to the hinge region of the adult wing, but not in cells of the wing pouch that give rise to the majority of the wing blade. In newly eclosed adult flies, the unopened wings are strongly fluorescent (Fig. 3*A*). Newly opened wings exhibit a layer of evenly spaced fluorescent cells sandwiched between the dorsal and ventral surfaces of the wing blade (Fig. 3*B*). As the wings tan over the course of an hour or two, the fluorescence gradually fades and disappears. Because *GAL4–30A* and *UAS-GFP* are linked to chromosome II, we have constructed a chromosome carrying both transgenes and have established a homozygous stock. This strain permits study of the effects of other genes or transgenes on GFP fluorescence in the wing.

Expressing *UAS-PKAc* simultaneously with *UAS-GFP* reveals a disordered pattern of fluorescent cells within the newly opened wing blade. Fluid-filled blisters contain large numbers of floating cells, many of which are in aggregates (Fig. 3*C*). Some cells are round, differing from each other in diameter by about a factor of 2; most are larger and lamellar or spindle-shaped. In some wings, fluid becomes trapped and eventually melanizes if the blister does not break. In others, as the cuticle begins to harden and tan, fluid drains from blisters into the thorax, carrying along single fluorescent cells but not aggregates. The fluorescent cells in wings expressing *UAS-PKAc* fit the descriptions of hemocytes of different types (12–14). The larger cells seem to be lamellocytes that are known to participate in melanotic tumor formation in pathological conditions.

The Wing Phenotype Caused by *UAS-PKAc* Transgenes Is a Result of Expression in Hemocytes Rather Than Wing Epithelium. Because *GAL4–30A* expression is not confined to hemocytes, we have tested the possibility that the wing phenotype caused by *UAS-PKAc* transgenes may not be caused by expression in hemocytes. A collection of independent insertion strains, in which *GAL4* is located on either chromosome II or III, was screened for strains that express *UAS-GFP* in the cells in newly opened wings. Of 81 strains tested, 5 showed GFP expression in these cells. When these five strains were crossed to *UAS-PKAc* strains, three caused early larval death and the other two produced flies with unopened, blistered, or collapsed wings and melanotic tumors as well as blackened pupae and blackened pharate adults.

We also tested the possibility that we have failed to observe a low level of *GAL4–30A* expression in epithelial cells that secrete the cuticle of the wing blade and that this expression is responsible for the effect of PKAc. We used *GAL4–30A* to overexpress α_{PS2} integrin, which causes a dominant wing-blistering phenotype when expressed in the wing pouch using *GAL4–684* (15), and observed no effect on wing morphology. We also used *GAL4–684* and *GAL4–71B* (11) to express PKAc in the wing pouch and observed strong blistering of the wing blade accompanied by enlarged veins and a disrupted venation pattern, quite unlike the effect obtained when *GAL4–30A* is used to express PKAc.

Thus, the phenotypic effects of ectopic PKAc driven by *GAL4–30A* would seem not to be caused by subliminal expression in the wing epithelium but to be the consequence of PKAc expression in hemocytes. This conclusion is strengthened by the fact that, of the *GAL4* strains selected for expression in cells found in the wing after eclosion, both of those that survive to the adult stage show wing and melanotic tumor phenotypes when expressing PKAc.

Ablation of Hemocytes by a *UAS-Ricin A* Transgene. To further understand what role hemocytes might play in normal wing maturation, as well as to understand what effect PKAc expression might have on hemocytes, we attempted to ablate hemocytes from the wing by using a *UAS* transgene that expresses a modified Ricin A polypeptide. Ricin A is the catalytic subunit of ricin toxin, which kills cells by depurinating 28S rRNA. The Ricin A cDNA expressed by this transgene has been modified to block secretion. Moreover, in the absence of the ricin B component of the toxin, Ricin A cannot be internalized by neighboring cells, making cell death induced by transgene expression cell autonomous (16).

GAL4–30A-driven expression of *UAS-Ricin A* is lethal at 25°C, but at 18°C we have achieved partial ablation of hemocytes without lethality as evidenced by reduced fluorescence in newly open wings and fewer unevenly distributed fluorescent hemocytes. Newly eclosed flies expressing *UAS-Ricin A* open their wings completely, unlike flies expressing PKAc, and frequently the dorsal and ventral surfaces are not bonded together (Fig. 1*F* and Fig. 3 *D*, *F*, and *G*). Some wings show a nonuniform distribution of hemocytes, with bonding of dorsal and ventral surfaces where hemocytes are in high concentration and failure to bond where hemocytes are rare (Fig. 3*E*). Evidently, hemocytes are necessary for the normal bonding of dorsal and ventral wing surfaces and not for unfolding of the wing. On the other hand, PKAc expression in hemocytes interferes with wing unfolding and with normal bonding of the wing surfaces, causing blisters. In addition, flies expressing Ricin A do not have melanotic tumors, nor do their cuticles melanize abnormally over a period of several days, as do the cuticles of flies expressing PKAc. It is interesting that many of the hemocytes that survive Ricin A expression seem to be lamellocytes (Fig. 3*G*). Lamellocyte differentiation may be a response to the massive cell death that these flies have experienced.

Interaction of *PKAc* Transgenes with Other Genes/Transgenes. To further understand the action of ectopic PKAc in hemocytes, we sought genetic enhancement or suppression of the phenotype produced by a chromosome carrying both *GAL4–30A* and

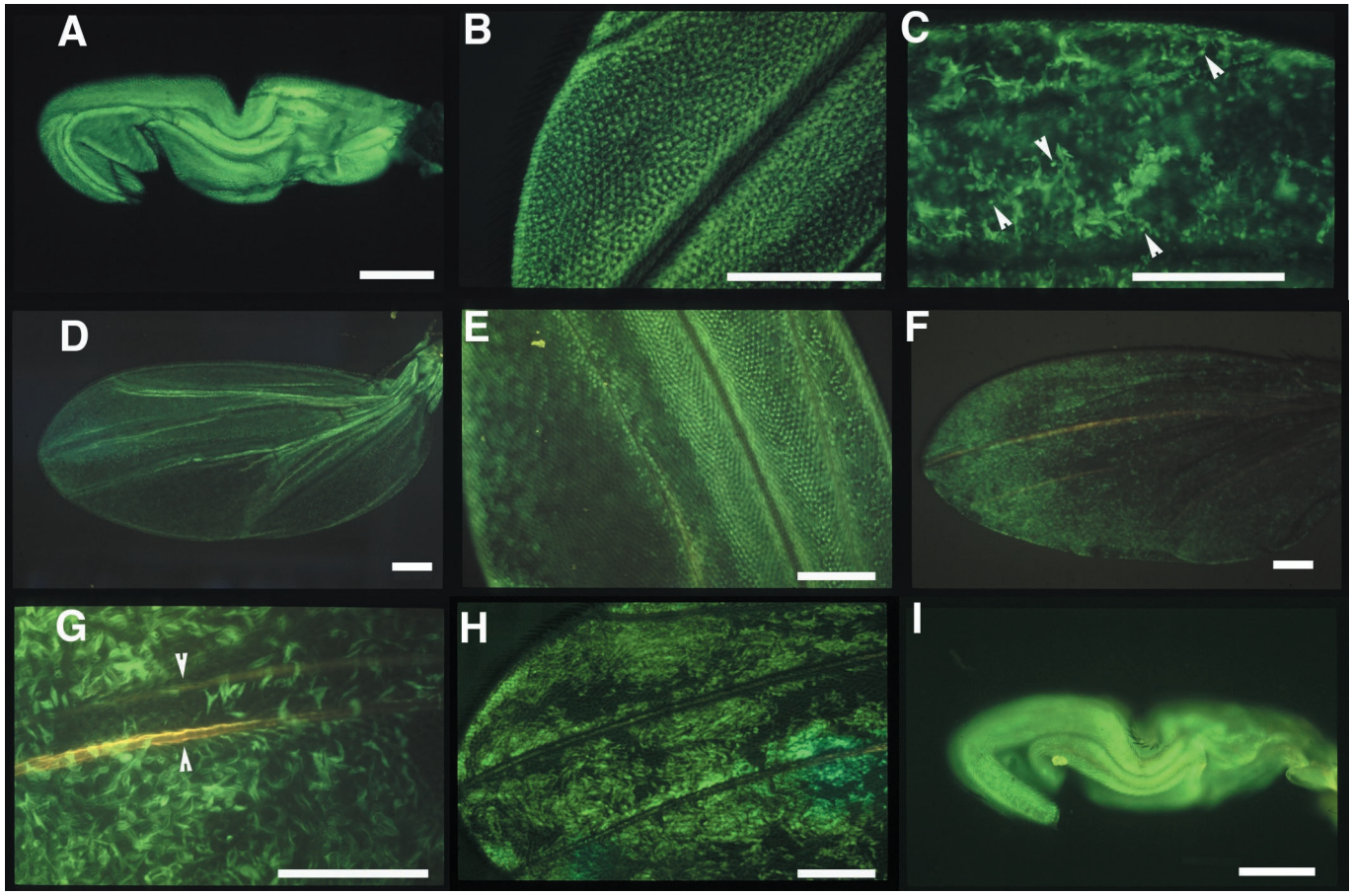

Fig. 3. Observations of GFP fluorescence. (*A*) Unopened wing of a *GAL4–30A, UAS-GFP* fly. (*B*) Newly opened wing of a *GAL4–30A, UAS-GFP* fly. (*C*) Newly opened wing of a *GAL4–30A, UAS-GFP*/+ *; UAS-PKAcF 5.2*/+ fly; note the large aggregations of hemocytes, most of which appear to be lamellocytes. Arrows mark some of the few round cells that can be identified. (*D–G*) Newly opened wings of *GAL4–30A, UAS-GFP*/*UAS-Ricin A* flies. In *D*, the coverslip was moved to show that dorsal and ventral surfaces can be displaced from their normal relationship to one another, creating the wrinkles in the wing blade. In *E*, partial ablation of hemocytes prevents bonding of the wing surfaces of the posterior wing blade (left), where hemocytes are out of focus, whereas bonding occurs in the central and anterior wing blade where hemocytes are abundant and in focus (right). In *F*, the wing is fully expanded and filled with hemolymph and some hemocytes; at higher magnification (*G*), many of the hemocytes appear to be lamellocytes. Note the separation of dorsal and ventral portions of the vein (arrows) in *G*. (*H*) Newly opened wing of a *GAL4–30A, UAS-GFP*/+ *; UAS-panΔN5*/+ fly. The dorsal wing blade and veins are in focus, whereas most of the hemocytes are not, because of the separation of the dorsal and ventral surfaces. In *D–H*, the low level of fluorescence required long exposures, causing some reflected background room light to illuminate portions of the wing blade and veins (*F–H*). (*I*) Unopened wing of a *bae*/*Y; GAL4–30A, UAS-GFP*/+ fly. [Bar = 0.2 mm.]

UAS-PKAc 15.3 transgenes. Endogenous PKAc is known to exert many of its long-term effects by phosphorylating cAMP response element-binding (CREB) proteins (17). Therefore, two genetic variants of *Drosophila* CREB, a heterozygous recessive lethal mutation, *creb B-17A*^{S162} (18) and a truncated dominant-negative *UAS-dCbz* transgene (19), were tested. Neither strongly affected the phenotype of *GAL4–30A, UAS-PKAc 15.3* flies as judged by comparing siblings produced by independent assortment of the CREB variant and *GAL4–30A, UAS-PKAc 15.3*.

Transduction of an activated JAK-STAT signal by the marelle/STAT92E transcription factor causes hematopoietic neoplasia in *Drosophila* (20, 21). Therefore, we tested the effect of two heterozygous *Stat92E* mutants (*Stat92E*^{06346} and *Stat92E*^{j6C8}) and found that neither strongly affected the phenotype of *GAL4–30A, UAS-PKAc 15.3* flies.

It was previously noted that ectopic expression of PKAc with certain *GAL4* drivers mimics phenotypes typical of a loss of Wingless function (ref. 6; J.A.K., unpublished observations). Therefore, we tested heterozygous recessive mutations of genes known to affect Wingless signal transduction or expression of Wingless target genes (22): *armadillo* (*arm*^{4}); *pangolin* (*pan*^{2}, *pan*^{3}); *groucho* (*gro*^{1}, *gro*^{C105}); and *nejire* (*nej*^{3}, *nej*^{Q7}, *p*[*w*$^{+}$]^{nej}). None strongly affected the phenotype of *GAL4–30A, UAS-PKAc 15.3* as judged by comparing siblings with or without the heterozygous mutant.

Subsequently, we tested *UAS-pan 24*, a transgene expressing the wild-type *Drosophila* homolog of the mammalian lymphocyte enhancer-binding factor 1 (LEF-1) and the related T cell factor (TCF; refs. 23–25). Coexpression of *UAS-pan 24* and *UAS-PKAc 15.3* resulted in strong suppression of the wing phenotype caused by PKAc (compare the wings of siblings in Fig. 1 *G* and *H*). However, fewer than the expected number of *GAL4–30A, UAS-PKAc 15.3*/*UAS-pan 24* flies emerged. These flies were weak and were trapped easily in the medium. In contrast, *GAL4–30A*/*UAS-pan 24* flies eclosed in expected numbers and seemed normal in phenotype, indicating a detrimental interaction between overexpressed PKAc and Pan.

We then used *GAL4–30A* to express a dominant-negative *pan* transgene, *UAS-panΔN*, known to block expression of Wingless target genes (23, 25). When development occurred at 25°C, most flies died as abnormally melanized pharate adults, an effect similar to the effect of expressing a strong *UAS-PKAc* transgene. At 21°C, many flies were able to eclose but were weak and

Table 2. Expression of PKAc produces dominant lethality in *bae* heterozygotes or hemizygotes

Genotypes of chromosomes			
		III	
I	II	*TM3, Sb*/+	or *Sal I, y*$^{+/+}$
		No. of flies	
bae/*y w*	*GAL4-30A, UAS-PKAc 15.3*/+	1	35
bae/*Y*	*GAL4-30A, UAS-PKAc 15.3*/+	0	30
y w bae/*y w*	*GAL4-30A, UAS-PKAc 15.3*/+	0	51
y w bae/*Y*	*GAL4-30A, UAS-PKAc 15.3*/+	0	38
y w/*y w*	*GAL4-30A, UAS-PKAc 15.3*/+	50	95
y w/*Y*	*GAL4-30A, UAS-PKAc 15.3*/+	55	97

became caught in the medium before opening their wings. Others had strongly blistered wings or had open wings that were flaccid, like those caused by Ricin A expression (Fig. 1*I*). Some flies had melanotic tumors in the head or wings (Fig. 1*I*), like flies expressing PKAc. Many flies had blood oozing from body joints or dried melanized blood around body joints. At 18°C, many flies eclosed with expanded wings and dorsal and ventral surfaces that could not bond together. These flies had disordered and fewer hemocytes than normal wings as judged by strongly reduced fluorescence (Fig. 3*H*).

***bae* and *UAS-PKAc* Interact to Produce Dominant Lethality.** The wings of *bae* flies contain hemocytes (Fig. 3*I*). We examined the effect of expressing PKAc in *bae* flies by using the *GAL4–30A, UAS-PKAc 15.3* chromosome and a mutant *P* element, *P*[*ry*$^{+}$*; Sal*I] (26). *P*[*ry*$^{+}$*; Sal*I], located on chromosome III, makes active P repressor but cannot make transposase. It assorts independently of *GAL4–30A, UAS-PKAc 15.3* to repress transcription of *GAL4* in one class of sibs and not the other, permitting any observed interactions with *bae* to be ascribed to PKAc expression (J.A.K. and C. I. Ho, unpublished data). Additionally, we examined interactions of ectopic PKAc with *bae* in two genetic backgrounds. The results of these crosses, presented in Table 2, demonstrate a lethal interaction. Surprisingly, ectopic PKAc interacts with *bae* to cause dominant lethality, for even *bae*/+ females die.

The possibility that overexpression of Pan might suppress the wing phenotype of *bae*/Y*; GAL4–30A*/*UAS-pan 24* flies was tested. The wings of these flies were identical to those of *bae*/Y flies.

Discussion

Role of Hemocytes in Normal Wing Maturation. Apparently, this is, to our knowledge, the first report of hemocytes in the wings of newly eclosed flies, because they were not described in a previous study of the final stages of *Drosophila* wing maturation made by transmission electron microscopy (4). Their presence must have been overlooked because of the debris created by death of the wing epithelium. The power of the *GAL4*/*UAS* system to express GFP specifically in hemocytes has now enabled their detection.

As demonstrated here, an apposition of dorsal and ventral wing surfaces occurs after eclosion. Two earlier appositions, followed by separations, of dorsal and ventral wing epithelia have occurred during pupal development (3). During each of these appositions, hemocytes are believed to secrete extracellular matrix (ECM) that binds the epithelia together. Subsequent separations are believed to be caused by proteolysis and phagocytosis of the ECM by hemocytes. Evidence from *Drosophila melanogaster* (3) and from *Manduca sexta* (27) indicates that components of the ECM are found in hemocytes during pupal development. Therefore, it is reasonable to propose that hemocytes persist between the wing surfaces after eclosion where they phagocytose apoptotic epithelial cells and secrete an ECM that binds dorsal and ventral wing blades together. As a result of the destruction of epithelial cells, this ECM would have to bind directly to the cuticle of the wing surfaces and may contain a protein with chitin-binding domains (28). Thus, it is likely that the death of the wing epithelia is apoptotic, contrary to previous conclusions (5).

Our identification of the fluorescent cells in normal wings as hemocytes is an inference based on the above mentioned studies of pupal development and on the detached fluorescent cells we observe in wings of flies expressing PKAc, Ricin A, or PanΔN. The latter cells fit previous descriptions of hemocytes. The association of PKAc expression with melanotic tumors, known to be caused by hemocytes, in various parts of the body strengthens this identification. The fluorescent cells in normal wings (Fig. 3*B*) are tightly bound in a strikingly precise array that makes them an integral part of the wing, as might be expected if their role is to secrete ECM. As such, they do not exhibit characteristics that readily identify them as hemocytes. However, PKAc, Ricin A, or PanΔN expression disrupts this cellular array and prevents bonding of dorsal and ventral cuticular wing blades without affecting synthesis of the cuticle that forms the wing, demonstrating that the fluorescent cells in the wing are distinct from wing epithelial cells.

Role of *bae* in Normal Wing Maturation. Fate mapping places the focus of *bae* gene activity in the anterior neuroectoderm, a location that could become part of either the brain or the ring gland (29) and distinct from the mesodermal origin of hemocytes (14). A striking feature of the data is that gynandromorphs either have both wings fully normal or fully mutant. This observation is consistent with a bilateral pair of nervous system primordia that interact in a submissive manner to establish the mutant wing phenotype in a nonautonomous manner (9, 10). Thus, the role of *bae* could be to control wing maturation by the release of a hormone that increases blood pressure, causing wing unfolding, and that activates hemocytes to perform their roles of phagocytosis and ECM synthesis. Wing inflation has been ascribed to an unidentified neuroendocrine factor different from the eclosion hormone (30). A phenotype very similar to that of *bae* is produced by ectopic expression of *UAS-dCBP(nej*$^{+}$*)* using *GAL4* strains expressed in specific central nervous system cells (31).

Effect of PKAc on Wing Maturation. Comparison of the effects of Ricin A and of PKAc on wing maturation indicates that ectopic PKAc does not simply inactivate hemocytes. Instead, it appears to substitute one normal function of hemocytes for another. Rather than carry out phagocytosis and ECM synthesis, hemocytes enter into an innate immune response in which lamellocytes are differentiated and crystal cells melanize target cells (32). Evidently, aggregation of lamellocytes within the wing blade interferes with wing expansion, and loss of normal hemocyte function interferes with bonding of dorsal and ventral surfaces. The observation that the effect of ectopic PKAc on the wing is suppressed by overexpression of Pan, the *Drosophila* homolog of mammalian blood cell transcription factors (lymphocyte enhancer-binding factor 1 and T cell factor), suggests that ectopic PKAc inhibits, or represses synthesis of, Pan, which in turn inhibits Wingless target gene expression. This conclusion is strengthened by the observation that ectopic expression of *UAS-dCBP(nej*$^{+}$*)* using *GAL4–30A* produces phenotypes similar to those caused by ectopic PKAc (31). Pan is bound and its transcriptional activity inhibited by dCBP (33). Expression of PanΔN, a dominant-negative inhibitor of Wingless target gene expression, elicits what seems to be a massive induction of the cellular innate immune response. Thus, the Wingless signal trans-

duction pathway may be involved in regulating a choice between the innate immune response and the apoptotic/ECM response (5).

The dominant–lethal interaction between ectopic PKAc and *bae* is intriguing. When and how death occurs needs closer examination, as does the cellular focus of *bae* activity. What role PKAc normally plays in regulating hemocyte behavior remains to be investigated. The association of a wing phenotype with altered hemocyte behavior should provide a means of identifying additional genes involved in hemocyte function during wing maturation.

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