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Misexpression Screen in *Drosophila melanogaster* Aiming to Reveal Novel Factors Involved in Formation of Body Parts

Nicole C. Grieder,*,[1] Ilias Charlafti,* Urs Kloter,* Herbert Jäckle,[†] Ulrich Schäfer[†] and Walter J. Gehring*

**Biozentrum der Universität Basel, Abteilung Zellbiologie, CH-4056 Basel, Switzerland and* [†]*Max-Planck Institut für Biophysikalische Chemie, Abteilung Molekulare Entwicklungsbiologie, D-37077 Göttingen, Germany*

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ABSTRACT

To identify novel factors that lead a fly imaginal disc to adopt its developmental fate, we carried out a modular dominant misexpression screen in imaginal discs. We have identified two factors that appear to change the fate of the respective body structure and appear to lead to the transformation of a body part. In one mutant line, notum tissue, normally derived from wing imaginal tissue, formed close to the site of the sternopleural bristles, which are leg disc derivatives. In the other line, the arista is transformed into a tubular structure, resembling an abnormal leg. We found that ectopic expression of *abrupt* was responsible for this potential transformation of the arista.

In *Drosophila melanogaster*, a considerable number of mutant flies, which lack a certain body structure or display a body structure at an ectopic position of the body, have been discovered. With the help of such mutants, key factors involved in the formation of specific body parts have been identified. For example, through powerful genetic screening procedures, mutants in the *bithorax* complex, which display transformations of body segments, have been recovered (Lewis 1978). Their molecular identification has led to the discovery of the *Hox* gene family and the homeobox (reviewed in Gehring 1993). In these mutants, the phenotype was mostly due to the loss of a certain homeotic gene activity. However, ectopic body structures can also be obtained by ectopic expression of those key genes (reviewed in Gehring 1993). Such examples are the fortuitous dominant mutation *Nasobemia* or *Antennapedia*^{73b} (*Antp*^{73b}), both of which are alleles of *Antp* and display the antenna-to-leg transformations (Gehring 1966; Garber *et al.* 1983). Molecular analysis of *Antp*^{73b} indicates that this phenotype is due to the misexpression of *Antp* (Schneuwly *et al.* 1987a). Ectopic or overexpression of a gene can be achieved by introducing *in vitro*-designed constructs into the fly. Ectopic body structures have also been gained in this way; for example, by heat-shock-mediated overexpression of *Antp* an antenna-to-leg transformation has been obtained (Schneuwly *et al.* 1987b). In recent years, the *Gal4-UAS* system has become the method of choice to force expression of genes and of *in vitro*-modified constructs, as it allows turning on transcription units in defined patterns and at defined times in development (Brand and Perrimon 1993). In this way, it has been possible to induce the formation of ectopic eyes in several locations of the fly body by inappropriate expression of the *eyeless* gene (Halder *et al.* 1995). Kim *et al.* (1996) have recovered wing tissue at several locations by forcing ectopic expression of *vestigial* (*vg*), a key gene in wing formation. Genome-wide over- and misexpression screening procedures have only recently become feasible in Drosophila. These techniques rely on modified *P*-element transposons, like the *EP* element, the *GS* element, or the *Mae-UAS.6.11* element (Crisp and Merriam 1997; Rørth *et al.* 1998; Toba *et al.* 1999). In these vectors, UAS-dependent transcription is directed outward from these *P* elements and into the neighboring genomic region in the presence of Gal4, leading to an overexpression of the gene in the vicinity of the *P*-element insertion site (*e.g.*, see Figure 1A). As *P* elements typically insert into the 5′ region of genes (Spradling *et al.* 1995), a random collection of such *P* elements will frequently allow overexpressing full-length coding sequences. Alternatively, antisense transcripts of neighboring genes are produced.

How a tissue acquires the identity of a limb or of a trunk region is a fundamental question in development. In *D. melanogaster*, the fly exoskeleton and its appendages derive from primordial groups of cells called imaginal discs, which are set aside during embryogenesis for later development during the larval and pupal stages. The way in which imaginal discs give rise to appendages (*e.g.*, legs and wings) and trunk regions (body wall) has become an experimental system, allowing the

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[1]*Corresponding author:* Biozentrum der Universität Basel, Abteilung Zellbiologie, Klingelbergstrasse 50-70, CH-4056 Basel, Switzerland. E-mail: nicole.grieder@unibas.ch

study of the establishment of fate determination. A tremendous amount of knowledge has accumulated about how the antero-posterior axis is subdivided and how the diversity of the fly segments is achieved in the embryo (reviewed in Cohen 1993; Gehring 1993; Mann and Morata 2000). However, there still must be unidentified key genes that contribute to the determination of body structures. Among them, there should be factors that relate the appendage-determining genes to the *Hox* selector genes, which are responsible for the antero-posterior diversity of the adult segments. Such links are only now being discovered (Gebelein *et al.* 2004). These selector genes also need to cooperate with signaling factors (Grieder *et al.* 1997; reviewed in Affolter and Mann 2001). For example, this was revealed in experiments identifying *Notch*, a prominent signaling molecule implicated in growth and differentiation, as a factor involved in appendage determination (Kurata *et al.* 1999).

Experiments, presented by Kurata *et al.* (1999) and subsequently by Katsuyama *et al.* (2005), have also shown that factors with a transdetermination potential can be discovered in ectopic expression screens. Such factors either are directly responsible for the change of cell identity or render cells capable of changing their identity (Kurata *et al.* 1999; Katsuyama *et al.* 2005). The transdetermination phenomenon was discovered by Hadorn (1963). He has shown that, following transplantation, predetermined imaginal disc cells spontaneously change their state of determination such that the transplanted discs develop different structures, which no longer correspond to their initial specification. Transdetermination is associated with tissue regeneration, dedifferentiation, and growth of the transplanted disc (Schubiger 1971). The mechanism underlying dedifferentiation has not yet been discovered. However, several more factors have been found to play a role in transdetermination. For example, a crucial role for intercellular signaling—mediated by factors like *wingless* (*wg*) and *decapentaplegic* (*dpp*), Drosophila Wnt and BMP2/4 homologs, respectively—is emerging from genetic studies and from transdetermination experiments (Kim *et al.* 1996; Maves and Schubiger 1998). It has been shown that ectopic expression of the signaling molecule *wg* in predetermined leg discs (during late third instar larval development) induces the formation of wing tissue, an experimental condition recapitulating in many ways the events associated with a classical transdetermination experiment (Sustair and Schubiger 2005). Recently, it has been shown that this transdetermination event is dependent on Jun kinase (JNK)-signaling-mediated regulation of the *polycomb* group genes, genes that are involved in gene silencing and maintenance of homeotic gene activity (Lee *et al.* 2005). A role for chromatin-interacting proteins has also emerged in a study investigating transdetermination of the eye (Katsuyama *et al.* 2005). Interestingly, many of these factors are also important for the specification of identity, as it occurs during development (reviewed in Maves and Schubiger 2003). This raises the question of whether transdetermination and developmental determination are related.

In summary, it is likely that a number of unidentified factors that are involved in fate determination and in transdetermination still exist. To elucidate the mechanisms of these processes at the cellular and molecular level, it is necessary that the function of such novel factors be revealed. To find novel factors, which confer identity to fly appendages and trunk regions, we embarked upon a genetic screen. Ectopic expression of selector genes leads to a transformation of the body. Therefore, novel factors that have potential as tissue- or appendage-defining factors should become immediately apparent in a dominant genetic misexpression screen in imaginal discs of *D. melanogaster*. They will be revealed as mutants displaying transformed body parts, since such factors will redirect the development of an imaginal disc into an allotypic structure.

In this article, we present a misexpression screen aiming at identifying novel factors that lead a fly imaginal disc to adopt its developmental fate. Among ~2800 independent *P*-element insertions, we found two lines that exhibited apparently transformed body structures. In one mutant, the dorsal part of the leg disc appeared dorsalized and a notum-like outgrowth was formed at the site of the sternopleura. In the other line, the arista appeared transformed into a tubular structure, resembling an abnormal leg.

MATERIALS AND METHODS

Drosophila strains: The *y w; P{w$^+$ dpp^{disc}-Gal4}/TM6B, Hu e Tb* line is described in Staehling-Hampton *et al.* (1994). The strains used to generate new *P{Mae-UAS.6.11}* insertions (Crisp and Merriam 1997) were described in Beinert *et al.* (2004). The *y w; P{w$^+$ UAS-argos.M}30-102-1; P{w$^+$ UAS-argos.M}30-85-1* (donation to the Bloomington Stock Center by A. Michelson), the *y w ; P{w$^+$ UAS-fringe.K}JK1* (Kim *et al.* 1995), and the *FM6, y^1 w^1 dm$^+$* balancer stocks were obtained from the Bloomington Stock Center. The *P{w$^+$ UASp-lacZ}1399-4* line (Rørth 1998) was obtained from Pernille Rørth. The *y w; P{y+, UAS- GFP}* stock (Gerlitz *et al.* 2002) was obtained from Konrad Basler. Generation of *y w; P{w$^+$ UAST-abrupt}42-6/TM6B* and *y w; P{w$^+$ UAST- abrupt}42-2/CyO* is described below. The *homothorax (hth)-lacZ* line is described in Pai *et al.* (1998). The *vgB-lacZ* line is described in Kim *et al.* (1996).

Crossing scheme: In a cross, typically three virgins of a *y w; P{w$^+$ dpp^{disc}-Gal4}/TM6B, Hu e Tb* stock were mated to one to three males of independent *y w P{Mae-UAS.6.11}* insertions (see Figure 1A) provided by Beinert *et al.* (2004). At least 20 flies of the F_1 *y$^+$ w$^+$* progeny were scored for mutant phenotypes in adults or in late pupal stages. Candidate *P*-element insertions were retested and balanced using the *FM6, y^1 w^1 dm$^+$* or a *y w; Kr^{If}/CyO$_{blue}$; TM6B, Hu e Tb/MKRS* balancers. Mutant flies were stored in acetic acid/glycerol (4 + 1). They were washed in 1× PBS before dissection and mounted in Faure's for image preparation. Due to storage in acetic acid/glycerol,

the red eye color often faded. For the "*abrupt*" control crosses, virgins of the *w; wg^{Sp}/CyO; P{w$^+$ dpp^{disc}-Gal4}/TM6B, Hu e Tb* genotype were crossed to *y w/Y; P{w$^+$ UAST- abrupt}42-6/TM6B* or *y w/Y; P{w$^+$ UAST- abrupt}42-2/(CyO)* males to obtain abrupt-overexpressing progeny; for some of the antibody-staining experiments, *UAS-GFP* (Gerlitz *et al.* 2002) was crossed into the *B2149* background. Similarly, *hth-lacZ* (Pai *et al.* 1998) was crossed into a *B2149* background, and *vgB-lacZ* (Kim *et al.* 1996) was crossed into the *dpp^{disc}-Gal4* background.

Molecular analysis: *P{Mae-UAS.6.11}*-element insertions were mapped by iPCR as described in Beinert *et al.* (2004). Alternatively, a modification of the Exelixis inverse polymerase chain reaction (iPCR) method (Thibault *et al.* 2004) was employed. In this protocol, genomic DNA was digested with either *Rsa*I or *Msp*I restriction enzymes. After religation, the following primers were used for amplification of the insertion site fragments: primers TGGGAATTCGTTAACAGATCCAC (reverse), CAGCTGCGCTTGTTTATTTG (forward), ACACA ACCTTTCCTCTCAACAA (Sp1), and GAATTGTCGCTCCG TAGACGA (5′P forward2) for the 5′-end and primers CAAT CATATCGCTGTCTCACTCA (ry4), CCTTAGCATGTCCGTG GGGTTTGAAT (ry1), GCGAGTACGCAAAGCTTGGC (Sp6), and TCAATACGACACTCAGAATACTATTC (Spep4) for the 3′-end. The obtained DNA fragments were sequenced on an ABI Prism 310 Genetic Analyzer using primers TAAGTGTA TACTTCGGTAAGCT (5′P) and GCATACGTTAAGTGGAT GTCTCT (3′P), and the obtained sequences were BLASTed (Altschul *et al.* 1997) against the genomic Drosophila sequence (Adams *et al.* 2000; Celniker *et al.* 2002) and submitted to the EBI database. More detailed protocols are available upon request.

Antibody staining: Antibody stainings were carried out as described in Grieder *et al.* (2005). The following antibody concentrations were used: rat monoclonal antibody 7E8A10 anti-Embryonic Lethal, Abnormal Vision (Elav) [Developmental Studies Hybridoma Bank (DSHB)], 1:30; rabbit anti-Abrupt (Hu *et al.* 1995; a gift from Stephen Crews), 1:500; the monoclonal antibody 4D4 anti-Wg, 1:50 (DSHB); rat anti-Distal antenna (Dan), 1:30 (Emerald *et al.* 2003; a gift from Steve Cohen); guinea pig anti-Eyegone (Eyg), 1:200 (Aldaz *et al.* 2003; a gift from Natalia Azpiazu), rabbit anti-Spalt Major (Salm), 1:30 (Kühnlein *et al.* 1994; a gift from Reinhard Schuh), rabbit anti-Hth, 1:400 (Pai *et al.* 1998; a gift from Y. Henry Sun). The goat anti-mouse Alexa488 or Alexa568 antibody (Molecular Probes, Eugene, OR) was used at 1:400; donkey anti-rat or mouse Cy5 Fab fragments (Jackson ImmunoResearch, West Grove, PA) were used at 1:400.

***In situ* hybridization:** *In situ* hybridizations on whole-mount imaginal discs and on chromosomes were carried out as described in Grieder *et al.* (2005).

Transgenes: To generate pPUAST-abrupt (ab), the *ab* coding sequence was recovered as a *Mfe*I–*Asp*718 fragment from cDNA clone RE25924 (BACPAC Resource Center) and cloned into the *Eco*RI and *Asp*718 site of pPUAST (Brand and Perrimon 1993). This pPUAST-abrupt construct 42 was introduced into *y w* flies by *P*-element transformation (Rubin and Spradling 1982).

RESULTS

Design of the screen: When Czerny *et al.* (1995) carried out misexpression studies in imaginal discs using *UAS-eyeless* and *UAS-twin of eyeless* lines, they found that the *dpp^{disk}*-enhancer-driven *Gal4* line (Staehling-Hampton *et al.* 1994) was one of the most suitable driver lines to obtain ectopic eye structures. This *Gal4* line is expressed during the larval stages of Drosophila development in a subset of imaginal disc cells (Staehling-Hampton *et al.* 1994; Figure 5, B and C). Using this line, disc transformations can be obtained in a highly reproducible fashion and at high frequency. We tested this line by combining it with *UAS* constructs forcing expression of several *Hox* genes. The results of these experiments confirmed that the expected transformations are obtained (data not shown). Thus, we considered this line well suited to be used in our screen for transformation phenotypes. We carried out a modular dominant misexpression screen in imaginal discs utilizing the *P*-element collection generated by Beinert *et al.* (2004), which employs the *P{Mae-UAS.6.11}* element (Crisp and Merriam 1997; Figure 1A; materials and methods). The *P{Mae-UAS.6.11}* element has the advantage of being marked with *yellow*$^+$. As our driver *Gal4* line is marked with mini-*white*$^+$ and the responding *P{Mae-UAS.6.11}* fly collection is in a *y w* mutant background (Beinert *et al.* 2004), progeny containing both lines can be easily identified among *y w* flies, as they appear *y*$^+$ *w*$^+$*;* [they can be discriminated from non-expressing flies, which are *y w*$^+$ (containing *dpp^{disc}-Gal4*) or *y*$^+$ *w* (containing the *P{Mae-UAS.6.11}* element; Figure 1A]. To our convenience, the *dpp^{disc}-Gal4* line was located on the third chromosome and was balanced over a chromosome marked with the *Tb* mutation (materials and methods); this allowed unequivocal separation of late pupae and pharate adults in expressing (Tb$^+$) and nonexpressing (Tb$^-$) populations.

The screen reveals two lines with a potential transformation phenotype: The *y w P{Mae-UAS.6.11}* collection had been assembled aiming to target X chromosomal genes (Beinert *et al.* 2004). However, we also screened *P*-element insertions on autosomes; a total of 2810 lines were screened (materials and methods). We found 2 lines that showed potential transformation phenotypes (2/2810 or 0.7%): line *B1164* and line *B2149* (Figure 1, B–E, G). Line *B1164* consistently displayed an outgrowth at the position of the sternopleura (Figure 1B). Morphologically, this outgrowth looked like notum tissue. In addition, this mutant had abnormal legs, as well as abnormal wings (Figure 1B; Figure 2.12). In particular, the proximal parts of the wings and the legs were shortened. The other line (*B2149*) displayed an apparently transformed arista (Figure 1, C and G). The arista was growing out as a short tube-like structure (Figure 1G). Morphologically, it resembled the legs of this mutant, which were also abnormal (Figure 1D). As this mutant did not eclose, we did not score the wing phenotype.

Destructive phenotypes were about nine times more frequent than the potential transformation phenotypes: Among the screened *P{Mae-UAS.6.11}* lines, we observed other mutant phenotypes, which could be termed "destructive." These lines appeared not to transform the tissue into another tissue characteristic of another

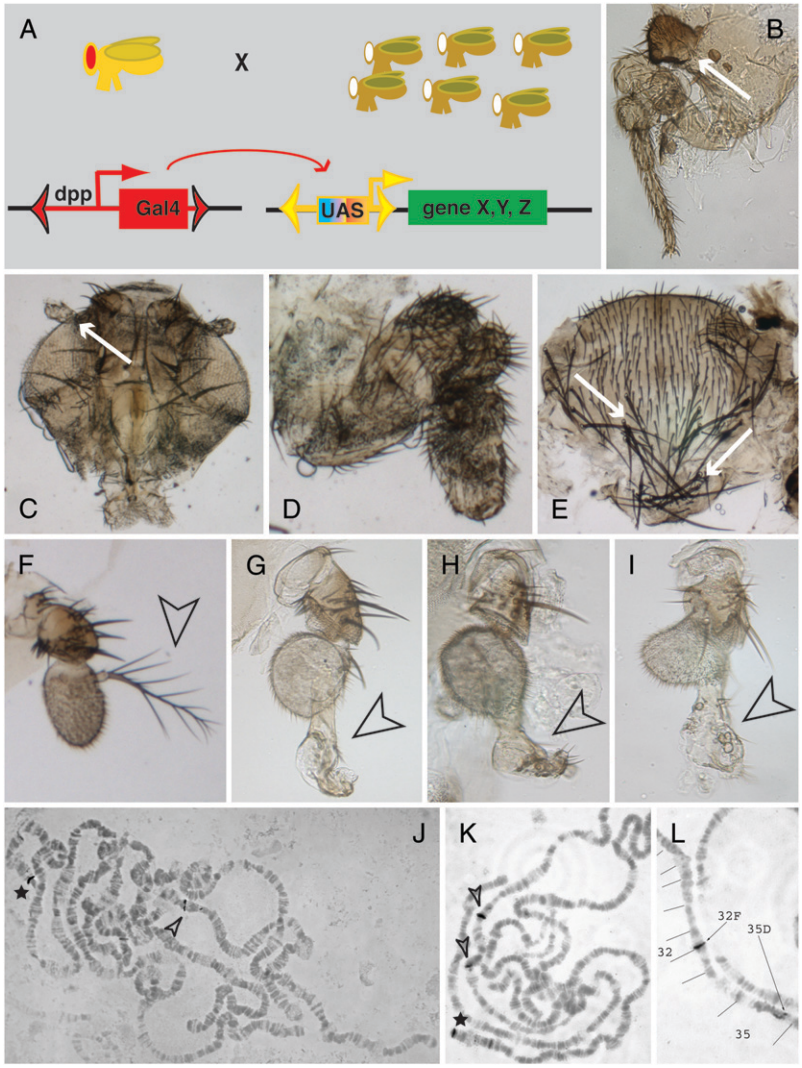

Figure 1.—Screen design: phenotypes of mutants exhibiting transformations, *B1164* and *B2149*. (A) *Gal4* is transcribed in *dpp*-expressing cells. The transgene is marked with *mini-white*. The *yellow*$^{+}$ transgene contains GAL4-binding sites (*UAS*) and upon Gal4 binding and activation directs transcription outward into the genomic region; chromosome is represented as the black line, *P* element as red line or yellow line; not drawn to scale. (B) Outgrowth of notum tissue (arrow) on the sternopleural plate between the leg and the wing of a fly expressing *B1164* under *dpp*^{disc}*-Gal4* control. (C) Head. (D) Leg. (E) Notum of a fly expressing *B2149* under *dpp*^{disc}*-Gal4* control. Arrow in C points at transformed arista; arrows in E at supernumery macrochaetes. (F) An antenna of an Oregon-R fly. (G) Antenna of a fly containing the *P{Mae-UAS.6.11}B2149* element and the *dpp*^{disc}*-Gal4* line. (H) Antenna of a fly expressing *abrupt* using the *dpp*^{disc}*-Gal4* line in combination with *P{w*$^{+}$ *UAS- abrupt}42* (on chromosome 2). (I) Antenna of a fly expressing *abrupt* using the *dpp*^{disc}*-Gal4* line in combination with *P{w*$^{+}$ *UAS- abrupt}42* (on chromosome 3). Arrowhead in F points at the position of arista. Arrowheads in G–I point at the position of the transformed arista. (J) *In situ* on salivary gland chromosome of a *B2149* larva with a *yellow* probe labeling the endogenous *yellow* gene (asterisk) and the insertion of the *P* element on chromosome 2 at 32C (arrowhead). (K and L) *In situ* on salivary gland chromosome of a *B1164* larva with a *yellow* probe, labeling the endogenous *yellow* gene (asterisk in K) and the insertions of the *P* element on chromosome 2 at 32F and 35D (arrowhead in K and arrows in L).

location, *e.g.*, a wing into a leg; rather, they displayed abnormal structures, *e.g.*, loss of veins. Nevertheless, it cannot be determined *a priori* whether these lines cause a gain or a loss of structure or function, respectively. First, the *P* element may produce an antisense message of a given gene and cause a loss-of-function phenotype. Second, a loss of structure, *e.g.*, a loss of vein, could also be an indication of a gain of another structure, *e.g.*, intervein tissue. Of all the lines screened, 170 lines (170/2810 or 6%) produced mutant phenotypes. We scored mainly abnormalities on the wings, the legs, and the antennae, but not on eyes or the fly trunk. The abnormalities that we observed ranged from subtle disturbances of the wing venation pattern to the inability to open wings and early pupal lethality. The insertion site of some lines could not be determined; other lines got lost during the rescreening procedure. All the lines for which the inserts were sequenced are listed in supplemental Table 1 (http://www.genetics.org/supplemental/). Examples of the mutant phenotypes are shown in Figures 2–4; mutant wings are shown in Figure 2, mutant legs in Figure 3, and mutant heads in Figure 4.

Mutant phenotypes are reproduced in independent hits: Although we were looking mainly for ectopic formation of body structures, the lines displaying abnormal body structures were useful in estimating the general reproducibility of the *P{Mae-UAS.6.11}*-induced phenotypes and thus served as a control for our screening procedure. We expected that insertions into the same loci would lead to similar if not identical phenotypes. To find out whether this was indeed the case, we determined the insertion sites of all lines possible (materials and methods; supplemental Table 1 at http://www.genetics.org/supplemental/). Sequencing of the *P{Mae-UAS.6.11}* element flanking sequences readily revealed that several genes were hit more than once. The associated phenotypes were alike or similar in the sense of allelic series. For example, lines containing a *P* element that had inserted upstream and in a position driving transcription of *broad* were found three times to exhibit similar shortening of legs (Figure 3.15). As

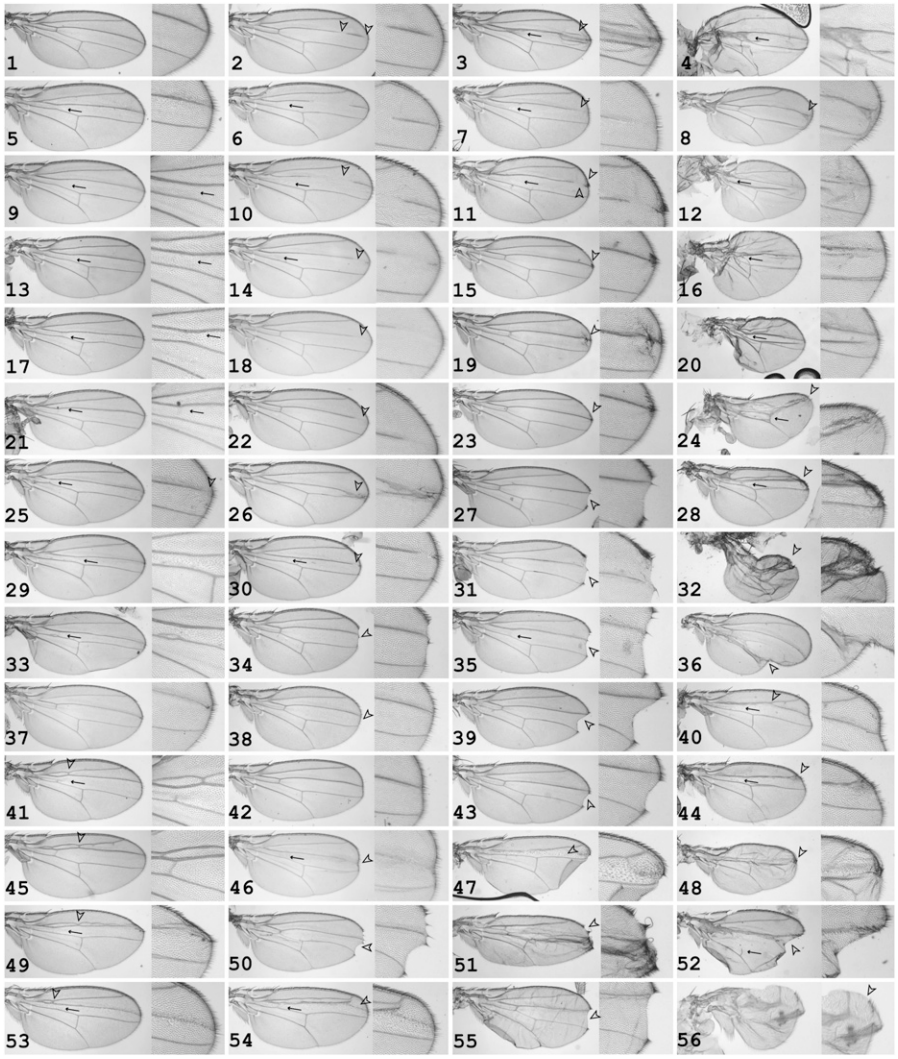

Figure 2.—Wing phenotypes. Entire wings are shown on the left and close-ups of mutant features are shown on the right. Some mutant features are highlighted with arrows and arrowheads; for additional description of phenotypes, see supplemental Table 1 at http://www.genetics.org/supplemental/. L, longitudinal vein; cv, cross vein. (1) Oregon-R. (2) *B0915*: arrowheads point at gaps in L3. (3) *B0079*: anterior cv malformed (arrow), L3 thickened, few wing blisters (arrowhead). (4) *B1513*: wing shortened (mainly proximal), vein fusions (arrow). (5) *B0613*: anterior cv occasionally missing (arrow), L3 often not extending to margin, wing appears rounder distally. (6) *UAS-argos*: cv missing (arrow), L3 gapped. (7) *B0891*: wing shortened distally, L3 often not extending to margin (arrowhead), anterior cv sometimes missing (arrow). (8) *G1060*: vein fusions, L3 enlarged at distal end (arrowhead), margin can be affected. (9) *B0193*: anterior cv missing (arrows). (10) *B0911*: L3 gapped (arrowhead), anterior cv can be missing (arrow). (11) *B1512*: anterior cv missing (arrow), L3 gapped, margin defect, ectopic bristles (arrowheads). (12) *B1164*: wing shortened proximally (arrow points in direction where anterior cv should be, but anterior cv is absent, phenotype variable). (13) *B2022*: anterior cv missing (arrows). (14) *B0256*: anterior cv missing (arrow), L3 not extending to margin (arrowhead). (15) *B0326*: ectopic bristles close to wing margin (arrowhead). (16) *G1365*: wing shortened proximally (arrow). (17) *B0176*: anterior cv missing (arrow). (18) *B0748*: L3 not extending to margin (arrowhead). (19) *G1286*: L3 affected at distal end, surrounding intervein tissue not flat (arrowhead). (20) *G1430*: wings shortened, anterior cv missing (arrow). (21) *B0611*: anterior cv missing (arrows). (22) *B0625*: L3 not extending to margin (arrowhead). (23) *B0538*: ectopic bristles close to wing margin (arrowhead). (24) *B1505*: central wing domain missing (posterior and submarginal cell), some margin bristles affected (arrowhead), arrow points toward posterior cv. (25) *B2008*: anterior cv missing (arrow). (26) *B2038*: blistered wings, first posterior cell narrower, vein defects, ectopic bristles on wing blade (arrowhead). (27) *B0538*: some notched wings (arrowhead). (28) *B1368*: vein fusions (arrow), reduction of first submarginal and posterior cell (arrowhead), wings appear shorter. (29) *B1112*: first posterior cell narrower, anterior cv missing (arrow). (30) *G1477*: typically anterior cv missing (arrow), L3 gapped at end (arrowhead). (31) *UAS-fringe*: wing notched (arrowhead). (32) *G1554*: central intervein cells absent, vein fusions (arrowhead). (33) *G1326*: anterior cv affected (arrow), central intervein cells appear narrower. (34) *B0621*: wing appears shorter, roundish, sometimes notched (arrowhead points to margin). (35) *B0995*: anterior cv missing (arrow), wing notched (arrowhead). (36) *B0960*: veins affected (arrowhead points to L5), first and second cell affected. (37) *B0751*: first submarginal cell narrower at distal end. (38) *B0211*: wing shortened distally, roundish (arrowhead). (39) *B1506*: notched (arrowhead). (40) *B1193*: submarginal cell almost absent, vein fusions (arrowhead), anterior cv missing (arrow). (41) *B0756*: anterior cv missing (arrow), submarginal cell affected (arrowhead). (42) *B0531*: wing shortened at distal end, roundish. (43) *B0016*: wings can be notched (arrowhead). (44) *B0929*: vein defects (arrow and arrowhead), first and submarginal cell narrower or fused. (45) *B1022*: occasionally anterior cv missing or vein fusions in submarginal cell (arrowhead). (46) *B0404*: wing shortened, roundish (arrowhead), veins affected (arrow), L3 may not extend to margin. (47) *B0323*: L3 absent, hairs in first/submarginal cell misoriented (arrowhead), vein defects. (48) *B0469*: submarginal and first cell missing, arrowhead points to margin where fused veins end. (49) *B0066*: venation defects mainly around L2 and L3 (arrow and arrowhead). (50) *B0519*, notched wings (arrowhead). (51) *B2026*: notched wings (arrowhead). (52) *G1179*: first posterior cell missing or fused (arrowhead), venation defects (arrow to posterior cv). (53–54) *B2036*: first submarginal cell and L3 affected, anterior cv missing (arrow), vein fusions (arrowhead). (55) *B0969*: notched wings (arrowhead). (56) *B2032*: wing vein missing, margin bristles missing (arrowhead).

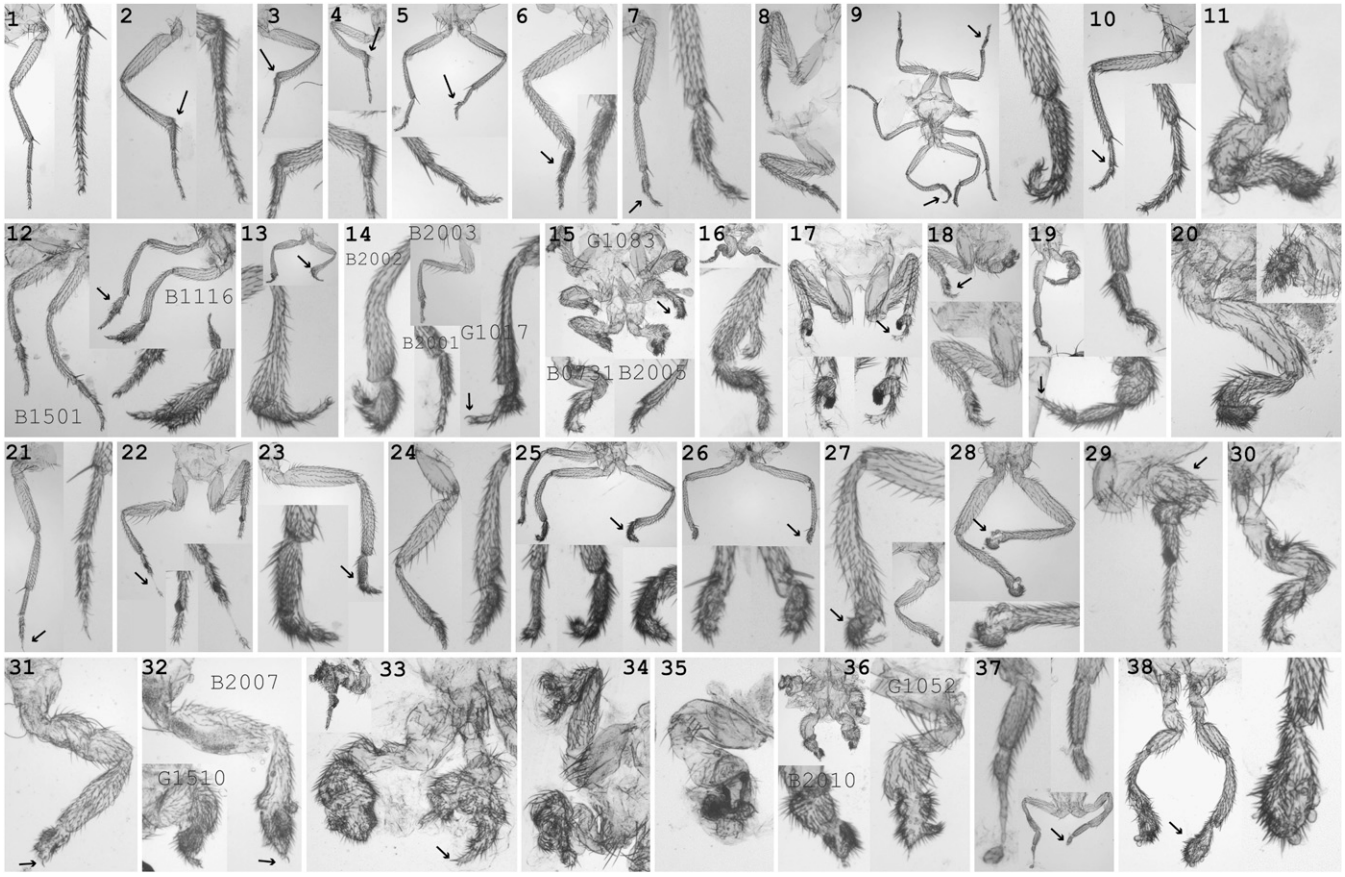

Figure 3.—Leg phenotypes. Insets show a close-up of the most affected leg region. Arrows typically point at the most prominently affected part of leg or claw. (1) Oregon-R. (2) *B2028*: legs bent at tarsi, mainly third legs. (3) *B2030*: slightly bent third legs at tarsi. (4) *B1350*: legs slightly bent at tarsi. (5) *G1153*: shortened tarsi, mainly third legs. (6) *G1477*: tarsi shortened. (7) *G1326*: tarsi shortened, one claw. (8) *B1522*: tarsi shortened. (9) *B0469*: tarsi shortened. (10) *B1368*: tarsi shortened. (11) *G1179*: legs deformed and shortened. (12) *B1501* and *B1116*: tarsi shortened. (13) *B1510*: tarsi shortened, thickened. (14) *B2001*, *B2002*, *B2003*, and *G1017*, tarsi shortened. (15) *B2005* and *G1083*: legs deformed and shortened (arrow points at claw). (16) *G1022*: legs deformed and shortened, claws sometimes absent; close-up in inset is from another leg of the same genotype (outgrowth/split within leg). (17) *B0323*: tarsi truncated, no claws (arrow). (18) *B1505*: legs shortened and deformed, often no claws. (19) *G1430*: tarsi or entire leg deformed, often only one claw (arrow). (20) *G1341*: legs deformed and shortened, often one claw missing. (21) *B1193*, tarsi truncated, claw often missing (arrow). (22) *B0942*: tarsi truncated, claws sometimes present. (23) *B1512*: mainly tarsi affected, shortened. (24) *B0960*: tarsi truncated, claws often missing. (25) *B0611*: tarsi shortened (arrow), claws absent. (26) *B0462*: tarsi truncated, claws often missing. (27) *B1508*: tarsi truncated, claws often missing. (28) *B1509*: tarsi truncated, typically no claws. (29) *B2037*: legs shortened, mainly proximal. (30) *B0731*: leg deformed, tarsi shortened, claws often present. (31) *G1554*: leg shortened, claw sometimes missing (arrow points at claw). (32) *B2007* and *G1510*: legs affected, mainly tarsi shortened, mainly one or no claw. (33) *B0183*: extreme shortening and malformation of leg, sometimes split legs, one claw often present. (34) *B0472*: legs shortened, tarsi truncated, sometimes split, often no claws. (35) *B0699*: legs deformed, tarsi truncated, no claws. (36) *B2010* and *G1052*: pronounced leg shortening, no claws. (37) *B1511*: tarsi truncated, no claws. (38) *B0617*: legs deformed, mainly tarsi truncated, no claws.

another example, independent lines containing an insertion at 8F9 close to *nej* and *CG15321*, showed similar truncations of legs (Figure 3.36; supplemental Table 1 at http://www.genetics.org/supplemental/). In the third example, the *P*-element insertions were associated with strange phenotypes, such as problems in unfolding the wings, and were isolated several times in connection with *CG14628* (supplemental Table 1 at http://www.genetics.org/supplemental/). In addition, this analysis confirmed that most of these *P{Mae-UAS.6.11}* elements were inserted upstream of known transcripts and led to overexpression of the respective gene. As expected, some *P{Mae-UAS.6.11}* lines seemed to be associated with putative antisense transcripts. This was the case, *e.g.*, for line *B1509*, in which antisense transcripts of *CG2023* appear to be made. *PUAST* transgenes should also confirm that the insertion in the respective gene was sufficient to cause the observed mutant phenotypes. We used transgenic lines for *fringe* and *argos* in a control experiment (materials and methods). When crossed to the *dpp^{disc}-Gal4*, their progeny showed very similar mutant phenotypes like the genomic *P{Mae-UAS.6.11}* insertions [Figure 2.6 (*UAS-argos*); Figure 2.2 (*B0915*); Figure 2.31 (*UAS-fringe*); and two examples for *B0538* in

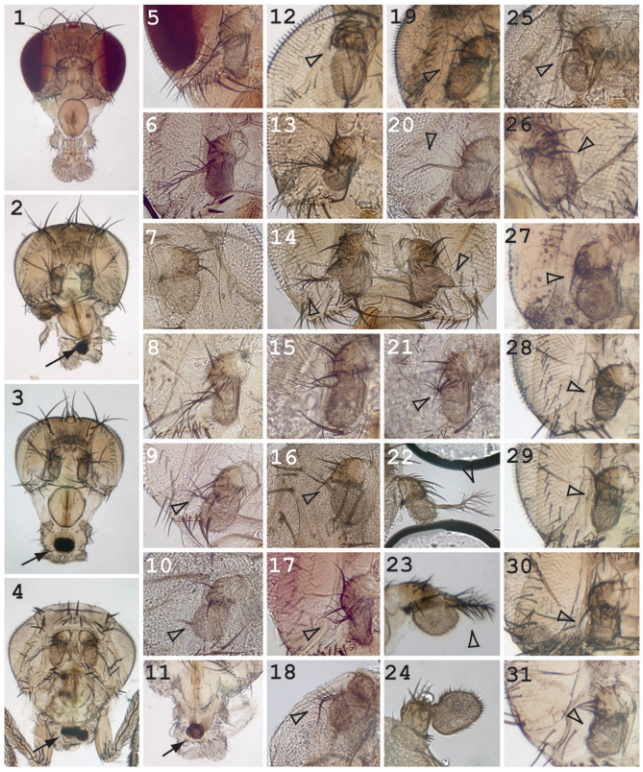

FIGURE 4.—Head phenotypes: (1–4) whole heads and (5–31) antennal regions. Arrows and arrowheads point to a particular abnormality of arista or an antennal segment, except in 2–4 and 11, where the arrows point at dead cells within the medi-proboscis. (1) Oregon-R. (2) *G1082*. (3) *G1156*. (4) *B1501*. (5) Oregon-R. (6) *B1510*. (7) *B1511*. (8) *B0942*. (9) *B0469*. (10) *B0968*. (11) *B1522*. (12) *B2012*. (13) *G1179*. (14) *B1512*. (15) *B0617*. (16) *G1373*. (17) *B0995*. (18) *B0699*. (19) *G1017*. (20) *B0462*. (21) *B0323*. (22) *G1554*. (23) *B0216*. (24) *B2011*. (25) *G1341*. (26) *B1505*. (27) *B1508*. (28) *B0079*. (29) *B0911*. (30) B0193. (31) *B1509*.

Figure 2.23 and 2.27; supplemental Table 1 at http://www.genetics.org/supplemental/]. Insertions in genes that have never been targeted by a *P* element were not recovered. However, some that have rarely been hit or not been hit with a *UAS*-tagged construct have been found. One example of such a case is line *B0729*. Considering the large number of *P*-element screens that have been carried out, and considering the fact that *P* elements show insertion site preferences, this is not surprising (see SPRADLING *et al.* 1999).

In line *B2149*, the *P{Mae-UAS.6.11}* element is inserted upstream of *abrupt*: Since the potential transformation phenotype was due to Gal4 expression in the *B1164* and the *B2149* flies, the gene of interest would have to be in the neighborhood of Gal4-binding sites, and hence of the *P{Mae-UAS.6.11}*-element insertion sites. The *P* elements present in *B1164* and *B2149* mapped genetically to chromosome 2. Therefore, to find out which genes were causing the transformation phenotypes, the *P{Mae-UAS.6.11}* insertion sites were determined. We found that the *P* element in line *B2149* was inserted just a few nucleotides upstream of the first exon of the *abrupt* gene (Figure 5A; supplemental Table 1 at http://www.genetics.org/supplemental/), encoding for a nuclear BTB–zinc-finger protein (HU *et al.* 1995). The *P* element was inserted such that the 5′-end of the *P* element was oriented toward the *abrupt* gene. Thus Gal4 activation should lead to overexpression of *abrupt* and, consequently, the potential transformation of the arista might be induced through overexpression of *abrupt*. The insertion site at 32C was also confirmed by *in situ* hybridization to salivary gland chromosomes (Figure 1J). Chromosome *in situ* hybridization to salivary gland chromosomes of line *B1164* revealed two *P*-element insertion sites, one at 35D and the other at 32F (Figure 1, K and L). iPCR reactions on line *B1164* were not straightforward; a sequence for only one of the *P*-element insertion sites was obtained by iPCR; that site was found in a nonannotated *3S18/Belsazar* element on chromosome 2 (data not shown). *B1164*'s molecular analysis will be presented elsewhere (N. C. GRIEDER and W. J. GEHRING, unpublished results).

***abrupt* is overexpressed in Gal4-expressing *B2149* discs:** Our iPCR analysis suggested that *abrupt* should be overexpressed in *B2149* discs when combined with a *Gal4* line, in our case, dpp^{disc}*-Gal4*. To find out whether *abrupt* was indeed overexpressed in a *dpp*-like pattern of such discs, we carried out whole-mount *in situ* hybridizations (MATERIALS AND METHODS). To this end, we used a digoxigenin-labeled *abrupt* antisense riboprobe and a sense control probe on imaginal discs of genotype *B2149;* dpp^{disc}*-Gal4* as well as on control *y w* discs (Figure 5; MATERIALS AND METHODS; data not shown). As already shown by HU *et al.* (1995), *abrupt* is widely expressed in wild-type imaginal discs (also see Figure 5, D and E; data not shown); interestingly, *abrupt* expression seems confined to the region anterior to the morphogenetic furrow in the eye disc (Figure 5D; HU *et al.* 1995). In discs of individuals containing both dpp^{disc}*-Gal4* and *B2149*, *abrupt* was found to be overexpressed in a *dpp*-like expression pattern (Figure 5, G and H), whereas the control sense probe did not show any pattern (data not shown). The Abrupt protein pattern was similar to the *abrupt* transcript pattern (HU *et al.* 1995; Figure 5, I and J, red); Abrupt protein also was excluded from cells posterior to the morphogenetic furrow, as confirmed by double staining with an antibody against Elav (Figure 5I, green) and was readily expressed in a *dpp*-like pattern in *B2149;* dpp^{disc}*-Gal4* discs (Figure 5, L and M).

***abrupt* expression is sufficient for the potential arista transformation:** We wanted to find out whether the insertion of the *P{Mae-UAS.6.11}* into the *abrupt* locus and consequently the *abrupt* misexpression was sufficient to cause the potential arista transformation. To this end, we cloned an abrupt cDNA into PUAST (BRAND and PERRIMON 1993) and generated transgenic lines (MATERIALS AND METHODS). A *UAS-abrupt* transgene on both the second and the third chromosome yielded transformed aristae when crossed to the dpp^{disc}*-Gal4* line

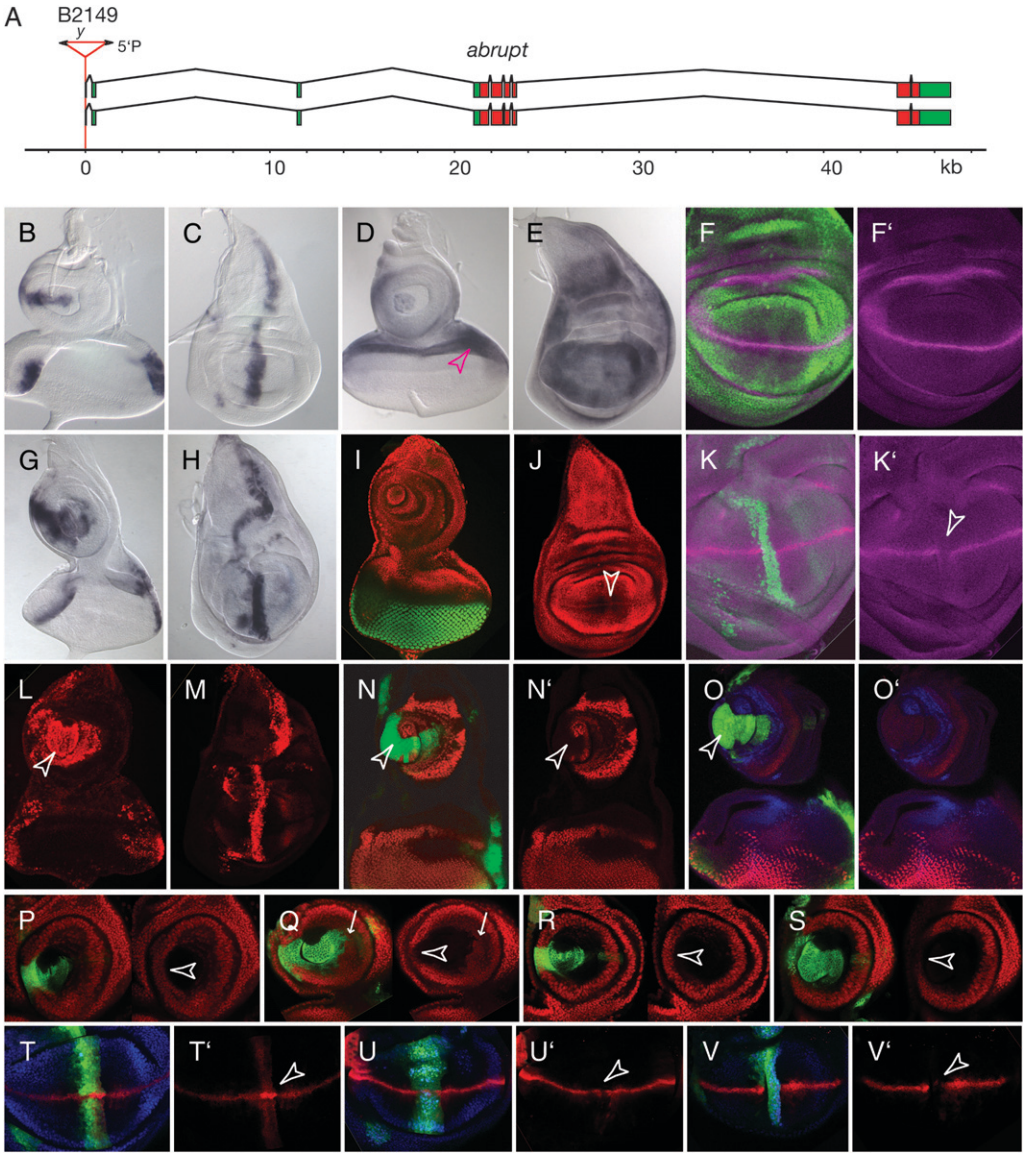

FIGURE 5.—*abrupt* is overexpressed in transformed antennal discs. (A) Map of the *abrupt* locus. The *P{Mae-UAS.6.11}B2149* element (red, not to scale) is inserted just upstream of the first noncoding exon (green), which is ~20 kb away from the coding sequence (red). The alternatively spliced abrupt transcripts are almost identical. (B–V) Third instar imaginal discs. (B, D, G, I, L, N, and O) Eye-antennal discs. (C, E, F, H, J, K, M, and T–V) Wing discs. (P–S) Antennal discs. (B and C) Discs expressing lacZ RNA in the dpp^{disc} expression domain (*UAS-lacZ*; dpp^{disc}*Gal4*). Expression of abrupt RNA in control imaginal discs (D and E). (F) Control wing disc stained for Abrupt (green) and Wg (magenta, F and F′). (G and H) Expression of abrupt RNA in discs of larvae containing the *P{Mae-UAS.6.11}B2149* element and the dpp^{disc}*-Gal4* line. Since the expression levels in the *abrupt*-overexpressing cells apparently are very high, the color reaction developed very fast and was to be stopped before the endogenous expression pattern became clearly visible (G and H). (I and J) Expression of Abrupt (red) in *y w* discs. In the eye discs (I), the Abrupt protein, like the abrupt RNA (D), is confined to the anterior of the morphogenetic furrow (arrowhead in D). Cells posterior to the morphogenetic furrow are labeled with Elav (green in I). There is no overlap between Elav and Abrupt (I). (J) Arrowhead points to Abrupt expression at the wing margin. (K) Expression of Abrupt in wing disc expressing *B2149* under dpp^{disc}*-Gal4* control, stained for Abrupt (green) and Wg (magenta, K and K′) (*B2149; UAS-GFP/*dpp^{disc}*-Gal4*); arrowhead in K′ points to gap in Wg expression domain. (L and M) Expression of Abrupt in discs of larvae expressing *B2149* under dpp^{disc}*-Gal4* control; the Abrupt protein levels in the misexpressing discs are considerably higher than the endogenous Abrupt expression levels, which are only faintly visible. The same is observed for the RNA (G and H). (N and N′) Dan (red) is not expressed in Abrupt- and GFP-expressing cells (green in N) of the antennal discs (*B2149; UAS-GFP/*dpp^{disc}*-Gal4*). (R and S) Eyg (blue) and Salm (red) are not expressed in Abrupt-overexpressing antennal disc cells (O, same genotype as in N). Arrowheads in (N and O) point at *abrupt*-overexpressing wedge within the antennal disc. (P and Q) Localization and expression of the Hth protein (red) in antennal discs, expressing *GFP* (*UAS-CD8-GFP;* dpp^{disc}*-Gal4*, green) (P) or expressing *abrupt* (*B2149/UAS-CD8-GFP;* dpp^{disc}*-Gal4*) (Q); (P and Q, right) red channel (Hth); (Q) Arrowhead points to the reduction in Hth expression levels. The arrow points to cells that are surrounded by Hth-expressing cells, but do not express Hth. (R and S) Expression of *hth-lacZ* in antennal discs of larvae expressing either CD8-GFP (R) or CD8-GFP and *B2149* under dpp^{disc}*-Gal4* control (S); the *hth-lacZ* expression levels in the *abrupt*-misexpressing cells (arrowhead in S) are slightly lower than in control disc cells (arrowhead in R); (R and S, right) red channel (*hth-lacZ*). (T–V) Expression of *vgB-lacZ* in wing discs of larvae expressing either GFP (green in T) or GFP and *B2149* under dpp^{disc}*-Gal4* control (U and V); the *vgB-lacZ* expression levels are reduced in *abrupt*-overexpressing cells (arrowhead in U′ and V′). The discs are double stained for Abrupt (blue channel in T, U, and V); (T′, U′, and V′) only red channel corresponding to *vgB-lacZ* is shown.

(Figure 1, H and I). Therefore, *abrupt* is accountable for the change of the arista into a tube-like structure.

Antennal markers are suppressed in *abrupt*-overexpressing antennal discs: If *abrupt* expression transforms the antenna to a leg-like structure, then antennal markers should be suppressed in these antennal discs. Therefore we analyzed *B2149;* dpp^{disc}*-Gal4/UAS-GFP*-expressing eye-antennal discs for the expression of the

antennal markers Spalt Major (Salm) (Wagner-Bernholz *et al.* 1991), Eyegone (Eyg) (Jang *et al.* 2003), and Dan (Emerald *et al.* 2003). Indeed, the expression of all three markers was absent in the *abrupt*-overexpressing antennal tissue (Figure 5, N and O). This supports the idea that this tissue no longer has an antennal identity. A change in identity from antenna to leg is observed in the case of *homothorax* (*hth*) expression being compromised in antennal disc cells (Casares and Mann 1998; Pai *et al.* 1998). To find out whether a change of Hth expression could be accountable for the observed arista transformation, expression of a *hth-lacZ* line and localization of the Hth protein was analyzed in control and *abrupt*-overexpressing antennal discs (Figure 5, P–S). These experiments indeed revealed that Hth was not appropriately expressed in a subset of *abrupt*-misexpressing cells, which are part of the distal *hth* expression domain, but was normal in the more proximal *hth* expression domain (Figure 5, P and Q). In addition, in these experiments it is also clearly visible that it is the most central domain of the antennal discs that is predominantly changed in response to *abrupt* overexpression, as the domain expressing GFP (due to *dpp^{disc}-Gal4*), but not *hth*, is larger than in control antennal discs (Figure 5, P and Q). Although these findings are well in line with the idea that a transformation might take place due to a potential relative distal reduction of the *hth* expression domain (Emerald and Cohen 2001), a clear transformation to leg identities could not be confirmed independently by other molecular markers, since unambiguous distal leg markers are not available. Therefore, it is not entirely clear whether this tissue truly acquired a leg identity, either by morphological criteria or by molecular criteria, albeit the transformed aristae resemble the malformed legs of the same genotype. For example, *Antp*, which is renowned for its capacity for promoting the transformation of the antenna to a leg (reviewed in Gehring 1993), was not ectopically expressed in these antennal discs (data not shown).

The notum phenotype of the *abrupt*-overexpressing flies resembles a *Delta* loss-of-function phenotype: We observed that *B2149; dpp^{disc}-Gal4* flies not only display a potentially transformed arista and abnormal legs, but also display supernumery macrocheates on the notum (Figure 1E). This phenotype is reminiscent of a conditional *Delta* loss-of-function phenotype (Parks and Muskavitch 1993). Delta is a ligand of the Notch-signaling pathway (reviewed in Greenwald 2006). To find out whether *abrupt* overexpression can also mimic loss of Notch signaling in other tissues, we looked at the expression of *wg* in the wing blade primordium of the wing disc (Figure 5, F and K). It has been shown that *wg* expression in the wing blade margin is dependent on Notch signaling (Diaz-Benjumea and Cohen 1995; Doherty *et al.* 1996). Whereas *dpp^{disc}-Gal4*-mediated expression of a constitutively active Notch leads to ectopic expression of *wg* (Diaz-Benjumea and Cohen 1995), *dpp^{disc}-Gal4*-mediated expression of *abrupt* suppresses *wg* expression (Figure 5K). Similarly, we found that the expression of the Notch-signaling-dependent *vgB-lacZ* reporter (Kim *et al.* 1996 and Figure 5T) was compromised in *abrupt*-overexpressing wing discs (Figure 5, U and V). These results could indicate that *abrupt* can delimit *Notch* signaling in the wing blade tissue and suggests the possibility that *abrupt* is antagonizing or limiting *Notch* signaling. Alternatively, it could also indicate that the *abrupt*-overexpressing cells are incapable of expressing these genes.

DISCUSSION

In this study, we present the results obtained by screening a collection of flies, misexpressing an arbitrary set of genes within the *dpp* expression pattern of imaginal discs. Misexpression was driven by binding and activation of Gal4 to the *UAS* sequences, present in the *P* element used to generate this fly collection. We have screened these flies with the aim of finding mutant flies that develop body structures at ectopic positions. Our results suggest that these phenotypes are found, albeit at a low frequency. We recovered two lines that show a novel potential transformation phenotype. This corresponds to 0.7% of the collection. Since it is obviously easier to interfere with normal development and trigger malformation of an appendage than to induce an entire structure and its characteristic cell types at an ectopic position, it was expected that mutants with ectopic body structures would occur rarely and at a much lower frequency than abnormal appendages. This was indeed the case, as 6% of the fly collection produced flies exhibiting abnormal body parts. This frequency was also in the range as reported in previous screens (*e.g.*, Rørth *et al.* 1998). Most of the flies, however, were morphologically completely normal (~94%). This indicates that transformations of the body are generally not easily obtained and that overexpression of many genes is not detrimental to development. Also, few factors may have the potential to spatially allocate a body part, which in turn suggests that the observed phenotypes are significant. In this respect, it is noteworthy that both candidate genes of our screen, which cause the potential transformations, code for putative transcription factors (see below). The mutant phenotypes observed in abnormal legs, wings, or antennae were reproducible and characteristic of the respective gene, which was overexpressed by the respective insertion. This was verified in a few control crosses but also became clear by identifying several hits in the same locus. Finding such characteristic phenotypes among those lines poses the question of whether insertions, which show a similar wing or leg phenotype, genetically and molecularly interact with each other. Therefore, it will be interesting to test whether insertion lines that share a similar loss-of-structure phenotype as that of the two potential transformation

lines act downstream of these genes or affect similar pathways. For example, two lines [*G1365* (Figure 2.16) and *G1430* (Figure 2.20)] expose a wing phenotype similar to that of the transforming insertion line *B1164*. All of them display a proximal shortening of the wing blade. In one of these lines, *wg* signaling, which has a demonstrated role in determination and transdetermination events (reviewed in Maves and Schubiger 2003), might be affected. One line (*B2037*, Figure 3.29) displayed a leg phenotype similar to that of *B1164*: the proximal part of the leg was reduced and not shapely.

One of the lines, which led to a transformation phenotype, was *B1164*. It displayed notum tissue at the site of the sternopleura. The notum is a derivative of the wing imaginal disc (Bryant 1970) and the sternopleura, a derivative of the mesothoracic leg discs (Steiner 1976). Therefore, a transformation of the ventro-lateral body wall to the dorsal body wall seemed to have occurred in this mutant. The molecular analysis of this line was not straightforward, as it contains two P insertion sites. Its characterization will be presented elsewhere (N. C. Grieder and W. J. Gehring, unpublished results). Our unpublished results indicate, however, that the gene responsible for this transformation corresponds to the putative transcription factor Spalt Major, which is expressed at the right time in development of the wing imaginal disc to accomplish the proposed function in determination of dorsal tissue. It is interesting that the transformed part of the fly in *B1164* fate maps onto the upper medial quarter of the leg disc, the region of the disc with highest regeneration and transdetermination potential (Schubiger 1971). Therefore, in future transdetermination experiments it will be tested whether it also represents a transdetermination factor.

In the second line (*B2149*), the arista was potentially transformed due to expression of *abrupt*, a BTB–zinc-finger protein putatively involved in gene regulation (Hu *et al.* 1995). Morphologically, the transformed arista looked like an abnormal leg, similar to the ones present in these mutant flies. Since both the legs and the transformed arista looked rather abnormal, it could not be unambiguously determined whether a true transformation was present in this line. However, this seems possible on the basis of our molecular analysis: The three antennal markers Eyg, Salm, and Dan were absent in *abrupt*-expressing cells of the antennal disc. Also expression of Hth was slightly compromised at the distal edges of its expression domain, which might be sufficient to explain the observed potential transformation (Casares and Mann 1998; Pai *et al.* 1998; Emerald and Cohen 2001). Alternatively, it is possible that the tissue was not entirely transformed but rather was incapable of differentiating properly and so developed into a strange outgrowth, due to the failure to turn on the appropriate differentiation pathways. Such a function seems not impossible and would be expected of some factors involved in transdetermination. It has been shown that tissue regeneration is required prior to tissue transformation in the process of transdetermination (Schubiger 1971). Therefore, factors that promote or reverse the status of differentiation must exist. For example, cells that constitutively overexpress *Notch* overproliferate and are delayed in their differentiation (Kurata *et al.* 1999). Indeed, the notum phenotype of *abrupt*-overexpressing flies was strongly reminiscent of a partial *Delta* loss-of-function phenotype (Parks and Muskavitch 1993). This may indicate that *abrupt* can delimit *Notch* signaling in the wing disc and suggests the possibility that *abrupt* is antagonizing or limiting *Notch-Delta* signaling. It has been shown that *Delta*-mediated activation of the *Notch*-signaling pathway is required for allocation of the correct number of macrochaetes (Parks and Muskavitch 1993). This Delta-signaling event involves the transport of the Delta protein along filopodia (de Joussineau *et al.* 2003). In this context, it is interesting that a function for *abrupt* in endowment of the characteristics of dendritic morphology in class I neurons has been shown (Sugimura *et al.* 2004). In the absense of *abrupt*, the dendritic morphology in these neurons is more elaborate than it normally would be: more and more complex dendrites are forming. Ectopic expression of *abrupt* in class II–IV neurons led to a size reduction of their respective dendrites (Sugimura *et al.* 2004). Therefore, it is tempting to speculate that in *abrupt*-overexpressing wing discs, Notch signaling gets dampened by a shortening effect of Abrupt on filopodia, which are required for Delta's function in long-range lateral inhibition. Interestingly, Abrupt expression is elevated in the wing margin of developing wing discs where Notch signaling is required for *wg* and *vgB-lacZ* activation, albeit through *Serrate* (Diaz-Benjumea and Cohen 1995; Kim *et al.* 1996; Neumann and Cohen 1996; Figure 5K). Whether *abrupt* represents a true transdetermination factor and plays a role in differentiation, and whether it interacts molecularly with the Notch-signaling pathway, will be analyzed in future experiments.

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