**Dorsal wing, a locus that affects dorsoventral wing patterning in Drosophila**

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**SUMMARY**

The wing imaginal disc is subdivided into a dorsal and a ventral compartments. A new dominant homeotic mutation, *Dorsal wing* (*Dlw*), transforms ventral into dorsal compartment in heterozygotes. This phenotype is similar to one of the dominant phenotypes of *Polycomb* (*Pc*) mutants. In *Pc Dlw*/Pc+ Dlw1 double mutants, the transformation is greatly enhanced. The recessive phenotype of *Dlw* is the opposite to the dominant phenotype. Dlw+/Dlw somatic clones induced at any larval stage differentiate only ventral pattern on both wing surfaces. This effect is one of the somatic clone phenotypes of *trithorax* (*trx*) lethals. A similar dorsal-to-ventral transformation is observed in *Pc Dlw/Dlw* clones. Dlw+/Dlw clones have no effect elsewhere, except in the dorsal notum, which may differentiate extra macrochaetes. We propose that: (1) *Dlw* is required for the specification of dorsal compartment; (2) some genes of the *Polycomb* group act as negative regulators of *Dlw*, while some genes of the *trithorax* group act as positive regulators.

Key words: homeotic, *Dorsal wing*, *Polycomb*, *trithorax*, compartment, dorsoventral wing, *Drosophila*

**INTRODUCTION**

The *Drosophila* adult wing and hemi-notum are derived from a single imaginal disc, the wing disc. A fundamental process in imaginal disc development is the subdivision of disc into smaller subunits known as developmental compartments (Garcia-Bellido et al., 1976; Steiner, 1976; Struhl, 1977; Baker, 1978). Each compartment is defined by a cell lineage restriction boundary. As early as the cellular blastoderm stage, the wing disc is already subdivided into anterior and posterior compartments. Later, as the wing disc grows during larval development, wing-notum and dorsoventral segregations occur, subdividing the disc into eight developmentally distinct units. A key feature of compartmentalization process is that it occurs sequentially in a binary fashion. From the analyses of a number of homeotic mutations such as *bithorax* (*bx*), *postbithorax* (*pbx*) and *engrailed* (*en*), which cause compartment-specific transformations (Lewis, 1963; Garcia-Bellido and Santamaria, 1972; Morata and Lawrence, 1975; Morata and Garcia-Bellido, 1976; Adler, 1978; Kornberg, 1981; Tiong and Russell, 1986; Kornberg et al., 1985), Garcia-Bellido (1975) proposed that, at each compartmentalization event, a regulator gene known as a selector gene is switched on in one compartment and off in the other. It is the on/off states of the selector genes that maintain the compartmental distinctions. In support of this selector gene model, the *en* gene has been shown to be required in the posterior compartment of each segment. In the wing disc, the posterior compartment is transformed into the anterior identity in *en* mutants. In addition, the anteroposterior cell lineage restriction boundary is abolished (Lawrence and Morata, 1976; Lawrence and Struhl, 1982). The *wingless* (*wg*) (Morata and Lawrence, 1977) and *apterous* (*ap*) (Diaz-Benjumea and Cohen, 1993) genes have been proposed to be the selector genes for wing-notum and dorsoventral distinctions respectively.

In the light of the compartment hypothesis, a number of events must take place at each compartmentalization step. First, the two polyclones (Crick and Lawrence, 1975) must be defined. Little is known about this process except that their allocation is dependent on the position of cells within the disc. Second, the regulatory gene(s) must be activated or inactivated. Third, the established compartmental state must be maintained so that it is heritable throughout the rest of development. Some information is known about the establishment of the anterior and the posterior compartments: The event is part of the embryonic segmentation process in early embryos, which happens well before the formation of distinct imaginal discs (Cohen, 1990; Bate and Martinez Arias, 1991; Cohen et al., 1993). Little is known about the establishment of wing-notum and dorsoventral compartmental distinctions.

Current views on how the activated state of a homeotic gene may be heritably maintained involve autoregulatory mechanisms (Bienz and Tremml, 1988; Kuziora and McGinnis, 1988) and/or maintenance by the *trithorax*-group genes (*trx-G*), which act as positive regulators of homeotic genes (Capdevila and Garcia-Bellido, 1981; Ingham, 1983; Breen and Harte, 1993; Kennison, 1993; Sedkov et al., 1994). The inactive state may be maintained by the *Polycomb*-group (*Pc-G*) genes, which act as negative regulators (Lewis, 1978;

Current evidence suggests that the compartment boundaries may act as organizers for imaginal disc cellular behavior and gene expression. For instance, the *hedgehog* (hh) gene, which is normally expressed in the posterior compartment of the wing, when ectopically expressed in the anterior compartment, induces new axis formation and outgrowth (Basler and Struhl, 1994). Similarly, the *ap* gene is normally expressed in the dorsal compartment. When *ap* clones are induced by mitotic recombination in the dorsal compartment, ectopic dorsoventral axes and outgrowth are induced (Diaz-Benjumea and Cohen, 1993). In addition, in both situations, new gene activity is induced (Basler and Struhl, 1994; Williams et al., 1994; Blair et al., 1994). Therefore, it appears that cell-to-cell communication at the compartment boundary plays an essential role in organizing cellular behavior.

In this report, we present evidence that a new gene, *Dorsal wing*, is an integral part of the dorsoventral wing patterning system. It is required for the specification of the dorsal wing identity.

**MATERIALS AND METHODS**

*Drosophila* strains and culture conditions

The genetic variants used are described in Lindsley and Zimm (1992). *Int3R*Dlw1 is a gamma-ray induced mutation. *Df(3R)RXT13* (98F12-14;99B3-4) is an unpublished deficiency kindly provided by Dr R. Tearle (University of Adelaide, Australia); *Dp(3:1)B152*, *Dp(3:1)ca27* and *Dp(3:1)ca74* are described by Frisardi and MacIntyre (1984), and Kongswan et al. (1986). Briefly, *Dp(3:1)B152* is a terminal duplication of 3R from 98F14. *Dp(3:1)ca27* and *Dp(3:1)ca74* are derivatives of *Dp(3:1)B152*, deleting 98B4-5 to 99B10-C1 and 98F14 to 99C2-6, respectively. *Df(3R)tll*, 99F1-2; 100B4-5 was obtained from Dr J. Merriam. The map position of 3R of bristle markers used are: *Ki* (47.2), *M(3)p*124 (79.7), *Pr* (90.0) and *Bsb* (100.6).

Flies were raised on a yeast-sucrose-agar medium (Nash and Bell, 1968). All genetic crosses were performed in a 25°C growth chamber.

Clonal analyses

The genetic crosses used in the clonal analysis experiments and the genetic analyses will be described in the text. Eggs were collected at 24 hours intervals and mitotic clones were generated by irradiating larvae of appropriate age with 1 kR Gamma-ray from a Co60 source. Adult flies were collected daily and preserved in 70% ethanol. Mitotic clones were detected by screening wings and nota under a dissecting microscope at high magnification. Alternatively, wings and nota were processed as described below and screened under a compound microscope. In the wing blade, we screened for mitotic clones only at the anterior triple row marginal bristle region, because the markers used can be reliably scored only in bristles.

Preparation of adult cuticle

Flies were boiled in 1 M NaOH for 5 minutes to remove the internal soft tissues. They were then washed twice in distilled water and stored in 70% ethanol until mounting. The wing and notum were dissected free from the rest of the body and mounted between coverslips in Euparal.

Statistical testing

The penetrance (Pen) of ventral-to-dorsal wing transformation was calculated as the percentage of wings expressing at least one ectopic median chaete (a dorsal structure, mtr) in ventral compartment of the anterior triple row (mtr'). The expressivity (Exp) of the transformation is given as the mean number of mtr' per wing (± s.e.m.). The significance level of the difference between two genotypes was assessed at 5% level by Student's *t*-test. Under conditions where *Dlw1* homozygous tissue patches survived in the wing, the phenotype expressed was all or none, so that the results required no further quantitation or statistical testing.

**RESULTS**

Dorsal wing phenotype

*Dorsal wing* (*Dlw1*) was discovered in a screen for mutations affecting the wing margin. The dominant *Dlw1/+* phenotype is best interpreted as a ventral (V) to dorsal (D) wing transformation. The wild-type dorsoventral (D/V) wing compartment boundaries run along the wing margin. In the anterior wing margin, the dorsal compartment contains two bristle rows, the dorsal row (dtr) and the medial row (mtr) (Fig. 1A). The ventral compartment contains a single row (vtr) (Fig. 1C). In the *Dlw1/+* wing, the ventral compartment is partially transformed into the dorsal compartment, giving a quadruple row appearance, with mirror-image symmetry along the D/V boundary (Fig. 1D). All wings are affected, but only 10-30% of the ventral bristles are transformed. The bristle patterns on the notum and elsewhere in the fly appear normal.

Initial genetic analyses

Using *mwh*, *red* and *e* as markers, we have mapped *Dlw1* to 101.0 cM on chromosome 3R (based on 90/292 recombinants between *e* and *Dlw1*). Examination of polytene chromosomes revealed that the mutant is associated with *In(3R)Dlw1* (99A10-B1; 99F3-5), which corresponds well with the mitotic map position of *Dlw1*. The inversion homozygote dies as a first instar larva. These larvae all have normal cuticles. No attempt has been made to examine internal structures for defects.

Several lines of evidence suggest that the proximal inversion breakpoint at 99A10-B1 is responsible for the *Dlw1* phenotype. First, *Dlw1/Df(3R)tll* (a deficiency uncovering only the distal breakpoint) is viable and the phenotype is the same as *Dlw1/+. Second, *Dlw1/Df(3R)RXT13* (a deficiency uncovering only the proximal breakpoint) is lethal. In addition, *Df(3R)RXT13/+* has a weak semi-dominant phenotype. As shown in Fig. 1B, some bristles in the two dorsal rows may be absent, leaving gaps in the medial row. Ventral-like bristles may also appear in the medial row, giving a double row appearance with mirror-image symmetry along the D/V boundary. Although the phenotypes may be interpreted as partial transformation of dorsal into ventral wing, the effects could be due to another gene that is uncovered by the deficiency. These results are consistent with the view that the *Dlw* locus is located at 99A10-B1 (see below for additional evidence).

Gene dosage analyses

In order to determine if an extra dose of *Dlw1* has any effect on the D/V wing patterning, and to examine the nature of the *Dlw1* mutation, we crossed *Dp(3:1)B152, Dlw1/FM6* ; *Dlw1/TM6* females to *Dlw1/TM3* males. As shown in Table 1, three doses of *Dlw1* give wild-type wings.

An extra dose of *Dlw1* neither suppresses nor significantly
**Fig. 1.** Some *Dlw* mutant wing and notum phenotypes. (A) Wild-type dorsal view, showing the two bristle rows, dtr and mtr. (B) Dorsal view of *Df(3R)RXT13/+*. Note the partial D→V transformation (arrows) and the absence of dtr. The transformed structures are labeled with a prime. (C) Wild-type ventral view showing single ventral bristle row, vtr. (D) Partial V→D wing transformation in *Dlw*+/+, showing dorsal-type bristles (dtr’ and mtr’) mixed with normal ventral bristles (vtr) on the ventral side. (E) A dorsal *Dlw*+/*Dlw* clone with D→V phenotype. The four short bristles marked by the arrows are not part of the clone. (F) A ventral *Dlw*+/*Dlw* clone with a normal phenotype. All marginal bristles shown in the photograph are part of the clone. (G) *Dlw*+ alula with dominant bristle markers, showing a single row of ventral hairs (shortened due to *Kc, M, Pr* and *Bsb* mutations). (H) A *Dlw*+/*Dlw* clone spanning the dorsal-ventral boundary, with two rows of alula hairs, one dorsal and one ventral. Also note that the normally alternating short (dorsal) and long (ventral) posterior rows (pr) of hairs are of approximately equal length and in mirror-image symmetry. (I-J) *Dlw*+/*Dlw* notal clones, enclosed by dashed lines. (I) A clone with extra macrochaetes. (J) A clone with a wild-type bristle pattern.

**Table 1. Dosage studies on *Dlw*+/+

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<tr>
<td>100 (20)</td>
<td>17.2±4.4</td>
<td>100 (20)</td>
<td>20.0±6.5</td>
<td>0 (20)</td>
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The sample size is given in parentheses; penetrance (Pen) is given in percentage the fraction of wings expressing at least one dorsal-like bristle (mtr’) in the ventral compartment; and expressivity (Exp) is expressed as the mean number of transformed medial triple row chaetes (mtr’) in the ventral compartment per wing, ± s.e.m.
enhances the ventral-to-dorsal wing transformation in \( Dlw^{+/+} \) flies. In contrast, \( Dp(3;1)B152 \) (\( Dlw^{+} \)) rescues the lethal phenotype of \( Dlw^{+}/Dlw^{+} \) (and \( Dlw^{+}/Df(3R)RXT13 \), data not shown), and the surviving flies have wild-type wings, exhibiting no sign of ventral-to-dorsal wing transformation. Thus, \( Dlw^{+} \) has a dominant gain-of-function activity, but this phenotype appears to depend upon the physical location of a \( Dlw^{+} \) allele.

**Cell autonomy analyses**

We generated \( Dlw^{+/+}/Dlw^{+} \) clones in order to assess cell viability and phenotype in imaginal tissues. Progeny of the cross between \( Dlw^{+}/ru cu ca \) males and \( Ki p^{0} M(3)w^{124} Pr Bsb/TM6B \) females were irradiated. Mitotic recombination in \( Dlw^{+}/Ki p^{0} M(3)w^{124} Pr Bsb \) proximal to \( Ki \) generates \( Dlw^{+}/Dlw^{+} (Ki^{+} M^{+} Pr^{+} Bsb^{+}) \) clones; the \( M/M \) twin-spots die. In the same cross, \( ru cu ca/Ki p^{0} M(3)w^{124} Pr Bsb \) siblings act as controls.

As shown in Table 2, \( Dlw^{+}/Dlw^{+} \) clones are cell viable. In the wing blade, the homozygous dorsal clones (24 clones in all) show dorsal-to-ventral transformation. The two dorsal rows at the anterior triple row region are replaced by a single row with ventral bristle morphology (Fig. 1E). All ventral clones (13 total; Fig. 1F) differentiate only wild-type ventral bristles. The dorsal-to-ventral transformation also occurs in the posterior compartment, as can be judged from the appearance of a double row of elongated hairs on the alula (Fig. 1H). The wild-type alula has a single ventral row of these hairs (Fig. 1G). In control wings, 12 dorsal and 6 ventral clones were found, all of them with unaltered triple row pattern.

\( Dlw^{+}/Dlw^{+} \) clones survive elsewhere, but are wild-type in structure, except on the dorsal notum, where extra macrochaetae are sometimes found. These extra macrochaetae are formed at regions close to where normal macrochaetae are located. Of the 37 clones identified on the dorsal notum in the experimental series in Table 2, 14 had at least one extra macrochaetae (Fig. 1I). The remainder did not (Fig. 1J). The microchaetal pattern appeared undisturbed whether or not extra macrochaetae were found. In the haltere, the clonal phenotype was not determined since this organ lacks bristles.

We have done a preliminary clonal analysis of \( Dlw^{+} \), a gamma-ray induced recessive lethal allele. This allele was isolated over a viable hypomorphic \( Dlw \) allele which has a dorsal-to-ventral wing transformation. \( Dlw^{+}/Dlw^{+} \) somatic clones have phenotypic transformations in the wing and the notum indistinguishable from those of \( Dlw^{+}/Dlw^{+} \) clones (see Table 2). These results clearly demonstrate that the clonal phenotypes of \( Dlw^{+}/Dlw^{+} \) are loss-of-function effects.

**Location of \( Dlw \) at 99A10-B1**

In our initial genetic analysis, the dominant effect of \( Dlw^{+} \) on the ventral wing compartment was shown to derive from the proximal breakpoint (99A10-B1) of \( In(3R)Dlw^{+} \). We used clonal analysis to establish the cytological origin of the recessive effect on the dorsal compartment. \( Dp(3;1)ca74/Y; Dlw^{+}/TM6B \) males were crossed to \( Ki p^{0} M(3)w^{124} Pr Bsb/TM6B \) females, and the progeny were irradiated at 108±12 hours. Four \( Dlw^{+}/Dlw^{+} \) clones in the \( Dp(3;1)ca74/+; Dlw^{+}/Ki p^{0} M(3)w^{124} Pr Bsb \) daughters showed dorsal-to-ventral transformation in the wing, as did similar clones (4 total) in their +/Y brothers. \( Dp(3;1)ca74 \) covers only the distal breakpoint of \( In(3R)Dlw^{+} \), and does not suppress the \( Dlw^{+}/Dlw^{+} \) mutant phenotype. In contrast, \( Dp(3;1)ca27 \) which covers both breakpoints, completely suppresses the transformation in equivalent clones. As expected, \( Dp(3;1)ca27/+; Dlw^{+}/Dlw^{+} \) flies are viable and show no transformation. These results indicate that the cell-autonomous dorsal-to-ventral wing transformation in \( Dlw^{+}/Dlw^{+} \) clones has the same cytological origin as the \( Dlw^{+}/+ \) effect. We therefore presume that they are due to the same mutation.

**Interaction with Polycomb**

One of the dominant phenotypes of \( Pc \) mutants is the transformation of ventral to dorsal wing (Fig. 2A) similar to the \( Dlw^{+}/+ \) phenotype (Tiong and Russell, 1990). \( Pc \)-group genes act as negative regulators of homeotic genes. This suggests that the dominant haplo-insufficient ventral-to-dorsal wing transformation in \( Pc \) mutants may be mediated through the derepression of the \( Dlw \) locus. If this is the case, one might expect \( Pc \) mutants to interact with \( Dlw^{+} \). Indeed, Table 3 shows that in \( Pc^{+}Dlw^{+}/Pc^{-} Dlw^{+} \) double heterozygotes, the ventral-to-dorsal transformation is greatly enhanced. The number of mtr bristles per wing is 7.5±0.6 in \( Pc^{+}/+ \) and 26.1±4.4 in \( Dlw^{+}/+. \) In \( Pc^{+}/Dlw^{+} \) wings (Fig. 2B), the number is increased to 61.3±2.9. For comparison, the average number of mtr bristles (the equivalent structures in their normal dorsal location) is 83.1±2.6 in wild-type wings. Similar enhancement is seen in \( Pc^{+}/Dlw^{+} \) wings. These effects are in excess of additivity, and indicate interaction between the loci.

In the double heterozygotes, abnormalities are not confined to the anterior wing margin. The second longitudinal vein (L2), which is ventral in origin, is partially eliminated. L3 and the distal portion of L4 are of dorsal origin. In \( Pc/Dlw^{+} \) wings, ectopic veins may be present in the ventral compartment at

### Table 2. Cell autonomy analysis of somatic clones in wing imaginal tissues

<table>
<thead>
<tr>
<th>Clone genotype</th>
<th>Wing</th>
<th>Notum</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>D→V</td>
</tr>
<tr>
<td>( Dlw^{+}/Dlw^{+} )</td>
<td>1088</td>
<td>24</td>
</tr>
<tr>
<td>( Dlw^{+}/Dlw^{+} )</td>
<td>724</td>
<td>0</td>
</tr>
<tr>
<td>( Pc^{+}Dlw^{+}/Dlw^{+} )</td>
<td>736</td>
<td>9</td>
</tr>
<tr>
<td>( Dlw^{+}/Dlw^{+} )</td>
<td>766</td>
<td>0</td>
</tr>
<tr>
<td>( Dlw^{+}/Dlw^{+} )</td>
<td>50</td>
<td>5</td>
</tr>
</tbody>
</table>

Somatic clones were induced at 36±12 hours and 108±12 hours. Data from the two series are combined as they give qualitatively similar results. The \( Dlw^{+}/Dlw^{+} \) clones were induced only at 84±12 hours. N is the total number of wings or nota screened; D→V is the number of clones with dorsal-to-ventral transformation at the anterior triple row region; D is normal dorsal clones; V is normal ventral clones; AC is the number of dorsal notal clones with at least one extra macrochaetae; NC indicates normal notal clones.

### Table 3. Effect of \( Dlw^{+} \) on \( Pc \) wing phenotype

(quantitation method is given in Table 1)

<table>
<thead>
<tr>
<th>Allele(s)</th>
<th>( Dlw^{+}/Pc )</th>
<th>( Pc^{+} )</th>
<th>( Dlw^{+}/+ )</th>
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<tr>
<td>( Pc^{+} )</td>
<td>100 (20)</td>
<td>61.3±2.9</td>
<td>95 (20)</td>
</tr>
<tr>
<td>( Pc^{3} )</td>
<td>100 (16)</td>
<td>48.9±5.4</td>
<td>35 (20)</td>
</tr>
<tr>
<td>( Dlw^{+} )</td>
<td>100 (20)</td>
<td>26.1±4.4</td>
<td>8.2±4.8</td>
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</table>
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$Dlw$ specifies dorsal wing identity in $Drosophila$

these locations. At the posterior margin, long ventral alula hairs are reduced in number, and the long hairs on the posterior margin of the wing blade may be reduced to the length of dorsal hairs. We interpret all these effects as transformations to dorsal structure. The notum and the wing sensilla on both surfaces appear unaltered.

The $Pc$ mutant phenotype in the wing blade is not radically changed by an additional $Dlw^+$ dose (data not shown). As shown in Table 4, reducing $Dlw^+$ to a single dose does suppress $Pc$ mutant expression significantly. In $Dlw^+/Dlw^-$ clones, the $Pc$-dependent ventral-to-dorsal transformation is apparently absent. As shown in Table 2, four ventral clones induced in $Pc^{T1}/+$ background (after the cross $mwh Pc^{T1} red e Dlw^1/ru cu ca$ males to $Ki-p^b M(3)w^{124} Pr Bsb/TM6B$ females) show no mtr' bristles (Fig. 2D), as compared with all the $Pc^{T1} Dlw^1/Pc^+ Dlw^+$ wings, which show strong transformations. Nine dorsal clones are fully transformed, as they would be in a $Pc^+$ background (Fig. 2C). All control clones differentiated normally. The results show that the recessive effect of $Dlw^-$ precludes the interaction between $Pc^{T1}$ and the dominant effect of $Dlw^+$. In all probability, it is also epistatic to the ventral-to-dorsal transformation generated by $Pc^{T1}$ alone. This suggests that the ventral-to-dorsal effect of $Pc$ mutants is mediated through $Dlw^+$. Heterozygotes for strong $trx$ alleles have no apparent change in D/V wing patterning. However, the ventral-to-dorsal transformation in $Pc^{+/}$ wings is totally suppressed in a $trx^+$/background.

**Interaction with **$trx$**thorax**

The $trx$-group genes act as positive regulators of homeotic genes, which suggests the possibility that $trx$ might interact with $Dlw$. Indeed, Ingham (1985) showed that $trx^{2}/trx^3$ clones in the wing blade have effects similar to $Dlw^+/Dlw^-$ clones. We have confirmed this effect in 10 transformed $trx^{E2}/trx^{E2}$ dorsal clones (see Fig. 2F) and four untransformed ventral clones. Heterozygotes for strong $trx$ alleles have no apparent change in $Dlw$.

### Table 4. Effect of one dose of $Dlw^+$ on $Pc$ wing phenotype

<table>
<thead>
<tr>
<th>Allele(x)</th>
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<th>$Pc^+/+$</th>
<th>$Dlw^+$/$+$</th>
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<tr>
<td>$Pc^{T1}$</td>
<td>88 (60)</td>
<td>2.9±2.4</td>
<td>92 (37)</td>
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<tr>
<td>$Pc^3$</td>
<td>20 (30)</td>
<td>0.3±0.5</td>
<td>96.7 (30)</td>
</tr>
<tr>
<td>$Pc^3$</td>
<td>20 (30)</td>
<td>4.1±2.7</td>
<td>0 (30)</td>
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Cross: $Df(3R)RXT1$, $Dlw^+$/TM1 $\times Pc^{T1}$/TM3

Fig. 2. Phenotypic similarity between $Dlw$, $Pc$, and $trx$ mutants. (A) V→D wing transformation in $Pc^{T1}/+$. Three mtr' bristles are marked with arrows. (B) $Dlw^+/Pc^{T1}$ wing with enhanced V→D transformation. Note the quadruple row of bristles instead of the normal triple row. (C) A dorsal view of a $Pc^{T1} Dlw^1/Dlw^+$ clone with D→V phenotype. All marginal bristles shown are part of the clone and are transformed. (D) A ventral view of the same clone as shown in C. Arrows point to bristles that are not part of the clone (of the genotype $Pc^{T1} Dlw^1/+$), which retain ventral-to-dorsal transformation. (E) $Pc^{T1} Dlw^1/Dlw^+$ notal clone (enclosed by a dashed line) with extra macrochaetes. (F) A dorsal $trx^{E2}/trx^{E2}$ clone with D→V transformation. The indicated mtr and dtr bristles are outside the clone. The arrow indicates an ectopic dorsal vein 2.
DISCUSSION

The Dlw\(^{-}\) phenotypes

The Dlw\(^{-}\) mutation produces two distinct wing morphology phenotypes. First, a dominant partial transformation of ventral to dorsal wing structures is due to a gain-of-function of Dlw\(^{+}\) in the ventral compartment. This interpretation is supported by the observation that Dp(3;1)Dlw\(^{-}\)+/Dlw\(^{-}\)/Dlw\(^{-}\) shows the transformation at least as strongly as Dlw\(^{-}\)+. In addition, the effect is enhanced by Pc mutants, whose mutant expression is generally found to involve derepression of target loci. The dominant phenotype is reduced by a single dose of trx\(^{+}\) mutants, which generally have the converse effect. The second phenotype is the dorsal-to-ventral transformation of wing structures, seen in Dlw\(^{-}\)/Dlw\(^{-}\) tissue patches. This is a loss-of-function phenotype, suppressible by a Dlw\(^{+}\) duplication. The phenotype is identical to that of Dlw\(^{-}\)/Dlw\(^{-}\) somatic clones, and similar to the haplo-insufficient phenotype associated with a Dlw deletion. Dlw\(^{-}\)/Dlw\(^{-}\) tissue patches in the ventral wing show no evidence of ventral-to-dorsal transformation, so the wild-type allele must be ectopically expressed to generate the dominant effect in Dlw\(^{-}\)/+ flies. The dominant phenotype must not be due simply to lack of pairing between In(3R)Dlw\(^{-}\) and its wild-type homolog, since Dlw\(^{-}\) cannot exert its effect upon a transposed wild-type duplication. Dp(3;1)Dlw\(^{-}\)+/Dlw\(^{-}\)/Dlw\(^{-}\) and Dp(3;1)Dlw\(^{-}\)+/Dlw\(^{-}\)/Df(3R)RXT13 individuals are wild type in both dorsal and ventral compartments, so the expression of the dominant gain-of-function is only seen when a Dlw\(^{-}\) allele is present in its normal location on 3R. These effects might involve transvection (Lewis, 1954), which requires that homologous chromosomes interact (reviewed by Wu, 1993).

An alternative view is that the dominant phenotype in Dlw\(^{-}\)/+ does not involve ectopic derepression of Dlw\(^{+}\) in the ventral compartment, but rather acts via abnormal transcompartmental activity. This view is not supported by the observations that Dlw\(^{-}\) does not behave as an antimorph in genetic tests, and that Dp(3;1)Dlw\(^{-}\)+/Dlw\(^{-}\)/Df does not have a dominant phenotype like Dlw\(^{-}\)+, although it has an equivalent gene dosage. The definitive evidence must come from molecular analysis.

Our data suggest that, while the coding function of Dlw has been reduced (cis-inactivated) in In(3R)Dlw\(^{-}\), pairing of the inversion chromosome with its wild-type homolog produces ectopic trans-activation of the Dlw\(^{+}\) homolog. Specificity of the trans-interaction must depend upon Dlw sequences resident in both homologs. However, it is not clear whether the trans-activation results from dissociation of a normally trans-inhibitory component of Dlw\(^{+}\) from Dlw\(^{-}\), due to chromosomal breakage, or its acquisition of a trans-stimulatory component from another gene as a result of the rearrangement. Analogous transvection effects have been reported in a number of genes in Drosophila. The most precise parallel to the effect reported here is a series of mutations of the Sex comb reduced genes in the Antennapedia complex (Pattatucci et al., 1991). These gain-of-function/loss-of-function mutations are all associated with chromosome rearrangements.

Dlw\(^{-}\) homozygous clones on the dorsal notum sometimes show one or more duplicated macrochaetes. Such duplications are thought to arise when “lateral inhibition” of macrochaetal development among a group of potential macrochaete precursor cells is incomplete (reviewed by Simpson, 1990). Lateral inhibition involves cell-cell signaling using the Notch and Delta gene products (Wharton et al., 1985; Feohan et al., 1990; Heitzler and Simpson, 1991). A number of target genes respond to the signals, ultimately leading to restriction of the activity of the achaete and scute genes to the single macrochaete precursor (Romani et al., 1989; Cubas et al., 1991 Skeath and Carroll, 1991; Cubas and Modelell, 1992). The finding that Dlw\(^{-}\)/Dlw\(^{-}\) clones on dorsal notum sometimes have duplicated macrochaetes suggests a possible role for Dlw\(^{+}\) in the execution of lateral inhibition.

Genetic evidence for a dorsoventral wing boundary

Cell-lineage analysis has defined a strict D/V compartmental restriction along the wing margin in M(3)i\(^{55}\)/ flies (Garcia-Bellido et al., 1976), arising at a time which is equivalent to 84±12 hours wild-type time at 25°C (Steiner, 1976). However, this view is the subject of considerable debate (see Brower, 1985 for discussion), mainly because the restriction boundary is associated with a zone of non-proliferating cells (ZNC) described by O’Brochta and Bryant (1985). The ZNC, it is suggested, may act as a passive barrier to the migration of cells. Hence, in this view, the observed cell-lineage restriction may not mark a boundary that separates cell types that have adopted mutually exclusive developmental fates. Blair (1993) suggested that a true D/V compartmental boundary in fact exists within the ZNC, and he argued against the ZNC acting as a purely passive barrier to cell migration. Recent genetic and molecular evidence shows that the apterous gene expression coincides with the D/V boundary line (Diaz-Benjumea and Cohen, 1993; Blair, 1993; Williams et al., 1993). We have demonstrated that Dlw\(^{-}\)/Dlw\(^{-}\) clones on the dorsal wing transform dorsal to ventral, while, by virtue of an unusual genetic circumstance, Dlw\(^{+}\)/Dlw\(^{-}\) wing has ventral-to-dorsal transformation. Both transformations generate mirror-image symmetry around the postulated D/V compartmental boundary.

Dlw\(^{+}\) is required for the specification of dorsal wing identity

Loss-of-function Dlw homozygous clones on the dorsal wing surface autonomously transform dorsal to ventral, while ventral clones differentiate only ventral pattern. This strongly suggests that Dlw\(^{+}\) is required only in the dorsal wing compartment. The
haplo-insufficient phenotype of the deficiency also lends support to this conclusion. In addition, gain-of-function of Dlw+ in the ventral surface can partially channel ventral cells into a dorsal developmental pathway. This complementary effect of gain-of-function suggests that Dlw+ has a central role in establishing dorsal wing identity as distinct from ventral.

The on/off state of Dlw+ is maintained by the trx-G and Pc-G

The current view on the function of trx-G and Pc-G genes is that they maintain homeotic genes "on" and "off", respectively, perhaps by locking the chromatin structure into a heritable state (Reviewed by Paro, 1990). Our genetic evidence suggests that Pc+ and trx+ may regulate Dlw+ in this manner.

Pc exhibits, as one component of its phenotype, a dominant effect similar to that of Dlw+. In addition, a strong interaction is observed in Pc/Dlw/ clones double heterozygotes. This interaction, and probably the Pc effect alone, are absent from Dlw/ clones. In other words, the recessive loss-of-function aspect of the Dlw/ mutant is epistatic to the wing margin phenotype of a Pc mutant. This relationship implies that Pc precedes Dlw in a developmental pathway. These observations make Dlw a potential target for Pc+ repression in the ventral wing. A second set of observations with trx mutants suggests that Dlw+ is a potential target for trx+ activation in the dorsal wing.

Maintenance of expression states for genes of the bithorax complex (Lewis, 1978) and the Antennapedia complex (Lewis et al., 1980) requires positive regulation by trx-G genes and negative regulation by Pc-G genes. Our data suggest that an analogous mechanism is involved in D/V wing patterning, providing a heritable activity state for Dlw+, which may be required to maintain the integrity of the two compartments. Cell-lineage restriction at the D/V boundary is abolished in Pc mutants (Tiong and Russell, 1990), which suggests that Pc+ is required to maintain Dlw+ inactive in the ventral compartment. The proposed phenomenon parallels the role of Pc-G genes in maintaining the anterior boundaries of expression for the bithorax complex genes (Simon et al., 1990, 1992).

Juxtaposing cells of dorsal and ventral identities does not induce a new axis

In a study by Diaz-Benjumea and Cohen (1993), the ap/ap- clones internal to the dorsal wing compartment were found to induce ectopic wing margins and outgrowth. Cells within the clone differentiate in a ventral pattern, like the Dlw loss-of-function phenotype, while wild-type cells bordering the clone form dorsal structures. Based on this observation, they proposed that new wing margin and disc proliferation (leading to a new wing-axis) are induced when cells with dorsal and ventral identity are abutted. The phenomenon resembles the effect when the hedgehog gene is ectopically expressed in the anterior wing compartment (Basler and Struhl, 1994). However, Irvine and Wieschaus (1994) observed that particular fringe (fng) mutants can induce ectopic growth and margin formation without changing dorsoventral cell identities. Complementary to their observation, in this study (and in the studies of Ingham, 1985 and Tiong and Russell, 1990) there are several instances of anomalously juxtaposed dorsal and ventral wing patches, involving trx, Pc and Dlw mutants. No new margins, axial outgrowths, or partial deletions of normal margin were observed. The Irvine and Wieschaus (1994) work and our own studies indicate that specification of dorsoventral identities can be uncoupled from the interactions between dorsal and ventral cells (D/V signaling) that regulate growth and generate margin structures. Fig. 3 shows our view of the interactions among ap, fng, and Dlw. At the onset of dorsoventral compartmentalization, the D/V positional information switches ap on in the dorsal compartment and off in the ventral. The function of ap is to activate both fng, which is required for D/V signaling, and Dlw, which is required for dorsal identity specification. Margin formation requires D/V signaling between cells with different fng activity states, while the differentiation of distinct pattern elements is dependent on the activity state of Dlw. The maintenance of Dlw activity states may require trx-G and Pc-G genes. These proposed functions would account for the cellular behavior of ap-, fng- and Dlw- clones. Downstream in the genetic hierarchy, there is evidence suggesting that ap regulates vestigial and scalloped in the formation of wing margin (Williams et al., 1993). In the wing blade, the position-specific integrin genes mew and inflated are regulated positively and negatively, respectively, by ap (Blair et al., 1994); it is not clear if those functions require Dlw. In the context of the model, it is now important to demonstrate molecularly the interactions among ap, fng, and Dlw in dorsoventral patterning.

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