**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+/Dlw* 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 (Díaz-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(Dhw+), a gamma-ray induced mutation, Df3R(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 McIntyre (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. Df3R(tll), 99F1-2; 100B4-5 was obtained from Dr J. Merriam. The map position on 3R of bristle markers used are: Ki (47.2), M(3)w124 (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 medial 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 Dhw+/ 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 (Dhw+) was discovered in a screen for mutations affecting the wing margin. The dominant Dhw+/+ 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 Dhw+/+ 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 Dhw+ to 101.0 cm on chromosome 3R (based on 90/292 recombinants between e and Dhw+). Examination of polytene chromosomes revealed that the mutant is associated with In3R(Dhw+) (99A10-B1; 99F3-5), which corresponds well with the meiotic map position of Dhw+. 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 Dhw+ phenotype. First, Dhw+/Df3R(tll) (a deficiency uncovering only the distal breakpoint) is viable and the phenotype is the same as Dhw+/+. Second, Dhw+/Df3R(RXT13 (a deficiency uncovering only the proximal breakpoint) is lethal. In addition, Df3R(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 Dhw locus is located at 99A10-B1 (see below for additional evidence).

Gene dosage analyses

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

An extra dose of Dhw+ neither suppresses nor significantly
Fig. 1. Some \textit{Dlw} mutant wing and notum phenotypes. (A) Wild-type dorsal view, showing the two bristle rows, dtr and mtr. (B) Dorsal view of \textit{Df(3R)RXT13/+}. Note the partial D\textarrowright 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\textarrowright D wing transformation in \textit{Dlw1/+}, showing dorsal-type bristles (dtr’ and mtr’) mixed with normal ventral bristles (vtr) on the ventral side. (E) A dorsal \textit{Dlw1/Dlw1} clone with D\textarrowright V phenotype. The four short bristles marked by the arrows are not part of the clone. (F) A ventral \textit{Dlw1/Dlw1} clone with a normal phenotype. All marginal bristles shown in the photograph are part of the clone. (G) \textit{Dlw1} alula with dominant bristle markers, showing a single row of ventral hairs (shortened due to Ki, M, Pr and Bsb mutations). (H) A \textit{Dlw1/Dlw1} 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) \textit{Dlw1/Dlw1} notal clones, enclosed by dashed lines. (I) A clone with extra macrochaetes. (J) A clone with a wild-type bristle pattern.

<table>
<thead>
<tr>
<th>\textit{Dlw1/+}</th>
<th>\textit{Dp;Dlw1/+}</th>
<th>\textit{Dp;+/+}</th>
<th>+/-</th>
<th>\textit{Dp;Dlw1/Dlw1}</th>
</tr>
</thead>
<tbody>
<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>
</tr>
</tbody>
</table>

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 ca ca males and Ki p p M(3)w124 Pr Bsb/TM6B females were irradiated. Mitotic recombination in Dlw/Ki p p M(3)w124 Pr Bsb proximal to Ki generates Dlw/Dlw (Ki + M+ Pr+ Bsb+) clones; the M/M twin-spots die. In the same cross, ru ca ca/Ki p p M(3)w124 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 macrochaetal 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 Dw, a gamma-ray induced recessive lethal allele. This allele was isolated over a viable hypomorphic Dw 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 Dw at 99A10-B1

In our initial genetic analysis, the dominant effect of Dw 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/TM6 males were crossed to Ki p p M(3)w124 Pr Bsb/TM6 females, and the progeny were irradiated at 108±12 hours. Four Dlw/Dlw clones in the Dp(3;1)ca74/+; Dw/Y; Ki p p M(3)w124 Pr Bsb daughters showed dorsal-to-ventral transformation in the wing, as did similar clones (4 total) in their +/+ 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 Dw/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 Dw/+ 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 Dw locus. If this is the case, one might expect Pc mutants to interact with Dw. Indeed, Table 3 shows that in 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/Dlw and 26.1±4.4 in Dw/+.

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

<table>
<thead>
<tr>
<th>Clone genotype</th>
<th>N</th>
<th>D→V</th>
<th>D</th>
<th>V</th>
<th>AC</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dlw/Dlew</td>
<td>1088</td>
<td>24</td>
<td>0</td>
<td>13</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>Dlw/Dlew</td>
<td>724</td>
<td>0</td>
<td>12</td>
<td>6</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>PcDlew/Dlew</td>
<td>736</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Dlew/Dlew</td>
<td>766</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Dlew/Dlew</td>
<td>50</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>4</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 Dw/Dlew 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 macrochaete; NC indicates normal notal clones.

Table 3. Effect of Dw on Pc wing phenotype (quantitation method is given in Table 1)

<table>
<thead>
<tr>
<th>Allele(s)</th>
<th>Pen</th>
<th>Exp</th>
<th>Pen</th>
<th>Exp</th>
<th>Pen</th>
<th>Exp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pca</td>
<td>100 (20)</td>
<td>61.3±2.9</td>
<td>95 (20)</td>
<td>7.5±0.6</td>
<td>100 (20)</td>
<td>26.1±4.4</td>
</tr>
<tr>
<td>Pca</td>
<td>100 (16)</td>
<td>48.9±5.4</td>
<td>35 (20)</td>
<td>1.1±1.9</td>
<td>100 (20)</td>
<td>8.2±4.8</td>
</tr>
</tbody>
</table>
1653Dlw 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 \( P_c \) 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 \( P_c \) mutant expression significantly. In \( Dlw^+/Dlw^+ \) clones, the \( P_c \)-dependent ventral-to-dorsal transformation is apparently absent. As shown in Table 2, four ventral clones induced in \( P_c^{T1}/+ \) background (after the cross \( mwh P_c^{T1} red e Dlw^1/cu ca \) males to \( Ki p^* M(3)w^{124} Pr Bsb/TM6B \) females) show no mtr' bristles (Fig. 2D), as compared with all the \( P_c^{T1} Dlw^1/Dlw^1 \) wings, which show strong transformations. Nine dorsal clones are fully transformed, as they would be in a \( P_c^+ \) background (Fig. 2C). \( P_c^{T1} Dlw^1/Dlw^1 \) clones in the notum have phenotypes similar to \( Dlw^1/Dlw^1 \) clones (24 normal and 7 with extra macrochaetes; Fig. 2E). All control clones differentiated normally.

The results show that the recessive effect of \( Dlw^1 \) precludes the interaction between \( P_c^{T1} \) and the dominant effect of \( Dlw^1 \). In all probability, it is also epistatic to the ventral-to-dorsal transformation generated by \( P_c^{T1} \) alone. This suggests that the ventral-to-dorsal effect of \( P_c \) mutants is mediated through \( Dlw^+ \).

### Interaction with \( trithorax \)

The \( trx \)-group genes act as positive regulators of homeotic genes, which suggests the possibility that \( trx \) might interact with \( Dlw^1 \). Indeed, Ingham (1985) showed that \( trx^{2}/trx^{3} \) clones in the wing blade have effects similar to \( Dlw^1/Dlw^1 \) 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 D/V wing patterning. However, the ventral-to-dorsal transformation in \( P_c/+ \) wings is totally suppressed in a \( trx/+ \) back-

### Table 4. Effect of one dose of \( Dlw^+ \) on \( P_c \) wing phenotype

<table>
<thead>
<tr>
<th>Allele(s)</th>
<th>( P_c/Dlw^+ ) Pen Exp</th>
<th>( P_c/+ ) Pen Exp</th>
<th>( Dlw^+/+ ) Pen Exp</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_c^{T1} )</td>
<td>88 (60) 2.9±2.4</td>
<td>92 (37) 6.0±4.0</td>
<td>0 (40) 0</td>
</tr>
<tr>
<td>( P_c^3 )</td>
<td>20 (30) 0.3±0.5</td>
<td>96.7 (30) 4.1±2.7</td>
<td>0 (30) 0</td>
</tr>
</tbody>
</table>

Fig. 2. Phenotypic similarity between \( Dlw, \ P_c, \) and \( trithorax \) mutants. (A) \( V\rightarrow D \) wing transformation in \( P_c^{T1}/+ \). Three mtr' bristles are marked with arrows. (B) \( Dlw^1/Pc^{T1} \) wing with enhanced \( V\rightarrow D \) transformation. Note the quadruple row of bristles instead of the normal triple row. (C) A dorsal view of a \( P_c^{T1} Dlw^1/Dlw^1 \) clone with \( D\rightarrow V \) 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 \( P_c^{T1} Dlw^1/+ \)), which retain ventral-to-dorsal transformation. (E) A \( P_c^{T1} Dlw^1/Dlw^1 \) notal clone (enclosed by a dashed line) with extra macrochaetes. (F) A dorsal \( trx^{E2}/trx^{E2} \) clone with \( D\rightarrow V \) transformation. The indicated mtr and dtr bristles are outside the clone. The arrow indicates an ectopic dorsal vein 2.
Table 5. Genetic interaction between \( \text{trx} \) and \( Dlw^d \)
(quantitation method is given in Table 1)

<table>
<thead>
<tr>
<th>Allele</th>
<th>( \text{trx}/\text{TM3} \times \text{Dlw}/\text{TM6} )</th>
<th>( \text{Dhw}^+ )</th>
<th>( \text{trx}^+ )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{trx}^{E2} \times \text{Dlw}^d )</td>
<td>37.5 (22) 0.7±1.0</td>
<td>100 (18) 18.6±5.0</td>
<td>0 (20) 0</td>
</tr>
<tr>
<td>( \text{trx}^d \times \text{Dlw}^d )</td>
<td>55.0 (20) 0.9±1.1</td>
<td>100 (20) 12.9±5.4</td>
<td>0 (16) 0</td>
</tr>
</tbody>
</table>

ground (data not shown). The somewhat stronger ventral-to-dorsal transformation found in \( Dlw^d/ \) is also suppressed in a \( \text{trx}^+ \) background, although not completely (Table 5). Perhaps surprisingly, there is no effect of one dose of \( \text{trx}^{E2} \) on the dorsal-to-ventral transformation in \( \text{Df}(3R)\text{RXT13}^+/ \) wings (data not shown); we had expected \( \text{trx} \) mutations to enhance the mutant phenotype.

In several regards, the \( Dlw^d \) mutant interacts with, and shares phenotypic features with, both \( \text{Pc} \) and with \( \text{trx} \) mutants, suggesting that its function is related to or dependent on the functions of \( \text{Pc} \)-group and \( \text{trx} \)-group genes.

**DISCUSSION**

**The \( Dlw^d \) phenotypes**

The \( Dlw^d \) 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 derived from the observation that \( \text{Dp}(3;1)\text{Dlw}^+/\text{Dlw}^d/\text{Df} \) allele must be ectopically expressed to generate the dominant phenotype. These effects are thought to arise when “lateral inhibition” of macrochaetdevelopment among a group of potential macrochaete precursor cells is incomplete (reviewed by Simpson, 1990). Lateral inhibition involves cell-cell signaling using the \( \text{Notch} \) and \( \text{Delta} \) gene products (Wharton et al., 1985; Fehon 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 \( \text{achaete} \) and \( \text{scute} \) 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 \( Dlw^d/ \) 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 wing compartmental restriction along the wing margin in \( \text{M}(3)i^{55} \) 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 \( \text{apterous} \) gene expression coincides with the D/V boundary line (Diaz-Benjumea and Cohen, 1993; Blair, 1993; Williams et al., 1993). We have demonstrated that \( Dlw^d/ \) clones on the dorsal wing transform dorsal to ventral, while, by virtue of an unusual genetic circumstance, \( Dlw^+ \) 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/Dlw1 double heterozygotes. This interaction, and probably the Pc effect alone, are absent from Dlw1/Dlw1 clones. In other words, the recessive loss-of-function aspect of the Dlw1 mutant is epistatic to the wing margin phenotype of a Pc mutant. This relationship implies that Pc precedes Dlw1 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

**Fig. 3.** A model for D/V signaling and dorsal identity specification. → denotes positive regulation; ← denotes negative regulation; ↔ denotes cell-cell communication.

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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