white$^+$ Transgene Insertions Presenting a Dorsal/Ventral Pattern Define a Single Cluster of Homeobox Genes That Is Silenced by the *Polycomb*-group Proteins in *Drosophila melanogaster*

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ABSTRACT

We used the *white* gene as an enhancer trap and reporter of chromatin structure. We collected *white*$^+$ transgene insertions presenting a peculiar pigmentation pattern in the eye: *white* expression is restricted to the dorsal half of the eye, with a clear-cut dorsal/ventral (D/V) border. This D/V pattern is stable and heritable, indicating that phenotypic expression of the *white* reporter reflects positional information in the developing eye. Localization of these transgenes led us to identify a unique genomic region encompassing 140 kb in 69D1–3 subject to this D/V effect. This region contains at least three closely related homeobox-containing genes that are constituents of the *iroquois* complex (*IRO*-C). *IRO*-C genes are coordinately regulated and implicated in similar developmental processes. Expression of these genes in the eye is regulated by the products of the *Polycomb*-group (*Pc*-G) and *trithorax*-group (*trx*-G) genes but is not modified by classical modifiers of position-effect variegation. Our results, together with the report of a *Pc*-G binding site in 69D, suggest that we have identified a novel cluster of target genes for the *Pc*-G and *trx*-G products. We thus propose that ventral silencing of the whole *IRO*-C in the eye occurs at the level of chromatin structure in a manner similar to that of the homeotic gene complexes, perhaps by local compaction of the region into a heterochromatin-like structure involving the *Pc*-G products.

THE product of the *white* (*w*) gene is necessary for the deposition of pigments in the compound eye of *Drosophila melanogaster*. The expression of *white* is extremely sensitive to position effects, which can be observed when the gene is relocalized by germ line transformation as a *P*[*w*$^+$] (Hazelrigg *et al.* 1984; Levis *et al.* 1985) or a *P*[mini-*white*$^+$] transgene (Pirrotta 1988).

Three types of effects are observed with *white*$^+$ derivative transgenes. Most frequent is the homogeneous reduction of pigmentation throughout the entire eye (Levis *et al.* 1985; Pirrotta 1988). Less frequently, pigmentation is randomly reduced or absent in certain ommatidia (variegated or unstable position effect; Henikoff 1994). In rare cases, the reduced or absent pigmentation in certain ommatidia is distributed according to a reproducible pattern.

The variegated pigmentation patterns are usually observed when *P*[*w*$^+$] transgenes are inserted in the proximity of heterochromatic loci (Hazelrigg *et al.* 1984; Levis *et al.* 1985; Wallrath and Elgin 1995), resulting in position-effect variegation (PEV; reviewed in Henikoff 1990; Reuter and Spierer 1992; Singh 1994). These patterns are not heritable, as neither siblings derived from the same parents nor the two compound eyes of the same individual display identical mosaic patterns. *P*[*w*$^+$] transgenes harboring *cis*-regulatory sequences of *Polycomb*-group (*Pc*-G) target genes, including Polycomb responsive elements (PREs; Simon *et al.* 1993), have been shown to frequently exhibit a similar variegation, although inserted at euchromatic sites (Fauvarque and Dura 1993; Chan *et al.* 1994; Gindhart and Kaufman 1995; Zink and Paro 1995). This variegated phenotype is also distinct from classical PEV, as it is modified neither by most genetic modifiers of PEV nor by the removal of the *Y* chromosome. However, this new kind of euchromatic variegation was shown to be sensitive to the dosage of *Pc*-G and *trithorax*-group (*trx*-G) gene products (Fauvarque and Dura 1993; Chan *et al.* 1994; Gindhart and Kaufman 1995; Zink and Paro 1995) and was therefore called developmental-regulator effect variegation (DREV; Fauvarque and Dura 1993).

Pc-G genes have been genetically isolated as a class of negative *trans*-regulators responsible for the maintenance —but not the initiation—of homeotic gene repression (for reviews see Paro 1993; Kennison 1995). The PC-G

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proteins act synergistically on homeotic gene regulation (Jürgens 1985). Five molecularly characterized members of this group (reviewed in Simon 1995) have been shown to be co-localized at a number of discrete sites on polytene chromosomes (Zink and Paro 1989; DeCamillis *et al.* 1992; Martin and Adler 1993; Rastelli *et al.* 1993; Lonie *et al.* 1994). The PC protein shares a domain homologous with the nonhistone heterochromatin-associated protein HP1 (Paro and Hogness 1991), which is necessary for PC attachment to the chromatin (Messmer *et al.* 1992). By analogy with the model proposed for heterochromatin formation in the case of PEV (Locke *et al.* 1988), it was thus proposed that PC-G proteins act negatively on their target genes by inducing locally a clonally inherited heterochromatin-like structure, thus ensuring the clonal maintenance of the transcriptional repression state of these targets (Paro 1990). Derived models are described in Pirrotta and Rastelli (1994); Orlando and Paro (1995); and Bienz and Muller (1995). It is likely that DREV depends on the local formation of a heterochromatin-like structure induced by multimeric complexes containing some or all Pc-G proteins.

Conversely, the *trx*-G gene proteins are required for continued transcriptional activation of homeotic genes (Mazo *et al.* 1990; Breen and Harte 1993). The molecularly characterized members of the TRX-G consist of a diverse set of proteins. While some proteins seem to be specific for regulating developmental genes (Chinwalla *et al.* 1995), others appear to have a more general role in gene activation (Tamkun *et al.* 1992; Farkas *et al.* 1994; Tsukiyama *et al.* 1994; Dingwall *et al.* 1995). It has been proposed that the TRX-G proteins maintain homeotic gene activity by keeping the DNA in an "open" chromatin configuration.

In contrast to PEV, DREV patterns can be stable and heritable. This reproducibility indicates that the phenotypic expression of the *white*$^+$ transgene is reflecting positional information at work in the developing eye. In most cases, the patterned expression displays an anterior-posterior gradient (Bhojwani *et al.* 1995; Sun *et al.* 1995). In rare cases, halolike patterns (Coen 1990) or dorsal-ventral (D/V) patterns can be observed (Levis *et al.* 1985; Hazelrigg and Peterson 1992; Irvine and Wieschaus 1994 and personal communication; Sun *et al.* 1995; Brodsky and Steller 1996).

The mini-*white*$^+$ gene, with its constitutive modest expression level (Pirrotta 1988), can be used as a reporter for the detection of both enhancer and silencer effects of *cis*-regulating sequences flanking its insertion point. These flanking sequences are supposed to direct the expression of the neighboring genes (Bellen *et al.* 1989; Bier *et al.* 1989; Wilson *et al.* 1989). Taking advantage of this property of mini-*white*$^+$ reporter genes, several groups have successfully performed screens for Drosophila genes that could be implicated in pattern formation (Bhojwani *et al.* 1995; Sun *et al.* 1995; Brodsky and Steller 1996). It is noteworthy that, as noticed by Bhojwani *et al.* (1995), over half of the chromosomal loci where various *P*[*w*$^+$] insertions display patterned *white* expression are binding sites for *Pc*-G gene products (Hazelrigg *et al.* 1984; Levis *et al.* 1985; Kassis *et al.* 1991; Fauvarque and Dura 1993; Sun *et al.* 1995). This suggests that the screen based on patterned expression of (mini-)*white*$^+$ reporters appears prone to detect genes that are targets for *Pc*-G-mediated developmental regulation.

We have undertaken the collection of transgenic lines displaying a dorsally restricted expression pattern in the adult eye. Our working hypothesis was that the study of different transgenic lines, all displaying the same stable eye pigmentation pattern, would allow us to identify genes whose expression is subject to common developmental regulators. Moreover, we thought that the study of lines showing a differential expression of *white*$^+$ reporter along the D/V axis of the eye would allow us to target our screen to genes that are involved in the establishment of the dorsal (*vs.* ventral) identity of ommatidial clusters in the adult eye. Localization of these transgenes, genetic analysis of the phenotypes induced by their insertion, and analysis of developmental expression patterns of the reporter genes allowed us to identify a single genomic region, 69D, showing the D/V effect. This region includes several homeobox-containing genes, coordinately regulated and implicated in similar developmental processes. In the developing eye, ventral repression of these genes is regulated by the *Pc*-G gene products, suggesting that regulation of the region we have identified may be exerted at the level of chromatin structure.

MATERIALS AND METHODS

Fly strains and culture: All strains were maintained on standard culture medium at 18°, 20°, or 25°. All variants used are described in Lindsley and Zimm (1992), except when stated in the article. All D/V transgenic lines described in this section were backcrossed with the *y w*^{67c23} stock for ten generations.

T3 and T81 (renamed iro^{T3} *and iro*^{T81}*):* These two strains contain an insertion of the *P*[*w*^{d1}] transgene. *P*[*w*^{d1}] carries the whole *white*$^+$ gene with a direct tandem duplication comprising the 5′ regulatory sequences and the first exon (Coen 1990). These strains were independently obtained after *P*-induced mobilization of an insertion of *P*[*w*^{d1}] (Coen 1990).

cre^1 *(renamed mirr*^{cre1}*):* We isolated this strain after mobilization of *P*[*lacW*] (Bier *et al.* 1989) with the stable source of transposase *P*[*ry*$^+$ Δ*2-3*](99B) (Robertson *et al.* 1988), designated hereafter as Δ*2-3*.

l(3)A5-3-42[1] (renamed mirr^{cre2}*), 35*^{d} *(renamed mirr*^{cre3}*), Sc5 (renamed mirr*^{cre4}*), 59-12 (renamed mirr*^{cre5}*), R's (renamed mirr*^{cre6}*), Sc2 (renamed iro*^{Sc2}*), B6.8 (renamed iro*$^{B6.8}$*), T's, and Sc4:* These strains, containing a *P*[*lacW*] insertion (Bier *et al.* 1989), were kindly provided by, respectively, the Bloomington Stock Center (Bloomington, IN); S. Kerridge, Laboratoire de Génétique—Centre Universitaire de Marseille, Marseille, France; M. Boube and D. Cribbs (who also provided *Sc2* and *Sc4*), Centre de Biologie du Développement Centre National de la Recherche Scientifique, Toulouse, France; P. Maroy, Department of Ge-

netics, Atlila Jozsef University, Szeged, Hungary; R. Cossard and R. Terracol, Laboratoire de Génétique du Développement et Evolution, Institute Jacques Monod, Paris, France; D. Dorer, Division of Biomedical Sciences, Meharry College, Nashville, TN. The *l(3)A5-3-42[1]* line is described in Hartenstein and Jan (1992).

K's: This strain harbors a *P*[*UAS-UbxW*] insertion (K. D. Irvine, personal communication). This transgene contains a p*UAST*-[*Ubx* IVa cDNA] fusion.

L's: This strain contains a *P*[*Mtn W*] insertion (L. Théodore, personal communication). This transgene harbors a functional *Metallothionein* (*Mtn*) transcriptional unit (Maroni *et al.* 1995).

J26.b16: This *hobo* enhancer trap line harbors the *H*[*pHLw2*] transgene (Smith *et al.* 1993). Enhancer trap lines *l(3)*jD3, *l(3)s2783*, *P*[w^+]*33*, *l(3)j2E11*, and *l(3)*j6C3, harboring a *P*[w^+] insertion in 68F2–3, 69F5–6, 70C, 70C5–6, and 70D4–6, respectively, were obtained from the Bloomington Stock Center.

Enhancer trap lines *11F3* and *59A*, harboring a *P*[w^+] insertion in, respectively, the *dptp69D* and the *Klc* gene (Desai *et al.* 1996), both located in 69D1–6, were obtained from C. Desai. We also obtained deficiencies affecting the corresponding genes (*Df(3L)8ex34*, *Df(3L)8ex25*, *dptp69D*[1], and A6B (Desai *et al.* 1996; C. Desai, personal communication) from the same source.

Cloning of transposon-flanking regions: DNA extraction from adult flies was performed as described by Junakovic *et al.* (1984).

Inverse PCR (I-PCR) procedure: Genomic DNA of iro^{T3} and iro^{T81} was digested, ligated, and treated as described in Delattre *et al.* (1995). Amplification reactions were performed on a Trio-Thermoblock Biometra Inc. (Tampa, FL) as follows: 35 cycles of 45-sec denaturation at 94°, 45-sec annealing at 45°, and 4-min extension at 72°, followed by 10 min at 72°.

The *P*-element-specific primers used were as follows (coordinates as in the *P*-element sequence; O'Hare and Rubin 1983): primer 1, P108-P89, 5′CGTCCGCACACAACCTTTCC 3′; primer 2, P414-P433, 5′ GGCTATACCAGTGGGAGTAC 3′; and primer 3, P31-7, 5′ CGACGGGACCACCTTATGTTATTTC 3′.

A *white*-specific primer, localized at position 5009–5028 in *white* coordinates (O'Hare *et al.* 1984; GenBank accession number X02974), was also used: primer 4, 5′CGAATGCTCTC TCCATGCTC 3′.

PCR, with primers 1 and 2, on iro^{T3} DNA (digested with *Eco*RI and ligated) allowed the amplification of a 1.2-kb DNA fragment flanking the 5′ insertion point.

Two successive PCRs—first with primers 1 and 2 on iro^{T81} DNA (digested with *Nde*2) and second with primers 3 and 4 on the preceding amplification product—allowed the amplification of a 0.7-kb DNA fragment flanking the 5′ insertion point.

Plasmid rescue procedure: Cloning by plasmid rescue was performed on iro^{Sc2}, $mirr^{cre2}$, and $mirr^{cre3}$ DNA digested with *Eco*RI; on $mirr^{cre1}$ and $mirr^{cre4}$ DNA digested with *Sac*II; and on $iro^{B6.8}$ digested with *Bgl*II according to Pirrotta (1986). This allowed the cloning of genomic DNA fragments (flanking the insertion point of *P*[*lacW*]) of 1.6 kb, 10 kb, 4.5 kb, 4.7 kb, 5.7 kb, and 2.0 kb, respectively, for iro^{Sc2}, $mirr^{cre1}$, $mirr^{cre2}$, $mirr^{cre3}$, $mirr^{cre4}$, and $iro^{B6.8}$. The same procedure was applied on *J26.b16* for the cloning of a 6.6-kb *Bam*HI fragment flanking the *H*[*pHLw2*] insertion point (Smith *et al.* 1993).

Sequencing of transposon-flanking regions: Clones containing the genomic DNA flanking $mirr^{cre2}$, $mirr^{cre3}$, $mirr^{cre4}$, and *DH1* insertions were sequenced in an ABI 373 automatic sequencer (ABI Adv. Biotechnologies, Inc., Columbia, MD) using a primer complementary to the *P*-element inverted repeat (IR = CGATCGGGACCACCTTATGTTATTTCATCAT).

A 0.5-kb *Cla*I-*Xho*I genomic DNA fragment obtained from *mirrλ1* genomic clone and including the 5′ end of *mirr* cDNA (McNeill *et al.* 1997; H. McNeill, personal communication), was inserted in pBluescript and sequenced using T3 oligonucleotide as primer.

Molecular mapping of D/V transgenes: Clones containing the genomic DNA flanking iro^{T3}, iro^{T81}, $iro^{B6.8}$, iro^{Sc2}, and *J26.b16* transgenes were used as probes to hybridize Southern blots containing *Eco*RI restriction fragments obtained by digestion of genomic DNA of the region (Gomez-Skarmeta *et al.* 1996; McNeill *et al.* 1997; R. Diez del Corral and J. Modolell, unpublished results). The restriction pattern of the transposon-flanking clones was then compared to that of genomic clones to determine the position of the insertions within the *Eco*RI restriction fragments. iro^{T3} is located upstream from the 5′ end of *ara* cDNA. iro^{T81} and $iro^{B6.8}$ are inserted in *ara* second and third introns, respectively. iro^{Sc2} is located upstream from the 5′ end of *caup* cDNA.

The exact positions of $mirr^{cre2}$, $mirr^{cre3}$, $mirr^{cre4}$, and *DH1* insertions, relative to *mirr* cDNA, were determined by sequencing cloned genomic DNA flanking the *P*[*lacW*] insertion site and a *mirrλ1* genomic subclone including the 5′ end of *mirr* cDNA (H. McNeill, personal communication). Sequence alignment revealed that $mirr^{cre3}$, $mirr^{cre4}$, and both $mirr^{cre2}$ and *DH1* are located 343 bp, 282 bp, and 474 bp upstream from the 5′ end of *mirr* cDNA, respectively.

Preparation of P1 DNA and Southern analysis: Bacterial clones containing single P1 clones (clones covering the 69C2–70A5 region: DS02752, DS00099, DS00285, DS07487, DS 08512, DS00044, DS08062, DS00298, DS00073, DS06094, DS07359, DS08991, DS04287, DS02826, DS00334, DS07050, DS04368, DS06456, DS00722, DS04746, and DS06041, Drosophila Genome Center, Berkeley, CA; Figure 3) were inoculated into 500 ml of Luria broth (LB) medium containing 25 μg/ml kanamycin and 1 mm IPTG (isopropyl 1-thio-β-D-galactopyranoside) and grown for ~6 hr at 37° (until OD_{550} = 1.3–1.5). Plasmid DNA was extracted according to the maxi-prep kit protocol (QIAGEN Inc., Chatsworth, CA). Southern blots of these P1 plasmids were hybridized with each flanking genomic DNA fragment cloned as probes.

***In situ* localization on polytene chromosomes:** Preparation of chromosome spreads and cytogenetic localization of transgene insertion sites of every transgenic line listed were performed as described in Fauvarque and Dura (1993). A 1.5-kb DNA fragment containing the sixth exon of the *white* gene (fragment *Sal*I +12725 to +14240 in *white* coordinates) labeled with biotin-dUTP was used as a probe.

Cloned DNA fragments flanking iro^{T3} and $mirr^{cre2}$ insertions were also labeled with biotin-dUTP and hybridized to chromosomes of the w^{1118} stock.

Methods of molecular biology: All standard molecular techniques (such as restriction digestion, agarose gel electrophoresis, Southern blotting, and hybridization) were performed as described in Maniatis *et al.* (1989).

Generation of $mirr^{cre1}$ derivatives: w^{1118}/*Y*; Δ*2-3*, *Sb*/*TM6*, *Ubx* males were crossed with w^{1118}; $mirr^{cre1}$ females. w^{1118}/*Y*; *Sb*, Δ*2-3*/$mirr^{cre1}$ F_1 males were then individually mated to w^{1118}; *TM3*, *Sb*/*T(2;3)apterous*^{Xa} females. Eye color was examined in the F_2 progeny: exceptional [white] F_2 males were individually mated to w^{1118}; *TM3*, *Sb*/*T(2;3)apterous*^{Xa} females. Sibling [white; Sb] F_3 male and female progeny were mated and, in their progeny, [white; Sb^+] individuals were scored for viability or phenotypical defects.

Histochemical staining: β-Galactosidase activity was detected in adult ovaries by 5-bromo-4-chloro-3-indolyl-β-d-pyranoside (X-gal) staining according to Lemaitre *et al.* (1993).

Imaginal discs and brains were dissected from late third-instar larvae and stained with X-gal as described by Lemaitre and Coen (1991).

Immunostaining of embryos: Embryos were stained with an antibody directed against β-galactosidase as described by

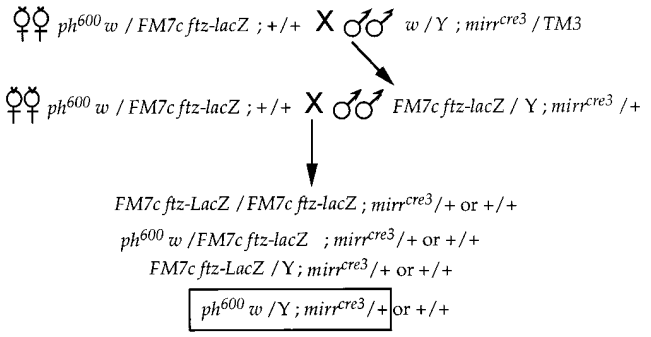

Figure 1.—Embryos immunostained for β-galactosidase. Stained embryos that do not display the *ftz* pattern have the boxed genotype.

Ingham and Martinez-Arias (1986). In control experiments on embryos of a w^{1118} stock, no staining was detected with this antibody.

***In situ* hybridization in whole embryos:** The *in situ* hybridization of (mini$^-$)*white*$^+$ or *lacZ* transcripts in whole embryos was performed as described by Bonneton and Wegnez (1995). The *white* antisense probe used was a 0.86-kb fragment (fragment *Sal*I +11866–+12725) including the fourth and fifth exon of the *white* gene (Delattre *et al.* 1995) and labeled with digoxigenin (Dig-dUTP). In control experiments on embryos of a w^{1118} stock, no staining was detected with this probe.

Testing for the effect of PEV modifiers: Females from the tested line were mated to males mutant for a modifier of PEV. The effect of these modifiers on eye pigmentation pattern was observed in males issued from this cross. Suppressors tested were *Su(var)2-101*, *Su(var)2-501*, *Su(var)205-5*, *Su(var)2-b4801*, *Su(var)2-b204*, *Su(var)2-b701*, *Su(var)2-b801*, *Su(var)2-201*, *Su(var)2-1001*, *Su(var)3-316*, *Su(var)3-103*, *Su(var)3-c1101*, *Su(var)3-902*, *Su(var)3-401*, and *Su(var)3-111*. Enhancers were *E(var)8*, *E(var)102-1*, *E(var)129-1*, *E(var)166-7*, *E(var)56-9*, *E(var)70-2*, *E(var)90*, *E(var)3-101*, and a duplication of *Su(var)3* in 21A. These modifiers of PEV were kindly provided by G. Reuter (Institute of Genetics, Martin Luther University, Halle, Germany) and J. Gausz (Institute of Genetics, Biological Research Center, Szeged, Hungary). Their effect on PEV was confirmed on w^{vco}.

To generate progeny with an extra *Y* chromosome (*XXY* females) or with no *Y* chromosome (*XO* males), females from the tested lines were mated to males with attached *XY*, w^{1118} (R. Levis; described in Dorer and Henikoff 1994).

Testing for the effect of *trx*-G or *Pc*-G gene mutations: To test the effect of a dosage reduction of *Pc*-G or *trx*-G gene products on eye pigmentation, females from the tested line were mated to males heterozygous mutant for the *Pc*-G or *trx*-G gene tested (mutant/Balancer). In the progeny, eye pigmentation patterns of sibling males that did or did not display the balancer chromosome marker were compared.

To test the effect of the ph^{410} mutation on *lacZ* expression patterns in larvae, ph^{410} *w* females were mated to *w*/*Y*; *P*[*lacW*]/*TM3* males. Mutant ph^{410} *w*/*Y*; *P*[*lacW*]/+ male and heterozygous ph^{410} *w*/+; *P*[*lacW*]/+ female larvae derived from this cross were stained with X-gal and compared for *lacZ* expression patterns.

Crosses allowing analysis of the effect of the ph^{600} mutation on $mirr^{cre3}$ expression pattern in embryos are described in Figure 1.

A *fushi tarazu-lacZ* (*ftz-lacZ*) fusion was used to trace the *X* chromosomes that do not bear the *ph* mutation. In the progeny of the first cross, males presenting the D/V pigmentation pattern with a Bar phenotype were crossed to ph^{600} *w*/*FM7c ftz-lacZ* females. F_2 embryos, immunostained for β-galactosidase, that do not display the *ftz* pattern are mutant for *ph* and bear one copy of the $mirr^{cre3}$ transgene (ph^{600} *w*; $mirr^{cre3}$/+ males).

RESULTS

***white*$^+$ transgenes showing a D/V restriction of *white* expression pattern co-localize in a single chromosomal region, 69D:** In the course of *P*[w^+] transgene-mediated mutagenesis, we have recovered three independent transgenic lines (iro^{T3}, iro^{T81}, and $mirr^{cre1}$; see Table 1) displaying a peculiar pattern of *white* expression in the adult eye: the pigmentation is normal in the dorsal half of the eye, but *white* expression is strongly or completely repressed in the ventral half, with a clear D/V boundary (Figure 2, A and C). The cytological localization of these insertions revealed that all three were clustered in the same chromosomal site, 69D1–3 (Table 1).

We systematically collected, from various sources, flies harboring a *white*$^+$-derived transgene with a similar D/V pattern (D/V transgenes). We thus obtained 11 other independent transgenic lines presenting this pattern in the adult eye (Table 1 and Figure 2). Differences in the extent of the pigmented area and in the pigmentation level can be seen among these transgenic lines. In most cases (iro^{T3}, iro^{T81}, $mirr^{cre1}$, $mirr^{cre2}$, $mirr^{cre3}$, $mirr^{cre4}$, $mirr^{cre5}$, $mirr^{cre6}$, iro^{Sc2}, *L*'s, *K*'s, and *J26.b16*), the pigmented area corresponds to the dorsal half of the eye, with a pigmentation level ranging from orange to red (Figure 2, A–D). However, two of the lines, $iro^{B6.8}$ and *T*'s, display a more limited pigmentation, gradually fading from the dorsal to the equatorial part of the eye (Figure 2, E and F). In most lines, *white* gene expression is completely abolished in the ventral half of the eye (ommatidia are white, Figure 2, C–F). In iro^{T3} and iro^{T81} lines (bearing a transgene containing the complete *white* gene sequence), the ventral half of the eye is yellow, with a ventral-posterior red sector and some additional mottling (Figure 2A). The *L*'s line also displays some mottling in the ventral half of the eye (Figure 2B). For every *P*[*lacW*] insertion that is homozygous viable, the pigmentation level is higher when the transgene is homozygous than when it is heterozygous.

The localization of these transgenes by *in situ* hybridization on polytene chromosomes of salivary glands was carried out using a fragment of the *white* gene as a probe. The 12 D/V transgenes considered here were found located in the same cytological interval of bands on the third chromosome: 69D1–3 (Table 1). We have cloned the genomic DNA flanking the insertion point of nine of the D/V transgenes. *In situ* localization of the genomic DNA fragments flanking two insertions (iro^{T3} and $mirr^{cre2}$) was performed on polytene chromosomes of the w^{1118} stock, which is devoid of *P*-element insertions. In both cases, the localization was identical to that obtained with the *white* probe on the corresponding transgenic lines.

Meiotic recombination rates between different D/V

TABLE 1

Origin and localization of D/V lines

Original name[a]	New name	Origin	Cytological localization
T3	iro^{T3}	This study	69D1–3
T81	iro^{T81}	This study	69D1–3
cre 1	$mirr^{cre1}$	This study	69D1–3
l(3)A5-3-42[1]	$mirr^{cre2}$	Hartenstein and Jan 1992	69D1–3
35[d]	$mirr^{cre3}$	S. Kerridge, pers. comm.	69D1–3
Sc5	$mirr^{cre4}$	M. Boube, pers. comm.	nd[b]
59-12	$mirr^{cre5}$	P. Maroy, pers. comm.	nd[b]
R's	$mirr^{cre6}$	R. Cossard, pers. comm.	69D1–3
Sc2	iro^{Sc2}	M. Boube, pers. comm.	69D1–3
L's	*L's*	L. Théodore, pers. comm.	69D1–3
K's	*K's*	K. D. Irvine, pers. comm.	69D1–3
B6.8	$iro^{B6.8}$	D. Dorer, pers. comm.	69D1–3
T's	*T's*	R. Terracol, pers. comm.	69D
J26.b16	*J26.b16*	Smith *et al.* 1993	69D1–3

[a] For a detailed description of the transgenes see materials and methods.
[b] Not determined.

insertions were estimated by the yield of [white] recombinants produced by females heterozygous for two different D/V transgenes. This analysis showed that there could be up to 1.5% recombination between the most distant transgenes (iro^{T3} and $mirr^{cre2}$), whereas no recombinants were obtained between iro^{T3}, iro^{T81}, and iro^{Sc2}. Intermediate recombination frequencies were obtained between other combinations of D/V transgenes when tested by pair (data not shown). D/V insertions are thus genetically separable, suggesting that the D/V phenotype was not the result of several $P[w^+]$ insertions into a single site.

By Southern blotting of genomic DNA from P1 contiguous clones covering the 69C2–70A4 chromosomal region (Drosophila Genome Project; Smoller *et al.* 1991; Hartl *et al.* 1994), we have mapped some transgene insertion points using the flanking genomic DNA frag-

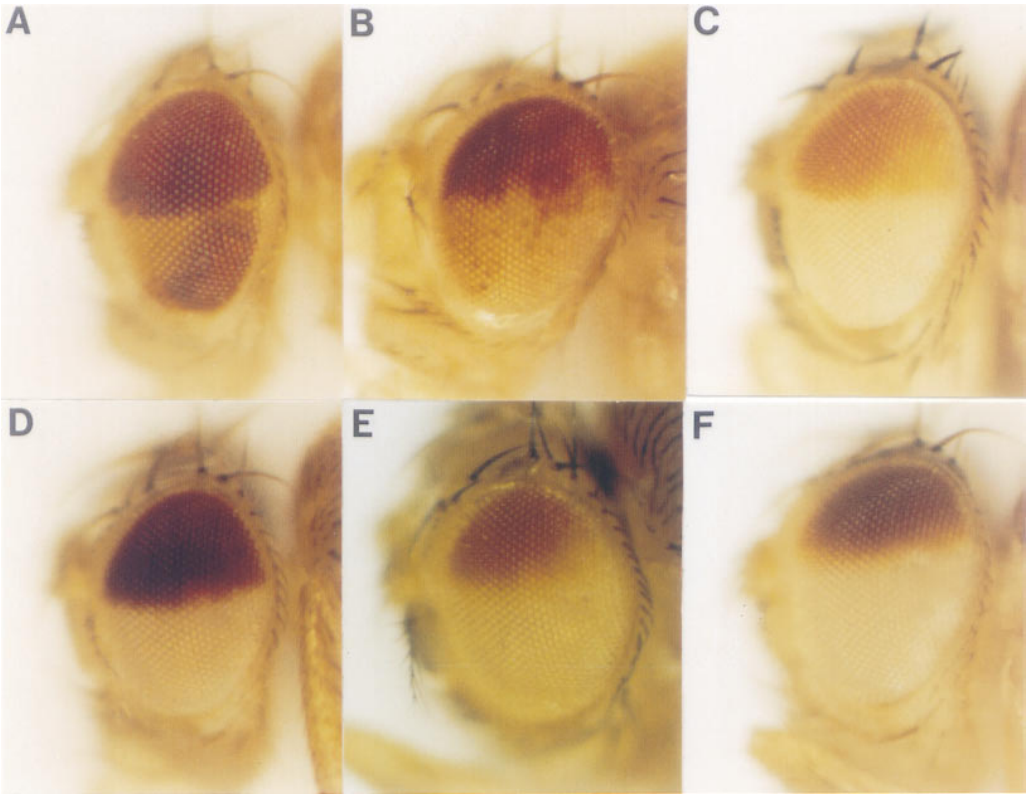

Figure 2.—Eye pigmentation pattern of some D/V transgenic lines. (A) iro^{T81}; (B) *L's*; (C) $mirr^{cre1}$; (D) iro^{Sc2}; (E) $iro^{B6.8}$; (F) *T's*. Orientation is anterior to the left and dorsal to the top. In A, a red sector appears ventrally on a yellow background. In B, mottling is visible in the ventral part of the eye. For all other D/V lines shown, all the ventral ommatidia are white.

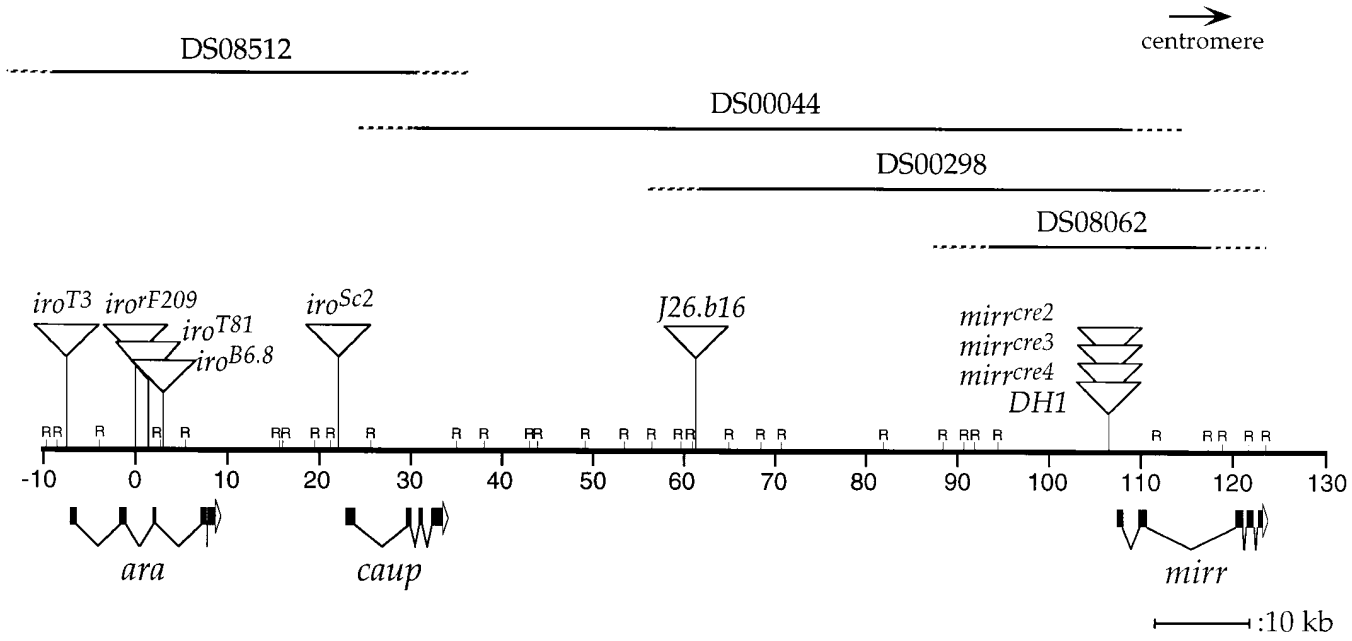

Figure 3.—Physical map of the D/V region. An *Eco*RI (R) restriction map of the D/V region is shown. The iro^{rF209} element insertion point has been taken as the origin of coordinates (Gomez-Skarmeta *et al.* 1996). The triangles indicate the positions of the D/V insertions. Overlapping lines above the map indicate P1 clones used to localize the insertions. The dotted lines indicate that the positions of the ends of cloned DNA in the P1 bacteriophages have not been determined. The exact size of the DNA linking the *ara/caup* and *mirr* regions has been determined from the data of a genomic walk of the region (R. Diez del Corral and J. Modolell, unpublished results). Arrows under the DNA line show the structure of the *ara*, *caup*, and *mirr* transcripts (Gomez-Skarmeta *et al.* 1996; McNeill *et al.* 1997). The position of the insertions was determined by restriction and Southern analysis or by genomic sequencing (see materials and methods). *DH1* is a *P*[*lacW*] insertion presenting a D/V pattern in the eye and initially mapped to cytological position 69C8–11 (Sun *et al.* 1995).

ments as probes. The insertion site of iro^{T3}, iro^{T81}, $iro^{B6.8}$, and iro^{Sc2} was thus restricted to a single P1 clone (Figure 3): DS08512 (hybridizing the 69D1 band). The genomic DNA flanking the $mirr^{cre3}$ insert hybridizes with three P1 clones (Figure 3): DS00044, DS08062 (hybridizing the 69D1–3 bands), and DS00298 (hybridizing the 69D2–3 bands). The localization of $mirr^{cre3}$ can thus be restricted to the 69D2–3 interval (as it is not included within the DS08512 P1 clone). Genomic DNA flanking *J26.b16* hybridizes the P1 plasmids DS00044 and DS00298 and is thus localized in 69D2–3.

Three homeobox-containing genes were recently isolated in the 69D chromosomal region. Two transcription units, *araucan* (*ara*) and *caupolican* (*caup*) were detected within the *iroquois* (*iro*) locus (Dambly-Chaudière and Leyns 1992; Gomez-Skarmeta *et al.* 1996). They encode related proteins that contain a novel class of homeodomain. Both proteins contribute to *iro* function. Another related gene, *mirror* (*mirr*), has been isolated in the 69D region (McNeill *et al.* 1997). It encodes a protein with a homeodomain very similar to those of ARA and CAUP, although the *iro* proteins are related more closely to each other than to MIRR (Gomez-Skarmeta *et al.* 1996). The similarity of *mirr* to *ara* and *caup* and its coincident location in 69D led to the identification of *mirr* as another member of the *iro* complex (Gomez-Skarmeta *et al.* 1996; McNeill *et al.* 1997).

P[*lacW*] insertions in *mirr* ($mirr^{P1}$ and $mirr^{P2}$), are expressed in the dorsal half of the eye (McNeill *et al.* 1997; Brodsky and Steller 1996). A *P*[*lacZ*] insertion in *ara* (iro^{rF209}; Gomez-Skarmeta *et al.* 1996) expresses *lacZ* in the dorsal half of the eye imaginal disc (Figure 7D). The size of the genomic region separating those two loci (*ara/caup* and *mirr*) was estimated at less than 75 kb, since *caup* and *mirr* probes hybridized with the DS00044 P1 clone. This has been confirmed (R. diez del Corral and J. Modolell, unpublished results) by a genomic walk between the *ara/caup* and *mirr* previously isolated genomic clones (Gomez-Skarmeta *et al.* 1996; McNeill *et al.* 1997) demonstrating that *mirr* is located 70 kb proximal to *caup*. The *ara*, *caup*, and *mirr* transcription units are thus included within a 140-kb genomic region (Figure 3). We mapped the D/V insertions for which genomic flanking DNA was cloned (except $mirr^{cre1}$). This showed that iro^{T3}, iro^{T81}, and iro^{B68} are inserted into *ara*; iro^{Sc2} into *caup*; and $mirr^{cre2}$, $mirr^{cre3}$, and $mirr^{cre4}$ into *mirr*. *J26.b16* insertion point is located between *caup* and *mirr* and does not affect a transcription unit previously described in that region. The D/V insertions (except *T*'s) that have not been localized on the molecular map have been linked to insertions in *iro* or *mirr* by genetic recombination or complementation studies: *L*'s and *K*'s, respectively, map close to iro^{T3}, and $mirr^{cre1}$, $mirr^{cre5}$, and $mirr^{cre6}$ are *mirr* mutants (genetic analysis of the DIV region and data not shown). In conclu-

sion, we found that *P*[*lacW*] insertions showing a D/V pattern are all clustered in a single genomic region of at least 140 kb.

To determine whether the "D/V effect" (ventral repression of *white*$^+$ transgenes' expression) extends beyond 140 kb, we analyzed the eye pigmentation patterns of eight enhancer trap lines harboring a *P*[w^+] insertion in the 68–70 interval (see materials and methods). One line located in 69C (*Sc4*) displayed a pigmentation restricted to the anterior-equatorial part of the eye. The other lines, located outside of the 69C–69D1–3 interval, had uniformly pigmented eyes. This study allowed us to confine the D/V effect distally to 69C and proximally to 69D3–6.

We have mapped, by functional complementation, deficiencies covering *dptp69D* and *Klc* genes, both located in 69D1–6 (Desai *et al.* 1996), with respect to deficiencies of the *IRO*-C (data not shown). This genetic analysis showed that the *dptp69D* and *Klc* genes are proximal to *mirr*. Moreover, a *dptp69D* genomic clone (Desai *et al.* 1996) hybridized to the P1 clone DS08062, but neither to DS00298 nor to DS00044 (Figure 3), confirming that the *dptp69D* and *Klc* genes are proximal to *mirr*. As *P*[w^+] insertions in *dptp69D* and *Klc* genes are not affected by the D/V effect, this effect is thus limited to the distal region of these genes.

Genetic analysis of the D/V region: Seven out of 14 D/V insertions are homozygous viable and do not lead to any visible phenotype in adults.

With low penetrance, the $iro^{B6.8}$ strain displays a wing phenotype resembling that of iro^{1} and iro^{rF209} homozygotes (Dambly-Chaudière and Leyns 1992; Gomez-Skarmeta *et al.* 1996; Leyns *et al.* 1996). More than 30% of $iro^{B6.8}/iro^{2}$ *trans*-heterozygous survive. They display outheld wings and either duplicated or missing thoracic bristles. $iro^{rF209}/iro^{B6.8}$ *trans*-heterozygous are viable, and some of them display outheld wings. This suggests that $iro^{B6.8}$ is a hypomorphic allele of *iro*, weaker than iro^{rF209}.

Six D/V insertions are homozygous lethal or semilethal and are allelic (Table 2). We have called the functional unit affected by these insertions *crépuscule* (*cre*; "twilight" in French). These insertions do not complement the lethality of the $mirr^{P2}$ insertion, an early larval homozygous lethal allele of *mirr* (Brodsky and Steller 1996; McNeill *et al.* 1997), which shows that they are alleles of the *mirr* gene. We have therefore renamed these alleles $mirr^{cre1}$–$mirr^{cre6}$.

The escapers homozygous for the $mirr^{cre1}$ insertion display thoracic macrochaetae duplications. We have generated transposase-induced [white] derivatives of $mirr^{cre1}$. Revertants for the $mirr^{cre1}$-associated defects were recovered this way, showing that the mutant phenotype is a consequence of the *P*-transgene insertion. Partly viable [white] derivatives of $mirr^{cre1}$ were also recovered. They have outheld wings with missing or reduced alulae and either loss or duplication of thoracic bristles (Figure 4A). These defects are similar to those displayed by escapers homozygous for $mirr^{cre5}$ or *trans*-heterozygous for some combinations of *mirr* lethal or sublethal alleles (Table 2 and Figure 4, B and C).

The wing and bristle phenotypes of *mirr* mutants are reminiscent of the dominant Dichaete (D) phenotype (Bridges and Morgan 1923). Previously described *D* alleles (D^1, D^3, and D^4) are not separable from chromosomal rearrangements (Table 2). We have seen that D^1 and D^3 do complement D^4 lethality. Recently, the dominant wing phenotype of *D* alleles, associated with the breakpoints in 70–71, was shown to be due to a mutation located in 70D and encoding a Sox-domain protein (Russell *et al.* 1996). The name *Dichaete* was retained for that gene. We have obtained from this laboratory (A. T. Carpenter, Department of Genetics, University of Cambridge, England, personal communication) two allelic lethals, *Sail1* (Sai^1) and *Sail2* (Sai^2), mapped to 69D (Table 2) and associated with a dominant outheld-wing phenotype. The *mirr* alleles fail to complement the lethality of D^1 and D^3 or the lethality of Sai^1 and Sai^2, whereas they did complement the lethality associated with D^4 or other *D* alleles affecting 70D (Table 2 and A. T. Carpenter, personal communication). The 69D–70D interval comprises thus at least four genes (*ara*, *caup*, *mirr*, and *D*) susceptible to giving dominant or recessive D phenotypes when mutated independently. We propose to rename the alleles affecting the *mirr* gene $mirr^{D1}$, $mirr^{D3}$, $mirr^{Sai1}$, and $mirr^{Sai2}$. The *mirr* gene would be implicated, notably, in wing and peripheral nervous system development, according to the phenotype of its mutations.

This complementation and phenotypic analysis, together with previous study on *IRO*-C (Gomez-Skarmeta *et al.* 1996), shows that the D/V region in 69D contains at least three different functional units (*ara*, *caup*, and *mirr*), consistent with the molecular data.

Developmental expression patterns: Expression patterns were analyzed for some representative D/V transgenes from oogenesis to larval stages.

Four lines containing the *lacZ* reporter gene were analyzed for β-galactosidase activity in ovaries. $mirr^{cre2}$ and $mirr^{cre3}$ display an identical expression pattern: *lacZ* expression is detected from the beginning to the end of oogenesis. At stage 10, it is restricted to the follicle cells surrounding the anterior-dorsal part of the oocyte, the region where the nucleus is located (data not shown). No expression was detected in ovaries of iro^{Sc2} and $iro^{B6.8}$ lines.

The expression pattern of D/V transgenes in embryo was visualized by *in situ* hybridization with an antisense RNA *white* probe (for $mirr^{cre1}$, $mirr^{cre3}$, $mirr^{cre6}$, iro^{Sc2}, $iro^{B6.8}$, iro^{T3}, iro^{T81}, *L*'s, *K*'s, and *J26.b16* lines) and, in addition, by immunodetection of β-galactosidase (for $mirr^{cre1}$, $mirr^{cre3}$, $mirr^{cre6}$, and iro^{Sc2} lines that carry the *lacZ* reporter; data not shown). The slight differences in staining observed between the two methods of detection may be due to

TABLE 2

Genetic interactions between lethal, sublethal, and some viable lines in the 69D region

Allele	$iro^{B6.8}$	iro^{rF209}	iro^{1}	iro^{DFM3}	iro^{2}	D^{4}	$mirr^{D3}$	$mirr^{D1}$	$mirr^{Sai2}$	$mirr^{Sai1}$	$mirr^{cre1}$	$mirr^{cre2}$	$mirr^{cre3}$	$mirr^{cre4}$	$mirr^{cre5}$	$mirr^{cre6}$
$mirr^{cre6}$					−			±[D]					±[D]			±
$mirr^{cre5}$					−					±[D]		±[D]	±[D]	±[D]	±[D]	
$mirr^{cre4}$					−	+	−	−				±[D]	−	−[a]		
$mirr^{cre3}$	+		+	−	−	+	−	±[D]	−	−	−	−	−[b]			
$mirr^{cre2}$					−	+	±[D]	±[D]		−	±[D]	−[a]				
$mirr^{cre1}$				−				±[D]			±					
$mirr^{Sai1}$					−	+	−	−	−	−						
$mirr^{Sai2}$					−	+	−	−	−							
$mirr^{D1}$					−	+	−	−								
$mirr^{D3}$	+				−	+	−									
D^{4}					+	−										
iro^{2}	+[ow]	±[i]+[ow]	±[i]+[ow]	−	−											
iro^{DFM3}	−			−												
iro^{1}	+		±[i]+[ow]													
iro^{rF209}	+[ow]	+[ow]														
$iro^{B6.8}$	+[ow]															

$mirr^{Sai1}$ is associated with In(3L)69D2–6;84E12–F3. $mirr^{Sai2}$ is mapped genetically to 69D. $mirr^{D1}$ and $mirr^{D3}$ are linked to In(3L)69D3–E1;70C13–D1. D^{4} is associated with T(2;3)21D;70–71. iro^{2} is a deficiency of the 69BD region including *mirr* and *iro* (Dambly-Chaudière and Leyns 1992; Gomez-Skarmeta *et al.* 1996; Leyns *et al.* 1996). [i], iro bristle phenotype (Dambly-Chaudière and Leyns 1992). [D], partial or complete Dichaete phenotype (outheld wings and absent or reduced alulae). [ow], outheld-wing phenotype with alulae present. +, viable; ±, semilethal; −, lethal.

[a] Almost total larval/pupal lethality.

[b] Total larval/pupal lethality.

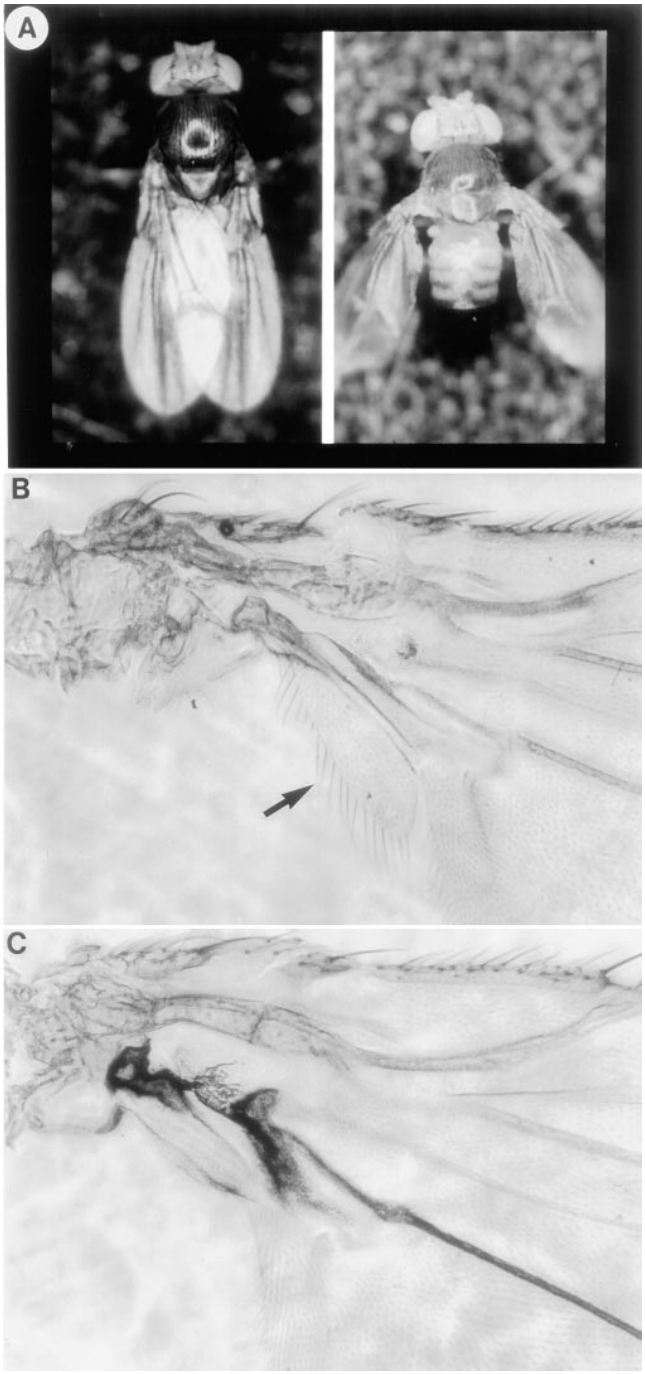

Figure 4.—Phenotypical defects of *mirr* mutant adults. (A) Wing and bristle defects of one viable [white] derivative of $mirr^{cre1}$: $mirr^{cre111}$: left panel, dorsal view of a w^{1118} adult showing wild-type wings and thoracic bristles; right panel, dorsal view of a $mirr^{cre111}$ adult displaying reduced size, outheld wings with missing alulae, and absence of some macrochaetes on the thorax. (B) High magnification of a wild-type wing hinge (Canton strain). The alula is indicated with an arrow. (C) $mirr^{cre2}$/$mirr^{cre4}$ adult wing-hinge defect. Severe distortion of the wing hinge and almost complete absence of alula are observed on both wings of $mirr^{cre2}$/$mirr^{cre4}$ escapers. Wings are oriented with proximal to the left and anterior to the top.

the perdurance of the β-galactosidase protein rather than to differences in the expression domain of the reporter genes tested. In fact, *in situ* hybridization achieved either with a *lacZ* or a *white* antisense probe on the $mirr^{cre3}$ line gave exactly the same pattern, therefore showing that the two reporters of the *P*[*lacW*] construct give the same expression pattern in the embryo, as previously described for transgenes subject to position effect (Kassis *et al.* 1991; Fauvarque *et al.* 1995).

For most lines, transgene expression is very dynamic and very specific. The expression patterns, although similar, differ from one line to the other, with common subpatterns shared by certain lines. In all cases, transgene expression is first detected very early in development and persists throughout embryogenesis. Expression patterns of *P*[*lacW*] insertions in *mirr*, *ara*, and *caup* reflect those revealed with the cDNA probes for the three corresponding genes (Gomez-Skarmeta *et al.* 1996; McNeill *et al.* 1997; J. L. Gomez-Skarmeta, personal communication). A representative for *ara*, *caup*, *mirr*, and also *J26.b16* expression is shown in Figure 5. Expression patterns of *K*'s and *L*'s insertions are hardly detectable (data not shown). It should be noted that throughout embryogenesis, iro^{T3} expression pattern was found to be mostly identical to that of the *white* gene (compare Figure 6A to 6B). iro^{T81} expression pattern is also identical to that of the *white* gene, with an additional strong staining in the primordia of the proventriculus. This shows that $white^{+}$ transgene expression in iro^{T3} and iro^{T81} mostly reflects that of the endogenous *white* gene.

We have studied *lacZ* reporter gene expression patterns in third instar larvae. Tissues expressing the reporter gene in each line are listed in Table 3.

For all D/V lines, *lacZ* expression in the eye imaginal disc reflects (mini^{-})$white^{+}$ expression pattern in the adult eye: β-galactosidase activity is detected only in the dorsal half of the disc (Figure 7, A–D). However, inside this domain, differences in the expanse of the β-galactosidase activity can be observed among the D/V transgenic lines. In fact, the higher the *lacZ* expression level, the closer the β-galactosidase staining is to the D/V border (for instance, compare Figure 7A to 7C). A gradual pigmentation pattern (from dorsal to equatorial) was also observed in some cases (Figure 2, E and F) in the adult eye.

In the wing imaginal disc, *lacZ* reporter is expressed in domains that are precursors of the notum and part of the dorsal hinge, including the precursor of the alula (Figure 7, E–H and data not shown). For certain lines ($mirr^{cre3}$, $mirr^{cre4}$, $iro^{B6.8}$, and iro^{Sc2}), *lacZ* expression is also detected in restricted areas in the wing pouch. These domains might correspond to the prospective longitudinal veins (respectively, proximal L1 and distal L3 veins for $mirr^{cre3}$ and $mirr^{cre4}$; L3 veins and proximal L1 and L5 veins for iro^{Sc2}; and L3 and L5 veins for $iro^{B6.8}$; Figure 7, E–G).

Most D/V lines do not show *lacZ* expression in leg

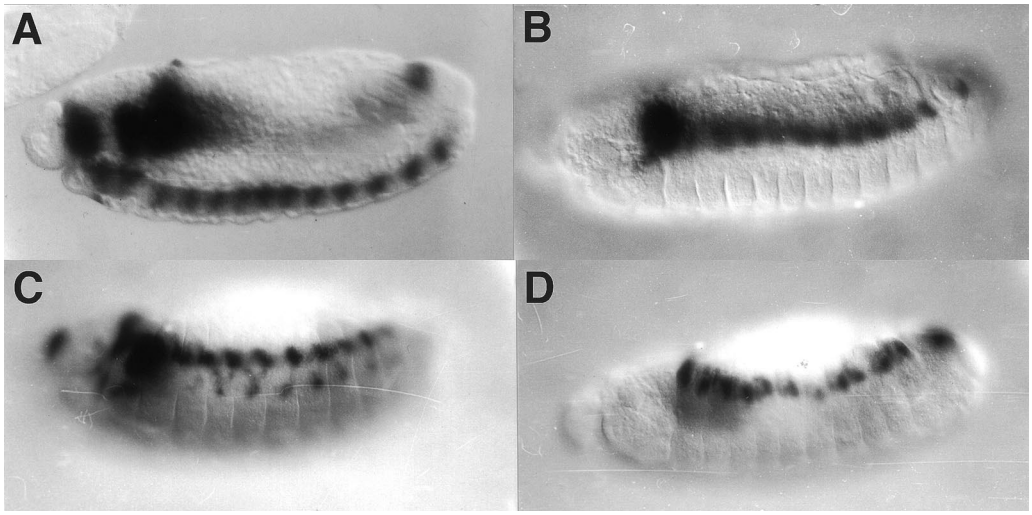

Figure 5.—D/V transgene expression patterns during embryogenesis. *In situ* hybridization, with a *white* antisense probe, on whole mount embryos. All embryos are shown at retracted germ band stage and are oriented anterior to the left and dorsal to the top. (A) $mirr^{cre3}$; (B) iro^{Sc2}; (C) $iro^{B6.8}$; (D) *J26.b16*. In A, expression of $mirr^{cre3}$ reporter gene is detected in the central nervous system (CNS), in the anterior part of each metameric unit of the ventral nerve cord, and in the brain. The reporter gene is also expressed in the proventriculus and in the dorsal epidermis of each segment. This expression pattern is identical to that revealed with a *mirr* cDNA probe (McNeill *et al.* 1997). In B, at the retracted germ band stage, iro^{Sc2} reporter expression domains are the same as *mirr*—except in the CNS, where there is no detectable staining. In C, when the germ band is retracted, $iro^{B6.8}$ reporter expression pattern is partly identical to that of iro^{Sc2} (no expression in the CNS)—but in addition, groups of cells are stained in the dorsal-lateral epidermis. In D, *J26.b16* transgene expression is very similar to that of iro^{Sc2}, but a weak staining is detectable in the ventral neural ectoderm.

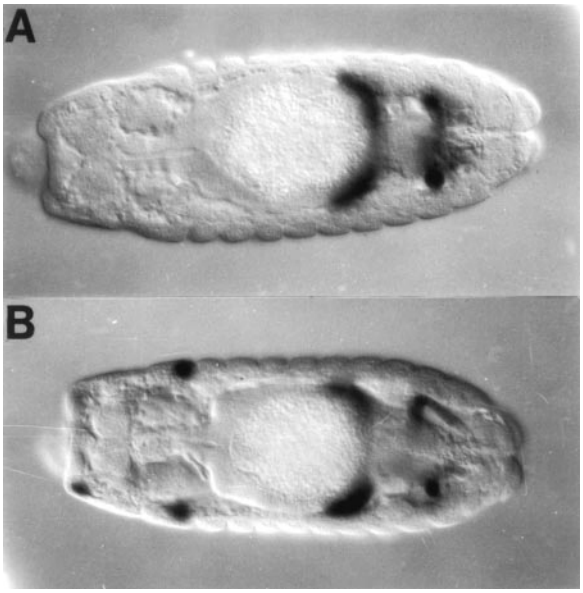

Figure 6.—iro^{T3} expression pattern during embryogenesis. *In situ* hybridization, with a *white* antisense probe, on whole mount embryos. Embryos are oriented anterior to the left, dorsal views. (A) Embryo of the Canton strain. At retracted germ band stage, endogenous $white^{+}$ expression is strong in the Malpighian tubules. (B) iro^{T3} embryo. iro^{T3} expression pattern is mostly identical to that of the *white* gene, since additional staining is detected only in the head.

imaginal discs except the iro^{Sc2}, $iro^{B6.8}$, and (weakly) *T*'s lines (Figure 7, I and J). For every line, *lacZ* is also expressed in other larval tissues in specific and similar patterns (Table 3 and Figure 7, K and L). The β-galactosidase accumulation patterns of *P*[*lacW*] inserted into *ara*, *caup*, and *mirr* mostly reflect the expression pattern of these genes in third instar larvae (Gomez-Skarmeta *et al.* 1996; McNeill *et al.* 1997 and data not shown).

The similarity of the expression patterns at all developmental stages suggests that the genes included in the D/V region may be implicated in common developmental processes and coordinately regulated.

Interaction with modifiers of variegation, *Pc*- and *trx*-G genes: The ventral silencing of (mini^{-})$white^{+}$ in D/V transgenic lines, which is sometimes associated with variegation and mottling (Figure 2, A and B), could be related either to PEV or DREV. Therefore, we tested the effect of mutations in genes involved in these phenomena on the expression pattern of the D/V transgenes.

The effect of 15 suppressors and 9 enhancers of PEV (listed in materials and methods) was tested on the iro^{T81} line pigmentation pattern. None of these mutations produced any effect on the D/V pattern. The effect of *Su(var)205-5* was also assayed on $mirr^{cre3}$ and iro^{Sc2} and did not affect pigmentation. The presence or absence of a *Y* chromosome, which is mainly heterochromatic, is also known to affect PEV (Gowan and Gay 1933). We have shown that, unlike PEV, the D/V pattern of $mirr^{cre3}$ or iro^{Sc2} lines was not influenced by variation in the number of *Y* chromosomes. Moreover, PEV decreases

TABLE 3

***lacZ* expression of D/V transgenes in larval tissues**

P[*lacW*] insertion	Eye-antennal disc	Wing disc	Leg disc	Haltere disc	Salivary glands	Brain	Proventriculus
$mirr^{cre1}$	++	++	−	+	−	+	+
$mirr^{cre2}$	++	++	−	+	−	+	+
$mirr^{cre3}$	++	++	−	+	−	+	+
$mirr^{cre4}$	++	++	−	+	−	+	+
$mirr^{cre6}$	++	+	−	+	−	+	+
$iro^{B6.8}$	±	±	+	−	−	−	+
iro^{Sc2}	++	++	+	+	−	+	+
J26.b16	±	+	−	±	+	−	+
T's	+	±	±	−	−	+	+

The intensity of β-galactosidase staining is given as ++, strong; +, intermediate; ±, weak; −, not detectable.

when breeding temperature of the flies increases and, reciprocally, increases when breeding temperature decreases. For iro^{T3} and iro^{T81} lines, we observed that, in contrast to PEV, the proportion of pigmented ommatidia increased when the flies were raised at 18° compared to 25°; the small ventral pigmented area is enlarged. This particular response to the elevation of temperature had previously been reported for *zeste* variegation (Chen 1948) and for insertions of transgene in which mini-*white*$^+$ expression was under the control of *polyhomeotic* or *AbdB* gene regulatory sequences (Fauvarque and Dura 1993; Zink and Paro 1995). Thus, the ventral repression of *white* expression in D/V strains is mediated by a mechanism different from the centric heterochromatin inactivation in PEV.

69D is a binding site for the product of at least four members of the *Pc*-G: PC, PH, PSC, and PCL (Zink and Paro 1989; DeCamillis *et al.* 1992; Martin and Adler 1993; Rastelli *et al.* 1993; Lonie *et al.* 1994). We therefore tested the effect of the mutation of several members of the *Pc*-G on the D/V pattern. Interactions with the alleles listed in Table 4 were studied with the iro^{T81} line. The effect of a *polyhomeotic* mutation (ph^{410}) was assayed on all D/V lines. In all cases, we observed that a reduction of the dosage of *Pc*-G gene products leads to a diminution of the ventral repression (Figure 8, A–D), which is completely relieved in some cases (*e.g.*, iro^{T81} in combination with Sce^1 or ph^{410}; Figure 8, A and C). In addition, the Pc^{16} mutation, which has no effect on the $mirr^{cre1}$ eye pigmentation pattern by itself, strongly enhances the derepressive effect of the ph^{410} mutation (data not shown). This suggests that *Pc* and *ph* products act synergistically for the ventral repression of $mirr^{cre1}$ expression in the eye, as they do for homeotic gene regulation (Dura *et al.* 1985). These results show that the ventral repression of D/V transgenes' expression in the 69D region is mediated by the *Pc*-G gene products and thus could be maintained by a mechanism analogous to DREV.

69D is a binding site for the *trx* gene products (Chinwalla *et al.* 1995). Mutations of *trx*-G gene members were analyzed for their influence on the D/V pigmentation pattern. *trx*-G mutations tested with iro^{T81} were *Df(2L)net- PMF* (including *kis*), kis^2, mor^1, trx^{E2}, *Df(3R)red-31* (including *trx* and *urdur*), *Df(2R)Ba-MP*, *Su(Pc)37D*, $Df(3L)kto^2$, Dll^3, brm^2, *Df(3L)fz-CAL5*, and *Df(3L)fz-D21* (both deficiencies, including *dev*). Two of these mutations produced a modification of the iro^{T81} pigmentation pattern: mor^1 and *Def(3R)red-31* reduce, respectively, weakly and strongly, the number of pigmented ommatidia in the ventral part of the eye (Figure 8, E and F). The eye pigmentation of $mirr^{cre1}$ was not significantly altered by the *trx*-G mutations tested (trx^{E2}, brm^2, mor^1, and *Df(3R)red-31*).

In another example of DREV, it has been shown that the number of pigmented ommatidia is dependent on the relative "balance" of *Pc*-G and *trx*-G gene products (Gindhart and Kaufman 1995). We have thus further investigated the influence of *trx*-G mutations by testing whether they can suppress the effect of *Pc*-G gene mutation on the D/V pattern. This was achieved by comparing the eye pigmentation pattern of ph^{410} *w*/*w*; iro^{T81}/+ and ph^{410} *w*/*w*; iro^{T81}/*trx*-G$^-$ females (mutations tested were mor^1, kis^2, trx^{E2}, and *Df(3R)red-31*). A partial suppression of the ventral derepression due to ph^{410} was observed in combination with mor^1 and *Df(3R)red-31*. Thus, relieving the ventral repression does not permit revealing of an effect of additional *trx*-G mutations (kis^2 and trx^{E2}) that could have been undetected in a ph^+ background because of a too strong repressive effect of PC-G proteins.

We have tested the effect of the ph^{410} mutation (which leads to a strong ventral derepression of mini-*white*$^+$ in the eye of D/V adults) on *lacZ* expression in $mirr^{cre3}$ third instar larvae. We compared the *lacZ* expression pattern of ph^{410} *w*/*Y*; $mirr^{cre3}$/+ males to that of sibling ph^{410} *w*/+; $mirr^{cre3}$/+ females (whose *lacZ* expression is identical to that of *w*/*w*; $mirr^{cre3}$/+ females). In these males, a clear ectopic staining was observed in the ventralmost part of the eye imaginal disc (Figure 9, A and B), but

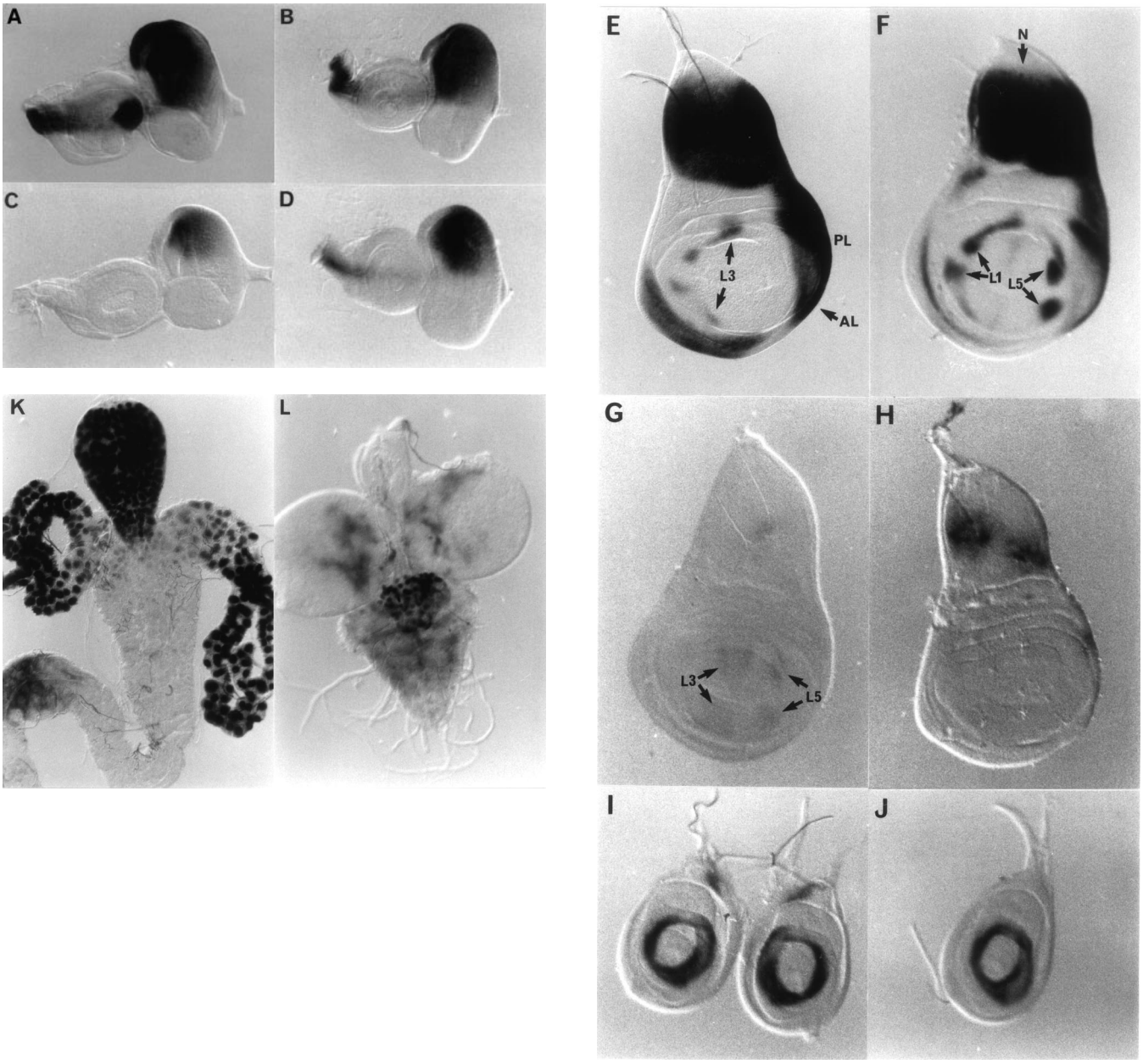

Figure 7.—Expression patterns of D/V transgenes at the third instar larvae. *lacZ* expression detected by X-gal staining of larval tissues. (A–D) Eye imaginal discs, oriented anterior to the left and dorsal to the top. *lacZ* expression is restricted to the dorsal half of the eye disc, as shown here for $mirr^{cre3}$ (A); iro^{Sc2} (B); *T*'s (C); and iro^{rF209} (D). (E–H) Wing imaginal discs, oriented dorsal to the top and posterior to the right. (E) $mirr^{cre3}$; (F) iro^{Sc2}; (G) $iro^{B6.8}$; (H) *J26.b16*. *lacZ* expression is detected in the prospective notum, N; alula, AL; pleura, PL; and in domains that may correspond to the prospective L1, L3, and L5 longitudinal vein regions (E–G). (I and J) Legs imaginal discs of iro^{Sc2} (I); and $iro^{B6.8}$ (J). (K) iro^{Sc2} proventriculus. (L) $mirr^{cre3}$ brain.

no effect of ph^{410} was detected in other larval tissues. This tissue-specific derepression of the *lacZ* reporter perfectly reflects mini-*white*$^{+}$ derepression in the eye of adults of the same genotype (see Figure 8D), showing that *ph* is required for the ventral repression of reporter genes' expression from the third larval stage onwards.

In embryos mutant for a member of the *Pc*-G genes, ectopic expression of homeotic genes first appears during germ band elongation (Struhl and Akam 1985; Wedeen *et al.* 1986; Dura and Ingham 1988; McKeon and Brock 1991; Simon *et al.* 1992). If *Pc*-G genes act on the D/V pattern in the same way that they act on the regulation of homeotic genes, they should be responsible for the maintenance, but not for the initiation, of this pattern. We thus tested the effect of a null mutation in *ph* (ph^{600}) on *lacZ* expression pattern in $mirr^{cre3}$ embryos. At the head involution stage (stage 15), the staining in the ventral nerve cord of these embryos is strongly decreased compared to ph^{+} embryos (Figure 9, C and D). No other effect of ph^{600} was detected, either

TABLE 4

Effect of *Pc*-G gene mutations on *iro*^{T81} eye pigmentation pattern

Gene name	Mutant allele	Effect on D/V pattern
polyhomeotic	*ph*^{410} and *ph*^{503}	++
Sex comb extra	*Sce*^{1}	++
Polycomb	*Pc*^{16}	+
Polycomb-like	*Pcl*^{15}	+
Additional sex comb	*Asx*$^{XF23\,a}$	±
polycombeotic	*E(Z)Su301*	±
Posterior sex comb	*Psc*^{1}	±
Enhancer of Polycomb	*E(Pc)*^{1}	±
extra sex comb	*esc*$^{r4\,a}$	±
Sex comb on midlegs	*Scm*^{D1}	±

The effect on eye pigmentation pattern is given as ++, very strong, leading to a nearly wild-type eye; +, strong, leading to a strong ventral depression that leaves a ventral-equatorial region unpigmented; ±, weak, indicating a weak increase of pigmented ommatidia in the ventral half of the eye.

[a] This allele is described on the World Wide Web in FlyBase at http://cbbridges.harvard.edu:7081/genes/.

before or after this stage or in other tissues. The same positive regulator effect of *ph* function on genes expressed in the central nervous system was previously described (Dura and Ingham 1988). Our result suggests that *ph* function is required, very likely indirectly, for the positive regulation of *mirr*^{cre3} expression specifically in the neural cells of retracted germ band embryos. In the case of homeotic gene regulation, the absence of *ph* product leads both to an ectopic expression of homeotic genes in the epidermis and to a diminution of expression in the ventral nerve cord from the extended germ band stage (Dura and Ingham 1988).

DISCUSSION

All *white*$^{+}$ transgenes showing a dorsal restricted expression of *white* localize in a single chromosomal region, the D/V region: Our results show that all the (mini^{-})*white*$^{+}$ transgenes displaying a D/V expression pattern, where the *white* gene is expressed in the dorsal half of the eye and repressed in the ventral part, are confined to a single genomic region, 69D1–3. This region seems to be unique, since no transgene showing the same pattern of *white* expression has, to our knowledge, ever been found elsewhere in the genome of Drosophila. In fact, recently, *P*[*lacW*] transgenic lines that express (mini^{-})*white*$^{+}$ strictly in the dorsal half of the eye have been isolated by others (Sun *et al.* 1995; Brodsky and Steller 1996; Choi *et al.* 1996; McNeill *et al.* 1997). These studies have allowed the isolation of eight independent *P*[*lacW*] insertions, which add to the 14 D/V inserts described in this study. All but two have been localized cytologically in 69D. We have shown that the two exceptions, *DH1* and *DH2* (Sun *et al.* 1995), previously mapped to 68C8–11, are both allelic to *mirr*, and

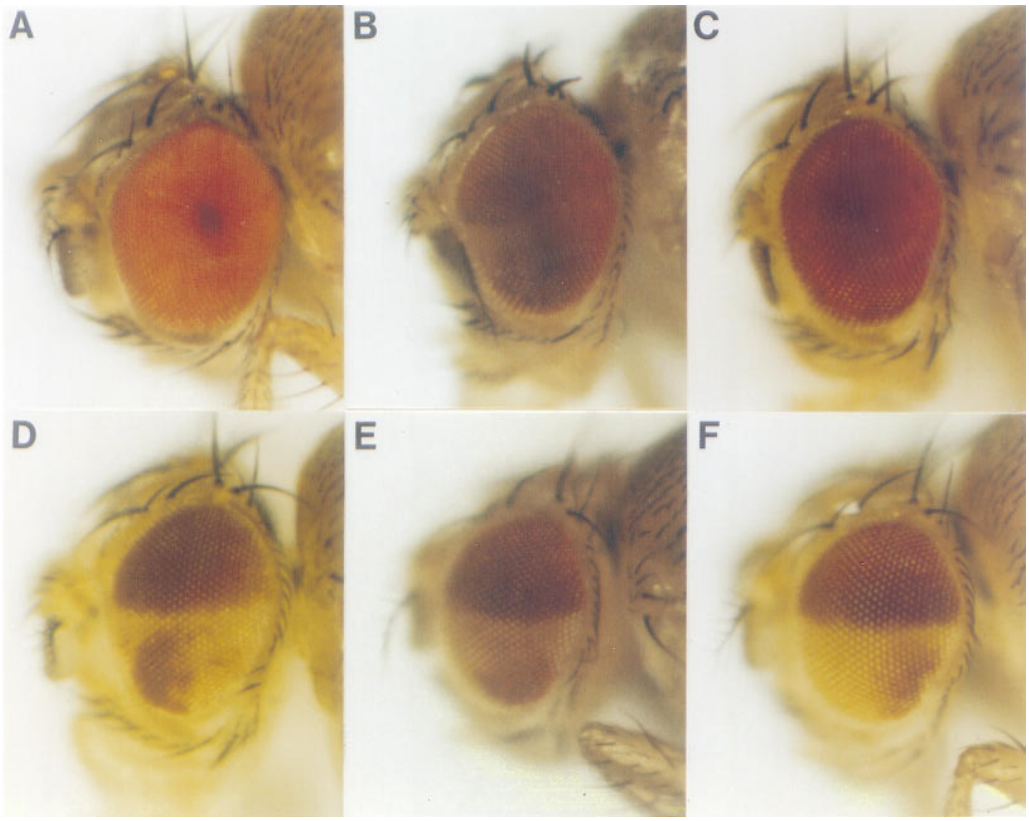

Figure 8.—Effect of mutant background for *Pc*-G and *trx*-G genes on the D/V eye pigmentation pattern. (A) ♂ *w*/*Y*; *iro*^{T81}/*Sce*^{1}. (B) ♂ *w*/*Y*; *iro*^{T81}/*Pc*^{16}. (C) ♂ *ph*^{410} *w*/*Y*; *iro*^{T81}/+. (D) ♂ *ph*^{410} *w*/*Y*; *mirr*^{cre3}/+. (E) ♂ *w*/*Y*; *iro*^{T81}/*mor*^{1}. (F) ♂ *w*/*Y*; *iro*^{T81}/*Df(3R)red-31*.

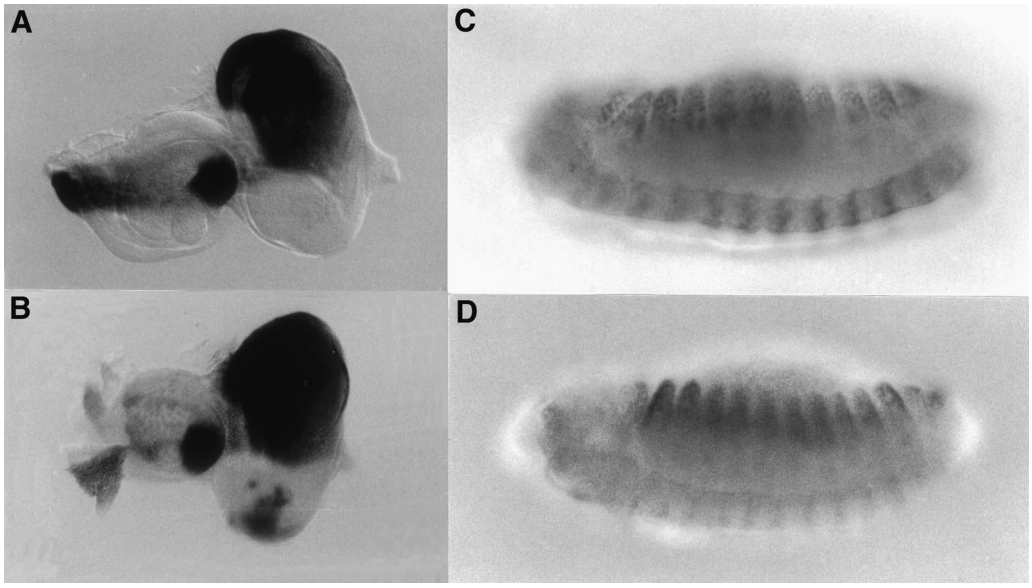

Figure 9.—*ph* function is necessary for maintenance of *mirr* expression pattern in embryos and larvae. (A and B) *lacZ* expression in third instar larvae eye imaginal disc of w/Y; $mirr^{cre3}/+$ male (A) and ph^{410} w/Y; $mirr^{cre3}/+$ male (B). In addition to the staining in the dorsal half in a wild-type background (A), an ectopic staining is detected in the ventral-most part of the disc in a ph^{410} background (B). Discs are oriented anterior to the left and dorsal to the top. (C and D) Immunostaining of β-galactosidase in whole mount embryos. All embryos are oriented with anterior to the left and dorsal to the top. (C) $mirr^{cre3}$ expression pattern in a ph^{+} background. (D) $mirr^{cre3}$ expression pattern in a *ph* null background. A strong decrease of staining is seen in the ventral nerve cord, when compared to C, at the retracted germ band stage.

we have molecularly localized *DH1* to the *mirr* upstream region (see Figure 3). The D/V phenomenon is not peculiar to one type of transgene, since $white^{+}$ or mini-$white^{+}$ in different *P* or *Hobo* constructs can respond to the ventral silencing.

Transgenes presenting the opposite dorsal/ventral expression pattern (*white* expression in the ventral half and repression in the dorsal half of the eye: V/D transgenes) have also been reported: $A^{R4\text{-}24}$ in 24CD on the second chromosome (Levis *et al.* 1985; Hazelrigg and Peterson 1992); three *P*[*LacW*] insertions in 24D (Brodsky and Steller 1996); and *35UZ-1* (Irvine *et al.* 1991) in 78A on the third chromosome (Irvine and Wieschaus 1994). We have obtained and localized two other V/D transgenes: an insertion of the $P[w^{d1}]$ element and a *P*[*lacW*] insertion (*VD164*) at 22E and 24CD, respectively, on the second chromosome. This suggests that, unlike D/V transgenes, V/D transgenes are not clustered in a single chromosomal site.

The mapping of the D/V insertions on a genomic walk covering *ara* to *mirr* revealed that they are all clustered in a region of about 140 kb. This region contains at least three transcription units (*ara*, *caup*, and *mirr*) that encode highly related homeoproteins. These genes are subjected to a similar developmental regulation, which favors the previously suggested idea that *mirr* belongs to the *IRO*-C (McNeill *et al.* 1997), thus defining a complex of at least three homeobox-containing genes. This indicates that the D/V effect (ventral repression of $white^{+}$ transgenes expression) should be exerted on a region having considerable size. This raises the question of the organization of the *cis*-regulatory sequences involved in the achievement of the ventral silencing (discussed below).

Transgenes inserted in the D/V region display similar but not identical developmental expression patterns: The study of the spatial-temporal expression patterns of our 14 D/V transgenes revealed that they are expressed throughout embryonic and larval development, in very specific expression patterns. Some of them are also expressed during oogenesis.

During embryogenesis, $mirr^{cre}$, iro^{Sc2}, $iro^{B6.8}$, and *J26.b16* transgenes display similar but distinct expression patterns. It is noteworthy that transgenes that have molecularly been shown to be inserted in or near distinct genes ($mirr^{cre3}$, $iro^{B6.8}$, and iro^{Sc2}) display similar expression patterns. These patterns could be imposed by the long-range effects of distinct regulatory elements on the promoter (sensitive to position effect) driving *white* expression in the *P*[*lacW*] transgene. However $mirr^{cre3}$, $iro^{B6.8}$, and iro^{Sc2} expression patterns are mostly identical to those of the cDNAs of the three corresponding genes (McNeill *et al.* 1997; Gomez-Skarmeta *et al.* 1996; J. L. Gomez-Skarmeta, personal communication). These expression patterns suggest that the genes in which, or in proximity to which, transgenes have inserted could play a role in morphogenetic movements and in dorsal epidermis and central nervous system determination. The case of the iro^{T3} and iro^{T81} transgenes (both inserted in *ara*) is peculiar, since their embryonic expression

patterns of *white*$^+$ are a combination of the *white* gene expression domain (as seen in wild-type controls) and of the *white*$^+$ expression directed by genomic regulatory regions flanking the insertion point. Therefore, during embryogenesis, the influence of genomic regulatory elements does not counteract the effect of *white* regulatory sequences included in the *P*[w^{d1}] construct. Thus, this transgene is not a reliable reporter for embryonic expression pattern. Conversely, in the adult eye, the D/V pattern observed in these lines suggests that a ventral silencing mechanism prevails on the effect of the *white* gene eye-specific enhancers.

All the D/V transgenes bearing the *lacZ* reporter display a spatially restricted and very similar expression pattern in most of the larval tissues. In the eye disc, *lacZ* expression is restricted to the dorsal half. Thus, there is a clear spatial correspondence between adult eye pigmentation and β-galactosidase staining in the eye imaginal disc. It should be noted that this is not always the case: Bhojwani *et al.* (1995) and Sun *et al.* (1995) have shown that patterned expression of *white*$^+$ in the adult eye is not always associated with a corresponding *lacZ* expression pattern in the eye imaginal disc of *P*[*lacW*] transgenic lines.

The expression pattern of D/V transgenes in the wing disc is mostly similar for all lines, apart from differences in restricted areas of this disc and differential levels of *lacZ* expression. The expression of this reporter is detected in domains of the disc suggesting that the affected genes may be implicated, notably, in the development of the dorsal thorax, wing hinge (including alula), and wing veins. This is compatible with the demonstration that *iro* acts very early in the establishment of sensory organ patterns by regulating the expression of *achaete* and *scute* proneural genes (Gomez-Skarmeta *et al.* 1996).

mirr^{cre} transgenes were also shown to be expressed during oogenesis in an antero-dorsal pattern. This may reflect the expression pattern of a gene putatively involved maternally in the establishment of the dorsal/ventral polarity of the embryo, thereby suggesting that *mirr* is involved in this process.

Thus, on the basis of similarity between the developmental expression patterns of D/V transgenes, it can be speculated that the genes in the D/V region belonging to the same *IRO*-C may be subject to the regulatory activity of common enhancer and silencer elements.

Genes of the *IRO*-C are involved in common developmental processes: The phenotypes observed for adult flies mutant for *mirr* or *iro* are in good agreement with the expression patterns in wing discs.

We have obtained six independent lethal or semilethal *P*[*lacW*] insertions (*mirr*^{cre1}–*mirr*^{cre6}) mutating the *mirr* gene. Adult survivors to hypomorphic *mirr* alleles display peculiar defects reminiscent of the Dichaete phenotype. We have shown that the lethality associated with the breakpoints in 69D–E of the D^1 and D^3 alleles was due to *mirr* inactivation. Two other lethals (*mirr*^{Sai1} and *mirr*^{Sai2}) displaying a dominant Dichaete-like partial phenotype were also found to be allelic to *mirr*. A dominant wing phenotype has been attributed to the breakpoints located in 70–71 cytological bands of *D* alleles (Russell *et al.* 1996). Our results suggest that breakpoints in 69D–E of D^1 and D^3 alleles might be responsible for a D phenotype by altering *mirr* function.

Viable mutations of *iro*, *iro*^{1} (Dambly-Chaudière and Leyns 1992; Leyns *et al.* 1996), *iro*^{rF209} (Gomez-Skarmeta *et al.* 1996; Leyns *et al.* 1996), and *iro*$^{B6.8}$ induce an outheldwing phenotype too, but in these cases the alula is not affected. These *iro* mutations also alter thoracic bristle patterning either when homozygous (*iro*^{1}; Dambly-Chaudière and Leyns 1992) or in *trans*-heterozygous combination with *iro*^{2} (this study and Leyns *et al.* 1996). From the analysis of the wing phenotypes, it appears that only *mirr* function is implicated in alula formation. A requirement for *iro* function in the formation of the alula was reported in cell clones lacking the *iro* function (Gomez-Skarmeta *et al.* 1996). This study was carried out with two deficiencies of the *iro* locus (*iro*^{DFM1} and *iro*^{DFM3}) known to delete *ara* and *caup*. However, we have shown that these deficiencies do not complement the lethality of *mirr*^{cre} alleles (Table 2). This is in accordance with a specific requirement, within the *IRO*-C, of *mirr* for alula formation. Nevertheless, we cannot rule out the possibility that a double mutant removing *ara* and *caup* (since it has been suggested that ARA and CAUP can functionally replace each other; Gomez-Skarmeta *et al.* 1996), but not *mirr* function, could also have a phenotype lacking allulae.

This genetic analysis of the D/V region and previous results concerning *IRO*-C demonstrate that this region contains at least two different functional units (*mirr* and *iro*) that are implicated in similar developmental pathways: notably, the development of the peripheral nervous system (bristle patterning) and the wing (hinge and vein formation).

The D/V region is a target of *Pc*-G and *trx*-G gene products: The D/V pattern was altered neither by modifiers of variegation genes nor by *Y* dosage. The effect of breeding temperature was the opposite of that exerted on PEV. All together, these results show that the ventral repression is achieved by a mechanism distinct from heterochromatin inactivation in PEV.

We have shown that, in most cases tested, a diminution of dosage of *Pc*-G gene products causes a ventral derepression of (mini$^-$)*white*$^+$ expression in the adult eye of D/V strains. Mutations in *Pc*-G genes have a synergistic effect on the regulation of their known target genes (Dura *et al.* 1985; Jürgens 1985; Adler *et al.* 1989; Campbell *et al.* 1995). We have detected such a synergistic effect on the D/V pattern, further indicating that each D/V transgene has inserted in, or in proximity to, a gene whose expression pattern is regulated by *Pc*-G gene products.

The fact that the Pc^{16} mutation leads to a ventral derepression of transgene expression in the iro^{T81} line but not in the $mirr^{cre1}$ line may be explained by a differential regulation of the two transgene insertion sites by the PC product. However, this result may simply reflect the difference in expression levels of the two transgenes (see Figure 2, A and C). The $P[w^{d1}]$ transgene in the iro^{T81} line may constitute a more sensitive detector than the mini-$white^{+}$ reporter for the detection of weak derepressive effects in the ventral half of the eye. This is further suggested by the fact that the ph^{410} mutation leads to a wild-type eye pigmentation in iro^{T81} and only to the apparition of mottling in the ventral part of the eyes of D/V lines bearing a mini-$white^{+}$ transgene (compare Figure 8C to 8D).

In the condition of our test (heterozygous mutant background), *trx*-G products have no detectable effect in the dorsal part of the eye. However, it is likely that these products have a role in the maintenance of *IRO*-C genes' expression in this domain. In fact, it has been shown, for example, that the effect of a *brahma* heterozygous mutant background is detectable, in the leg imaginal discs, only in a domain where the homeotic gene *Sex combs reduced* is ectopically expressed (Tamkun *et al.* 1992). Ventrally, an effect of TRX-G products is detectable in the eye of the iro^{T81} line in which $white^{+}$ reporter expression is not completely repressed, presumably because sequences internal to the transgene prevail over Pc-G-mediated repression (*e.g.*, *white* regulatory sequences in $P[w^{d1}]$). This ventral derepression and the additional derepression induced by a mutation in the *Pc*-G are suppressed by mutations in some *trx*-G genes.

The repressive effect of *Pc*-G gene products in the ventral part of the adult eye is already at work in the eye imaginal disc, as a diminution of *ph* product dosage leads to an ectopic expression of the *lacZ* reporter in the ventral-most part of this disc. This effect perfectly mimics that observed with *white* in the adult eye. However, no clear effect of *ph* gene dosage was observed in other larval tissues. We can assume that the silencing mediated by *Pc*-G gene products may be exerted and required only in the ventral part of the eye. In other larval tissues, the expression pattern of the genes included in the D/V region could be regulated by specific enhancers and may not involve a silencing mechanism.

From these results, we can conclude that the D/V pigmentation pattern is distinct from PEV but is related to DREV (Fauvarque and Dura 1993). 69D is a binding site for the product of some *Pc*-G genes (Zink and Paro 1989; DeCamillis *et al.* 1992; Martin and Adler 1993; Rastelli *et al.* 1993; Lonie *et al.* 1994), which suggests that the genes included in the D/V region are targets for the PC-G products. The accuracy of cytological localization on polytene chromosomes does not allow us to conclude that genes included in this region are targets directly *trans*-regulated by these products.

The D/V region might be regulated at the level of chromatin structure: Given our results concerning the size of the D/V region, the characteristics of the genes it includes, and its negative regulation by the *Pc*-G gene products, it is attractive to draw a parallel between this region and the homeotic gene complexes (*ANT*-C and *BX*-C). In fact, these complexes are of considerable size and are transcriptionally silenced by the products of *Pc*-G genes. Many different findings suggest that these proteins may act through local changes in chromatin conformation (Locke *et al.* 1988; Paro and Hogness 1991; Fauvarque and Dura 1993; Orlando and Paro 1993; Chan *et al.* 1994; Zink and Paro 1995). Current models of *Pc*-G target genes silencing over large distances evoke the possibility of a local heterochromatin-like structure inhibiting gene transcription (for recent reviews see Pirrotta and Rastelli 1994; Orlando and Paro 1995; Bienz and Muller 1995; Paro 1995).

By analogy with the homeotic complexes, we can speculate that the repression of genes included in the D/V region may be ensured by a mechanism of local compaction of the chromatin structure. On the basis of models proposed for the achievement of this compaction, two possibilities can be envisioned for the organization of the *cis*-acting sequences responsible for the D/V effect. The ventral repression may be under the control of a single *cis*-acting "inactivation center" exerting its effect over the entire D/V region. Alternatively, there may be independent silencer elements for each gene included in the region. These two hypotheses are not mutually exclusive. In fact, we can speculate that the silencing process is initiated at the putative inactivation center and then propagated to each locus by interaction between this sequence and a silencer element associated with each locus. Moreover, we can also speculate that the D/V region is delimited by boundary sequences, limiting the extent of the D/V effect. DNA segments that seem to specify functionally independent chromatin domains have been identified (Kellum and Schedl 1991; Corces and Geyer 1991; Galloni *et al.* 1993; Karch *et al.* 1994). Answering these questions would require identification and characterization of PRE(s) and/or boundary elements within the D/V region.

We can also ask if there is a functional requirement for the physical clustering of the IRO-C genes, which are all implicated at least partly in common developmental processes. We can speculate that this co-localization would have been maintained in the course of evolution because the chromosomal region in which these genes are included has acquired specific structural characteristics.

Thus, the D/V region could contain a complex of genes specifically regulated at the chromatin structure level. However, the regulation exerted by the *Pc*-G gene products (at least PH) on the expression of the D/V region is different from that exerted on homeotic gene expression. In fact, *Pc*-G products may not be required

during embryogenesis to keep the genes in the D/V region repressed in specific domains.

Maintenance, by *Pc*-G products, of *IRO*-C genes' dorsal restriction may be required for correct formation of the equator in the Drosophila eye: The Drosophila compound eye is composed of dorsal and ventral fields of photoreceptor clusters called *ommatidia.* The ommatidia in the dorsal half of the eye are the mirror image of those in the ventral region, establishing a global symmetry at the equatorial midline. The boundary where the dorsal and ventral fields meet is known as the equator. Ommatidial differentiation begins in the eye imaginal disc during the third instar larval period. A wave of differentiation sweeps across the disc from posterior to anterior. This wave is marked by an indentation, the morphogenetic furrow (MF), which separates the undifferentiated and differentiating regions of the disc (Thomas and Zipursky 1994). The mechanism of the establishment of the dorsal/ventral polarity and of the equator is still questioned. Some authors suggest that the equator could be positioned by global dorsal/ventral information (Baker and Rubin 1992; Ma and Moses 1995; Zheng *et al.* 1995), while others (Chanut and Heberlein 1995; Strutt and Mlodzik 1995; Wehrli and Tomlinson 1995; Jarman 1996) suggest that dorsal/ventral polarity is provided by cell to cell interaction during the progression of the MF. More recently, it has been shown that the global dorsal to ventral symmetry is determined independently of the local polarity of the ommatidia (Choi *et al.* 1996).

The dorsally restricted expression of *white*$^+$ transgenes in the eye suggests that *IRO*-C genes are involved in determining dorsal identity or in forming the D/V boundary. This is strongly supported by the recent finding that *mirr* plays a key role in forming the eye equator (McNeill *et al.* 1997). Moreover, at the third larval instar, the dorsal restriction of *lacZ* expression appears to be independent of furrow progression, as it is seen before and after the furrow position (Figure 7, A–D). This is confirmed by Brodsky and Steller (1996), who showed that D/V-specific patterns of *lacZ* expression in the eye disc are established prior to third instar and are maintained in a size-invariant manner until cell division in the disc has ended. This clearly indicates that D/V differences in positional identity exist prior to the MF progression. These differences have to be maintained until the end of the ommatidial differentiation, when the MF reaches the anterior margin of the eye disc. The juxtaposition of *mirr*-expressing and nonexpressing cells serves to define the equator position (McNeill *et al.* 1997). We have shown that the dorsally restricted expression of *white*$^+$ and *lacZ* reporters inserted in the *IRO*-C is relieved by mutations in the *Pc*-G genes. This raises the possibility that the PC-G product silencing effect plays a role in the maintenance of the D/V boundary of tissue polarity in the eye, established prior to the stage of ommatidia differentiation.

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