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Trans regulation in the *Ultrabithorax* gene of *Drosophila*: alterations in the promoter enhance transvection

Antonio Martínez-Laborda, Acaimo González-Reyes and Ginés Morata

Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma de Madrid, 28049 Madrid, Spain

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We report a genetic and molecular study of *Ubx*^{MX6} and *Ubx*^{195rx1}, two mutations in the *Ultrabithorax* (*Ubx*) locus which appear to have a strong effect on the activity of the homologous *Ubx* gene. These mutations show the characteristic embryonic and adult phenotypes of *Ubx* null alleles, and also fail to produce any detectable Ubx product. Yet, genetic and phenotypic analyses involving a large number of *trans* heterozygous combinations of *Ubx*^{MX6} and *Ubx*^{195rx1} with different classes of *Ubx* mutations, indicate that they hyperactivate the homologous gene. This effect is induced on wildtype or mutant forms of *Ubx*, provided that the pairing in the bithorax region is normal, i.e. these mutations have a strong positive effect on transvection. We also show that, unlike all the other known cases of transvection in *Ubx*, this is not *zeste*-dependent. Southern analyses indicate that *Ubx*^{MX6} is a 3.4 kb deletion, and *Ubx*^{195rx1} is an ~11 kb insertion of foreign DNA, both in the promoter region. We speculate that the region altered in the mutations may have a wildtype function to ensure *cis*-autonomy of the regulation of *Ubx* transcription.

Key words: homeosis/transvection/Ubx

Introduction

Homologous chromosomes are intimately paired in somatic cells of insects, as is most dramatically shown in the polytene chromosomes of *Drosophila* and other dipterans. The functional significance of this phenomenon is not clear, but its general occurrence may suggest a role in the control of gene expression. Furthermore, some recent reports suggest that somatic pairing may also occur in mammals, as indicated by the possibility of somatic recombination (Tartof and Henikoff, 1991).

Transvection is a phenomenon, originally defined by Ed Lewis (1954) (reviewed in Judd, 1988; Wu *et al.*, 1989; Tartof and Henikoff, 1991), that suggests a functional role for somatic pairing. Although a large body of evidence indicates that pairing is not a critical requisite for normal gene function—since rearrangements do not detectably affect gene activities—transvection points to the possibility of regulatory interactions between genes situated in homologous chromosomes (Judd, 1988; Tartof and Henikoff, 1991), and its study may contribute to elucidate the mechanism of action of *cis*-regulatory elements (Zachar *et al.*, 1985; Geyer *et al.*, 1990). It has been described for *white* (Zachar *et al.*, 1985), *decapentaplegic* (Gelbart, 1982), *brown* (Dreesen *et al.*, 1991), *yellow* (Geyer *et al.*, 1990) and some other genes, but it is in *Ubx* where it has been the most extensively studied (Lewis, 1954, 1955, 1982, 1985; Babu and Bhat, 1981; Babu *et al.*, 1987; Micol and García-Bellido, 1988; Castelli-Gair *et al.*, 1990; Mathog, 1990; Micol *et al.*, 1990). This gene offers several advantages for the study of transvection. (i) the genetics of *Ubx* are very well known (Lewis, 1978; Kerridge and Morata, 1982; Casanova *et al.*, 1985a) and there are many viable mutations that are easy to characterize phenotypically; (ii) there are many breakpoints known that alter the pairing in the *Ubx* region; (iii) its molecular structure is well known (Bender *et al.*, 1983; Weinzierl *et al.*, 1987; O'Connor *et al.*, 1988), which may open an avenue into the molecular analysis of transvection.

Ubx is a large gene, containing >100 kb of DNA (Bender *et al.*, 1983) including complex regulatory machinery. One transcription unit encodes all the functional proteins (Hogness *et al.*, 1985) and at least two *cis*-acting regulatory regions, *abx* and *bxd* (Casanova *et al.*, 1985a; Peifer and Bender, 1986), are involved in the spatial expression of the transcription unit. These two regions act from a long distance from the promoter of the transcription unit and have the properties of eukaryotic enhancers (Bender, 1985; Hogness *et al.*, 1985; Simon *et al.*, 1990; Müller and Schaffner, 1991). They control the activity of the *Ubx* gene in specific body regions (Casanova *et al.*, 1985a; White and Wilcox, 1985).

Lewis (1954) described transvection for some mutant phenotypes which are affected by the degree of pairing between homologous chromosomes; the phenotype of *trans* heterozygotes between *bx*^{34e} and *Ubx*^{1}, two mutant alleles at the *Ultrabithorax* locus, depends in part of the pairing of the chromosomal region where the *Ubx* gene resides. Flies of genotypes *bx*^{34e}/*Ubx*^{1} and *R*(*bx*^{34e})/*Ubx*^{1} (where R means a rearrangement preventing pairing in the *Ubx* region) exhibit different phenotypes, the latter being stronger. We now know that the partial rescue observed when pairing was normal could not have been due to any product originating from the *Ubx*^{1} chromosome, which is a null allele i.e. unable to produce functional protein (Weinzierl *et al.*, 1987). It follows that the functional level of the *Ubx* gene in the *bx*^{34e} chromosome depends on pairing, thus suggesting a functional cooperation between homologous genes.

Transvection at *Ubx* is not restricted only to combinations involving the *bx*^{34e} allele, which is a mutation in the *abx* regulatory element; it has also been described for other (*abx* and *bx*) mutant alleles in the same regulatory region and also for mutants in the *bxd* regulatory element (Lewis, 1982, 1985; Mathog, 1990).

Other cases have been described that can also be explained in terms of *trans* interactions between homologous chromosomes. For example, the dominant mutation *Contrabithorax*^{1} (*Cbx*^{1}) produces a partial transformation of the wing into haltere (Lewis, 1963; Morata, 1975; Casanova *et al.*, 1985b), caused by ectopic expression of the *Ubx*

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transcription unit in the wing cells, where it is not normally expressed. This abnormal expression is produced by a transposition of regulatory sequences within the *Ubx* gene (Bender *et al.*, 1983; Casanova *et al.*, 1985a; Karch *et al.*, 1985). It can be shown that in Cbx^{1}, the regulation of the two homologous *Ubx* genes is altered; the suppression of the *cis* activity of *Ubx* in $Cbx^{1}Ubx^{1}/+$ flies (Ubx^{1} is null) does not completely eliminate *Ubx* ectopic expression in the wing cells (and hence the *Cbx* phenotype), indicating an ectopic activation of the wildtype homolog. Furthermore, the *trans* heterozygote Cbx^{1}/Ubx^{1} shows less transformation than $Cbx^{1}/+$ because of the elimination of the *Ubx* function of the *trans* chromosome. Thus alteration of the *cis*-regulatory region of the Cbx^{1} chromosome has both a *cis* and a *trans* effect.

In conclusion, transvection is a general phenomenon within the *Ubx* gene. Although there are differences depending on the mutation under test, all the regulatory regions of the gene can be shown to be affected by pairing. In this paper we describe two new mutants in the *Ubx* locus which have a strong positive effect on transvection. The two mutations (a deletion and an insertion) map around the promoter region of the *Ubx* transcription unit. We propose that this region may act as a negative regulator of transvection.

Results

Isolation and molecular characterization of the mutations Ubx^{195rx1} and Ubx^{MX6}

The Ubx^{195rx1} allele was found after irradiation of a chromosome carrying the point mutation Ubx^{195}, in a strategy designed to isolate other mutations that rescue its null phenotype. Mutagenized chromosomes were tested over Ubx^{M4}, a weak ethylmethane sulphonate (EMS) induced-allele isolated by E.Sánchez-Herrero, that allows for adult viability. Heterozygous Ubx^{195}/Ubx^{M4} flies display a slight but clear transformation of haltere (a T3 appendage of the adult) towards wing (the corresponding appendage of T2). In addition, the first abdominal segment is partially transformed into thorax. A mutation called Ubx^{195rx1} was isolated that shows virtually wildtype phenotype over Ubx^{M4}.

The other mutation was X-ray induced on a chromosome already carrying the mutation $abd\text{-}A^{M1}$ [an amorphic allele of the *abd-A* gene (Morata *et al.*, 1983; Sánchez-Herrero *et al.*, 1985)] and named Ubx^{MX6} (Casanova *et al.*, 1987). This was described as an amorphic allele of *Ubx* in embryos, but showing moderate adult mutant phenotypes in combination with viable mutations of the *Ubx* gene (Casanova *et al.*, 1987).

The molecular characterization of the two alleles was carried out by Southern analysis and the results are illustrated in Figure 1. The Ubx^{MX6} mutation consists of a 3.4 kb deletion that includes the *Hin*dIII−*Eco*RI fragment harboring the two adjacent transcriptional start sites of *Ubx* (Saari and Bienz, 1987) and adjacent DNA.

The Ubx^{195rx1} allele carries an ~11 kb insertion in the same 1 kb *Hin*dIII−*Eco*RI fragment mentioned above. Further subdivision allows the localization of the insert in a smaller 317 bp fragment defined by the restriction sites of *Nru*I and *Mlu*I (Saari and Bienz, 1987). In addition, this mutation contains the Ubx^{195} point mutation as detected by

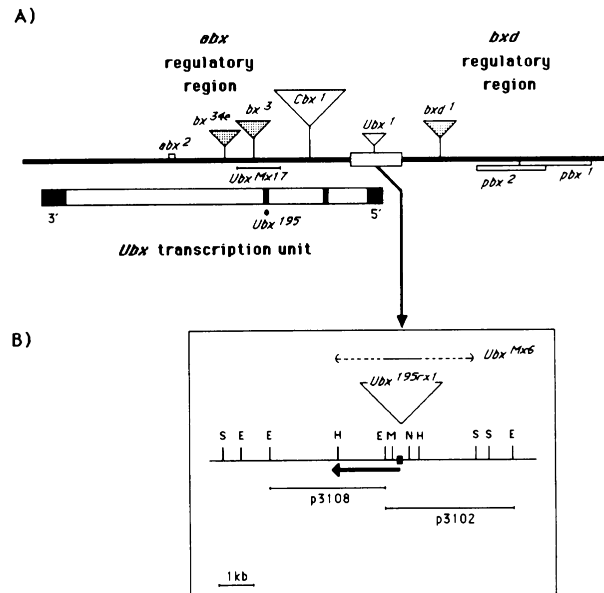

Fig. 1. (A) Molecular map of the *Ubx* gene showing the mutations used in this work. Deletions are represented as rectangles and insertions as triangles. The main *Ubx* RNA is shown below, the exons appearing as solid boxes. Cbx^{1} is an insertion of the DNA deleted in pbx^{1}. Ubx^{1} is a Doc transposon inserted in the 5′ untranslated region of *Ubx*. Ubx^{195} is a single base pair subtitution introducing a nonsense codon into the second microexon, and Ubx^{MX17} is an inversion within the *Ubx* transcription unit including the second microexon. The dotted triangles (bx^{34e}, bx^{3} and bxd^{1}) are gypsy insertions. (B) Molecular characterization of Ubx^{MX6} and Ubx^{195rx1}. Southern analysis showed abnormal restriction fragments when plasmids p3108 and p3102 were used. Restriction sites *Eco*RI (E), *Hin*dIII (H), *Mlu*I (M), *Nru*I (N) and *Sal*I (S) are indicated. The transcription start site is indicated by a black dot and the arrow shows the direction of transcription. In Ubx^{195rx1} the lesion was first located in the 1 kb *Eco*RI−*Hin*dIII fragment containing the transcription start site. A new *Sal*I fragment is detected with both p3102 and p3108, indicating that the mutation consists of an insertion of ~11 kb without any *Sal*I restriction site. More precise localization involved single and double digestions using *Mlu*I, *Nru*I and *Pst*I. The insert is located in a 317 bp fragment defined by *Mlu*I and *Nru*I, which also contains the transcription start. In Ubx^{MX6} the *Eco*RI and *Hin*dIII sites flanking the transcription start site are absent, and a new *Eco*RI−*Hin*dIII fragment of 2 kb appears with both p3102 and p3108. These results indicate a deletion of ~3.4 kb.

the *Alu*I restriction pattern in the second microexon genomic region (Weinzierl *et al.*, 1987).

Genetic characterization of Ubx^{195rx1} and Ubx^{MX6}

Neither of the two alleles possesses embryonic function. Both alleles, Ubx^{195rx1} and Ubx^{MX6}, are homozygous and hemizygous lethal. Ubx^{195rx1} mutant embryos show the characteristic syndrome described for *Ubx* null alleles (Lewis, 1978; Hayes *et al.*, 1984); parasegments 5 and 6 are transformed into parasegment 4 and there is a slight thoracic transformation of the A2−A7 abdominal segments. In the case of the Ubx^{MX6} chromosome, which is also deficient for *abd-A* function, we observed the sum of *Ubx* and *abd-A* mutant phenotypes, just as in $Df(3R)Ubx^{109}$, a deletion of both *Ubx* and *abd-A* genes (Morata *et al.*, 1983; Casanova *et al.*, 1987). The *trans* combination Ubx^{MX6} $abd\text{-}A^{M1}/Df(3R)bxd^{100}$, in which there is normal *abd-A* function, showed null *Ubx* phenotype.

In agreement with the embryonic phenotypes, Ubx^{195rx1} and Ubx^{MX6} homozygous embryos lack detectable levels of

Ubx protein as measured by a specific anti-Ubx antibody (White and Wilcox, 1984).

Neither of the alleles has imaginal function. We have studied the adult phenotype of the two alleles by generating hemizygous or homozygous cell clones for each of the alleles in heterozygous animals. The clones were produced by means of X-ray induced mitotic recombination as detailed in Materials and methods. Two examples are shown in Figure 2.

Mutant clones for Ubx^{195rx1} and Ubx^{MX6} were produced during the larval period, 72−96 h after egg laying. Many clones were found in all body regions. Those in the halteres, the three sets of legs and the abdominal segments were examined for homeotic transformations, as they derive from the body region where *Ubx* is expressed. For Ubx^{195rx1} we found a total of 22 clones in the haltere segment. All of them showed a complete transformation towards the homologous wing structure; those in the haltere appendage were transformed into wing, and those in the metanotum showed a mesothoracic transformation (Figure 2a). Since these clones were labelled with *mwh*, which marks individual trichomes, we could in many cases determine that all the cells were homeotically transformed. This is characteristic of null *Ubx* mutants (Morata and García-Bellido, 1976; Kerridge and Morata, 1982). Clones appearing in the third leg (12) exhibited a transformation into the homologous region of the second leg (Kerridge and Morata, 1982). In the posterior compartment of the second leg they differentiated normally, as expected because the *ppx* transformation appears only in early induced clones (Morata and Kerridge, 1981). Ubx^{195rx1} mutant clones did not materialize in the first abdominal segment, another typical feature of Ubx^- clones, probably because they are transformed into thorax, and thoracic development cannot proceed in an abdominal environment (Morata and García-Bellido, 1976). In the rest of the abdominal segments, Ubx^{195rx1} mutant clones differentiate normally.

The results obtained with Ubx^{MX6} clones closely parallel those of Ubx^{195rx1}; one example is shown in Figure 2b. The only difference is that mutant clones failed to appear in all the abdominal segments. This is because these clones are also mutant for *abd-A*, and this results in thoracic development of all the abdominal segments.

In summary, the results obtained with the clonal analysis strongly suggest that both Ubx^{195rx1} nor Ubx^{MX6} behave as null alleles for imaginal function.

Ubx^{195rx1} and Ubx^{MX6} partially rescue the phenotype of viable mutations in the Ubx locus

Four individual homeotic transformations are associated with the *Ubx* mutant syndrome (Casanova *et al.*, 1985a): *postprothorax* (*ppx*), *bithorax* (*bx*), *postbithorax* (*pbx*) and *bithoraxoid* (*bxd*), affecting the four compartments T2p, T3a, T3p and A1a respectively, which constitute parasegments 5 and 6. The *ppx* and the *bx* transformations result from lack of function of the *abx cis*-regulatory unit, and *bxd* and *pbx* are produced by a defect in the *bxd cis*-regulatory unit (Casanova *et al.*, 1985a). Some of these transformations are shown in Figure 1.

We have analyzed the phenotypes of Ubx^{195rx1} and Ubx^{MX6} in *trans* with several mutant alleles. We used the Ubx^{195} allele as a reference for the adult transformations because it is the parental chromosome of one of the mutations

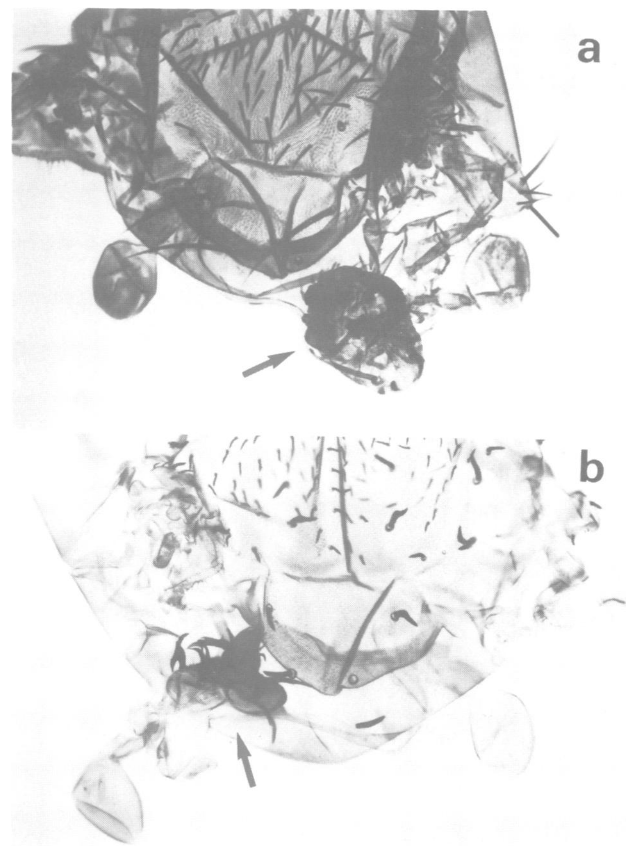

Fig. 2. (**a**) Clone (arrow) in the metanotum of Ubx^{MX6} homozygous cells marked with Sb^+, showing a mesothoracic transformation (see Materials and methods for details). (**b**) Large clone (arrow) of Ubx^{195rx1} hemizygous cells also showing mesothoracic transformation in the metanotum.

studied and because it carries a lesion consisting of a single base pair substitution (Weinzierl *et al.*, 1987) producing a stop codon that eliminates >90% of the *Ubx* proteins. Pairing is therefore not disrupted in combinations involving Ubx^{195} and so transvection should be normal. Furthermore, except for the *ppx* transformation, the phenotypes it produces are like those of null mutations such as Ubx^{1} and $Ubx^{9.22}$ (Kerridge and Morata, 1982).

Trans heterozygotes of Ubx^{195}, Ubx^{195rx1} and Ubx^{MX6} with abx^2, bx^{34e}, bx^3, pbx^1 and pbx^2, as well as with weak *Ubx* alleles affecting the entire domain (Ubx^{MX17}, Ubx^{M4} and Cbx^1), were constructed and their phenotypes were analyzed and quantified. Some of these results are illustrated in Figure 3. These can be summarized by saying that the homeotic transformations in the combinations of Ubx^{195rx1} and Ubx^{MX6} are much weaker than those of Ubx^{195}. The only exception is the *ppx* phenotype in which there is no difference from Ubx^{195}; the *ppx* transformation in Ubx^{MX6}/abx^2 is 48%, similar to the figures reported for strong *Ubx* alleles or the deletion of the gene (Casanova *et al.*, 1985a). In the case of Ubx^{195rx1} the penetrance of the *ppx* transformation is 5%, similar to that found for Ubx^{195} (Weinzierl *et al.*, 1987; our own results). This low penetrance in Ubx^{195} may indicate that this mutation retains some *Ubx* function, even though the phenotypes it produces in *trans* combinations with the other *abx* and *bxd* mutations are as strong as those produced by null alleles. We note that

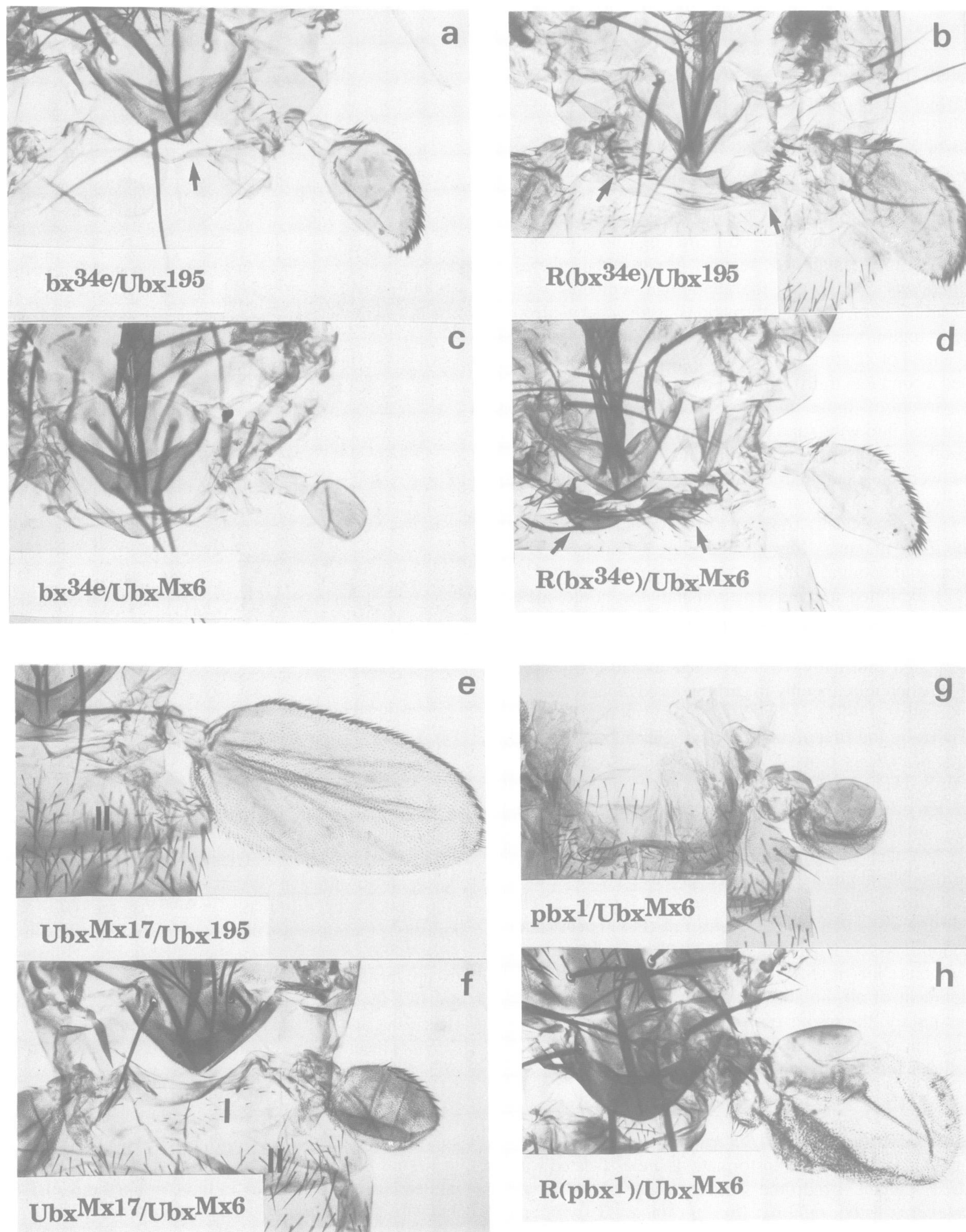

Fig. 3. Comparison of the phenotypes of some combinations of Ubx^{195} and Ubx^{MX6}. (**a**) bx^{34e}/Ubx^{195}: the haltere appendage is partially transformed towards wing, as indicated by its larger size and the presence of bristles characteristic of the anterior wing margin. Note (arrow) the metanotum, a narrow piece of cuticle completely devoid of bristles. (**b**) $R(bx^{34e})/Ubx^{195}$: the loss of transvection does not affect the transformation in the appendage; the difference is in the metanotum, which is partially transformed into mesonotum, as indicated by the thoracic bristles (arrows). (**c**) bx^{34e}/Ubx^{MX6}: the transformation in the haltere is virtually eliminated. (**d**) $R(bx^{34e})/Ubx^{MX6}$: the loss of transvection results in a transformation like that shown in panel b, including the thoracic bristles in the mesonotum (arrows). Comparison of panels c and d clearly shows the hyperactivity induced by Ubx^{MX6} on the bx^{34e} chromosome. (**e**) Ubx^{MX17}/Ubx^{195}: in this genotype, the haltere appendage is transformed into wing in the anterior (*bx* transformation) and posterior (*pbx*) regions. In addition, the first abdominal segment (*bxd* transformation) is missing. The second abdominal segment (II) is unaffected. (**f**) Ubx^{MX17}/Ubx^{MX6}: the haltere appendage is much more normal and the first abdominal segment (I) is restored. (**g**) pbx^{1}/Ubx^{MX6}: the haltere appendage is mildly transformed towards wing in the posterior region. (**h**) $R(pbx^{1})/Ubx^{MX6}$: the loss of transvection results in a strong transformation of the posterior haltere region into posterior wing.

the rescue is always greater in combinations containing *Ubx*^{195rx1}, which is able to rescue completely a strong allele like *bx*^{3}.

All these observations indicate that the *Ubx*^{195rx1} and *Ubx*^{MX6} mutations induce extra activity in the homologous *Ubx* gene. This excess of activity can also be noticed in comparison with combinations in which there is little or no transvection; the combinations of *Ubx*^{195} with *pbx*^{1}, *bxd*^{1}, *Ubx*^{MX17}, *Ubx*^{M4} and *Cbx*^{1}, in which pairing is normal, are as strong in the haltere transformation as the non-transvecting ones of the same viable alleles with *Df(3R)P9* or *Ubx*^{130}. Yet, the same alleles produce much weaker or wildtype phenotypes in *trans* to *Ubx*^{195rx1} and *Ubx*^{MX6} (see Figure 3e, f, g and h).

Ubx^{MX6} *and Ubx*^{195rx1} *hyperactivate a wildtype Ubx gene in trans*

A general feature of the *Ubx* gene is that it is haplo-insufficient, so that one dose of the gene is not able to produce a normal haltere; a *Ubx*$^{-}$/+ fly has enlarged halteres that contain one to five bristles, indicating a very mild transformation towards wing. We note (Figure 4) that *trans* heterozygotes of the two transvecting alleles show virtually normal halteres, indicating an increased activity of the wildtype *Ubx* gene in *trans*.

The extra activity of the Ubx gene in trans to Ubx^{195rx1} *and Ubx*^{MX6} *is pairing- but not zeste-dependent*

We have tested to what extent the extra activity seen in chromosomes in *trans* to the new *Ubx* alleles depends on pairing. To this end, several rearranged (R) chromosomes carrying mutations and breakpoints preventing pairing in the *Ubx* region were used. The basic observation is that the lack of pairing results in the loss of the rescue activity of the two new *Ubx* alleles, and is illustrated by the comparison of Figure 3c and d, and of Figure 3g and h. While the phenotype of *Ubx*^{MX6}/*bx*^{34e} is virtually wildtype, that of *Ubx*^{MX6}/*R(bx*^{34e}*)*, in which a translocation prevents normal pairing in the *Ubx* locus, exhibits a strong homeotic transformation. The dramatic difference between these two phenotypes emphasizes that the mutant *Ubx*^{MX6} gene induces a great deal of activity in the homologous gene and that this activity is entirely dependent on appropriate pairing. A similar observation can be made for the set of combinations of the two *Ubx* mutations with *pbx*^{1} and *R(pbx*^{1}*)* (Figure 3) and with *pbx*^{2} and *R(pbx*^{2}*)*.

We also note that the suppression of the haplo-insufficient phenotype of *Ubx*^{MX6} and *Ubx*^{195rx1} is pairing-dependent. For example, elimination of the pairing of *Ubx*^{195rx1} with the wildtype homolog in flies of genotype *DpP115*/+; *Df(3R)P115*/*Ubx*^{195rx1}, restores the typical haplo-insufficient phenotype of *Ubx* null alleles (Figure 4). In these flies, the wildtype allele of *Ubx* is in *Dp P115* in the first chromosome and unable to pair with the *Ubx*^{195rx1} gene.

Since in other combinations of the BX-C the partial complementation due to transvection depends on the normal function of the *zeste* (*z*) product (Kaufman *et al.*, 1973; Micol and García-Bellido, 1988), we tested whether the extra activity induced by our *Ubx* alleles also requires it. We used the null alleles *z*$^{a69-2}$ and *z*$^{a69-3}$ (Gelbart and Wu, 1982). To our surprise, the lack of *z* function does not affect the phenotype; the transformation observed in *z*$^{-}$;*bx*^{34e}/*Ubx*^{MX6} flies is like that of *z*$^{+}$;*bx*^{34e}/*Ubx*^{MX6}, essentially wildtype, although sometimes the halteres of flies of the *z*$^{-}$ combination are slightly bigger than in *z*$^{+}$. The same result was obtained for the combinations of the *Ubx*^{195rx1} mutation. This is the first case of a transvecting combination of *Ubx* that is not *z*-dependent, and suggests that the mechanism of *trans* interaction with which we are dealing here differs from that of normal transvection. As a control, we checked that transvection in the *Ubx*^{195} allele is *z*-dependent; the combination *z*$^{-}$;*bx*^{34e}/*Ubx*^{195} shows a phenotype stronger than that of *z*$^{+}$;*bx*^{34e}/*Ubx*^{195} and similar to that of *R(bx*^{34e}*)*/*Ubx*^{195}.

Discussion

Transvection in Ubx^{MX6} *and Ubx*^{195rx1}

We have characterized two lethal *Ubx* alleles with an unusual property. They are null mutations, unable to produce any functional product; yet, when they are normally paired with a *Ubx* gene that is able to function, give rise to an extra activity of the *trans* gene. No other known *Ubx* allele behaves in this way. Southern analysis indicates that in both cases a region of the *Ubx* promoter is affected. In one case a 3.4 kb sequence is deleted, and in the other an ~11 kb fragment is inserted in the same region (Figure 1). Elimination or alteration of these sequences of *Ubx* impedes transcription, but at the same time augments the activity of the homolog; transvection is increased. We have noticed this in all the combinations we have analyzed; the *Ubx* function in the *abx*^{2}, *bx*^{3}, *bx*^{34e}, *pbx*^{1}, *Cbx*^{1}, *Ubx*^{MX17} and *Ubx*^{M4} chromosomes is increased with respect to that of the same chromosomes in *trans* with regular null *Ubx* alleles. Moreover, we observe that the typical haplo-insufficient phenotype of null *Ubx* alleles (*Ubx*$^{-}$/+ flies show an engrossment of the haltere) is not present in *Ubx*^{MX6}/+ and *Ubx*^{195rx1}/+ flies, indicating that not only mutant *Ubx* genes, but also the wildtype shows increased activity.

Of the combinations that we have studied, those involving *bx*^{34e} and *pbx*^{1} are the best documented because we can study their function in *trans* with the different *Ubx* mutations in pairing and non-pairing conditions. These two mutations are caused by defects in *cis*-regulatory regions located far apart; *bx*^{34e} is a gypsy insert 60 kb from the 17 kb deletion causing the *pbx*^{1} phenotype. These alterations are located in regulatory sequences that have all the properties of transcriptional enhancers (Simon *et al.*, 1990; Müller and Bienz, 1991), and the fact that both *Ubx*^{MX6} and *Ubx*^{195rx1} suppress their mutant phenotype strongly indicate that their enhancers are substituting for the defective ones of the *bx*^{34e} and *pbx*^{1} chromosomes. Thus in the two *Ubx* alleles the alterations of the promoter region appear to have a long range effect. It is consistent with the current thinking (Geyer *et al.*, 1990; Müller and Schaffner, 1990) about transvection, that invokes the effect of enhancers of one gene on the transcription of the homolog.

One aspect worth noting is that neither of the two new alleles suppresses the early *ppx* phenotype (Casanova *et al.*, 1985a). This phenotype has been shown to result (Struhl, 1982) from an inappropriate activation of the *Scr* gene in the $T2_p$ compartment due to the loss of a repressing activity of *Ubx* at the embryonic period, before 10 h of development. Indeed, in *abx*^{2} mutants the second leg imaginal discs contain Scr protein which is not present in the wildtype

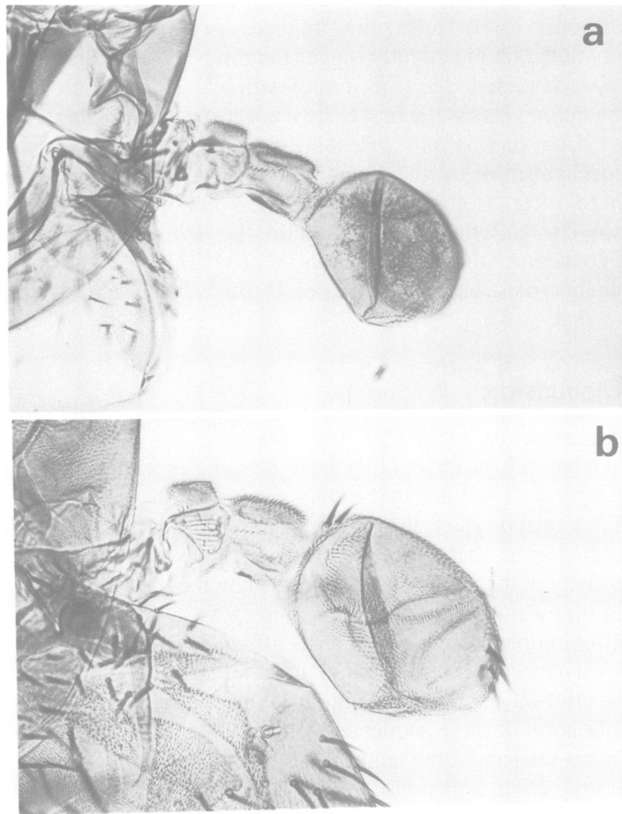

Fig. 4. Hyperactivation of the wildtype *Ubx* gene by Ubx^{195rx1} and its dependence on pairing. (**a**) $Ubx^{195rx1}/+$ haltere, showing suppression of the typical haploinsufficient phenotype of null *Ubx* alleles. (**b**) *DpP115/+;*Ubx^{195rx1}*/Df(3R)P115* haltere: the *Ubx* haplo-insufficient phenotype reappears due to the loss of pairing in the BX-C region.

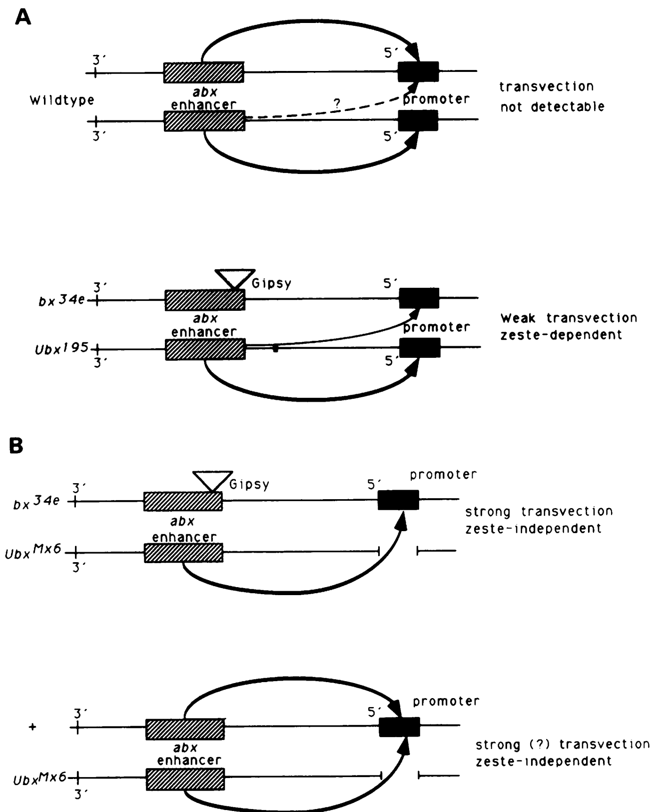

Fig. 5. Interpretation of some of the transvecting combinations involving bx^{34e}. In the wildtype (A, top), enhancer-like elements such as *abx* would act on their *cis* promoters. Any possible *trans* interaction would probably go unnoticed. In bx^{34e}/Ubx^{195} (A, bottom), the *abx* enhancer of the Ubx^{195} chromosome is able to transactivate weakly the *Ubx* transcription unit of the bx^{34e} chromosome, partially rescuing the mutant phenotype (compare Figure 3a and b). In bx^{34e}/Ubx^{MX6} (B, top) the same enhancer is able to act much more effectively on the bx^{34e} chromosome, producing a near wildtype phenotype (Figure 3c). The same *abx* enhancer of the Ubx^{MX6} gene can also act (B, bottom) on a wildtype *Ubx* gene producing an excess of activity that suppresses the haplo-insufficient phenotype (see Figure 4a and b).

(Littley *et al.*, 1990). As the *ppx* function is associated with the *abx* regulatory element which is also responsible for the *bx* transformation, suppressed by Ubx^{195rx1} and Ubx^{MX6}, we suppose that these are unable to suppress *ppx* because the hyperactivation of the homolog occurs after 10 h of development.

The combinations of bx^{34e} are of special interest, for they indicate that there are different levels of transvection. Compare the phenotypes of Ubx^{195}/bx^{34e} and $Ubx^{195}/R(bx^{34e})$ (Figure 3a and b), a typical case of transvection. There is a partial rescue of the $Ubx^{195}/R(bx^{34e})$ phenotype in the Ubx^{195}/bx^{34e} flies due to higher activity of the bx^{34e} chromosome. However, in the combinations of bx^{34e} with Ubx^{MX6} (Figure 3c) and Ubx^{195rx1}, the phenotype is virtually wildtype, despite the fact that the two mutations are null, indicating a much higher activity of the *Ubx* gene carrying the bx^{34e} mutation. Thus the two *Ubx* alleles cause a level of transvection which is much stronger than the normal one (Figure 5). Moreover, this elevated level of transvection does not depend on the presence of the zeste product, suggesting that the mechanism altered in the two new mutants differs from that operating in normal transvection.

The genetic properties of Ubx^{MX6} and Ubx^{195rx1} suggest a quick genetic method to detect lesions in the promoter region of preexisting *Ubx* null mutations, as they will rescue totally or partially the phenotype of strong combinations such as bx^3/Ubx^-.

A mechanism for preventing transvection?

Transvection was originally described for *trans* interactions in *Ubx* (Lewis, 1954) and involves a gene on one chromosome being able to influence expression of its paired homolog; this effect disappears if pairing is eliminated. Other *Drosophila* genes, including *w*, *br*, *dpp* and *y*, also show transvection. What is the significance of transvection? For most genes, including those that transvect, the lack of pairing does not have a detectable effect. A functional copy of *Ubx*, as in the case of $Ubx^{130}/+$ flies (non-transvecting, pairing at *Ubx* completely eliminated), is all that is needed to obscure any subtle difference with $Ubx^1/+$ ones (transvecting, pairing normal). By this sort of test, transvection appears to be of little significance. However, the detailed study of some cases of transvection may shed light on the regulatory mechanisms operating in large genes. The best documented cases of transvection involve interactions between mutant forms of a gene lacking some regulatory components. This is so not only for *Ubx*, but also for *yellow*. The latter is particularly interesting as there is a detailed molecular characterization of the transvecting combinations. Geyer *et al.* (1990) have demonstrated that a specific enhancer element of the *yellow* gene that cannot act in *cis* (because

a critical part of the promoter is deleted), can nonetheless activate the *trans* homolog, defective in the enhancer. This results in virtual complementation, yielding a wildtype fly. These authors postulate that there are two requisites for the phenomenon: one gene must be defective for the promoter and the homolog for the enhancer element. In normal circumstances, each enhancer will only act on its own promoter, and as long as one of the homologs is intact, *yellow* function will not be affected by lack of pairing. Only when the enhancer of one gene and the promoter of the other are defective, can the *trans* phenomenon take place. This requires physical proximity and hence it is pairing dependent.

Our results with the new transvecting *Ubx* alleles fit well with the model of Geyer *et al.* (1990). Our view about transvection in *Ubx* is schematized in Figure 5, for *bx*^{34e}, although it is probably applicable to the rest of the transvecting combinations of *Ubx*. We think that a critical element of transvection in *Ubx* is a region located at or near the promoter, which is defective in our two mutations. When present, the interaction between homologous chromosomes is weak (as in *bx*^{34e}/*Ubx*^{195} flies, Figures 3 and 5A) or non-existent, but in its absence, any properly paired *Ubx* homolog, whether mutant or wildtype, will become hyperactive (as in *bx*^{34e}/*Ubx*^{MX6} flies, Figures 3 and 5B). This is supported by the phenotype of *pbx*^{1} combinations [which are not rescued by transvecting *Ubx* alleles like *Ubx*^{1} or *Ubx*^{195} in which the region around the promoter is intact] but are partially rescued by *Ubx*^{MX6} and *Ubx*^{195rx1}.

The strong positive effect of *Ubx*^{195rx1} and *Ubx*^{MX6} on transvection can be explained by a phenomenon of promoter competition, as in the case of *yellow* (Geyer *et al.*, 1990). Normally, distant *cis*-regulatory elements of *Ubx* would interact with their own promoters, which are physically closer or are more accessible. When these enhancers are not able to act in *cis* because the promoter is missing or is functionally inactive, the action in *trans* would be permitted, giving rise to a *trans* interaction (transvection). The problem with this kind of explanation is that it is hard to imagine how a long range acting enhancer, *abx*, which is ~30 kb away from the *Ubx* promoter, can distinguish its own promoter from that of the homologous gene, especially in view of the intimate pairing of homologous genes in *Drosophila*; the observation that there is intracistronic mitotic recombination (Lawrence and Green, 1979) indicates that two homologous genes must be physically very close. Consider, for example, the lack of complementation in genotypes like *Ubx*^{195}/*bx*^{34e}, in which the normal *abx* enhancer of *Ubx*^{195} cannot act on the normal promoter of *bx*^{34e}, for if it did, it would have resulted in a wildtype phenotype. Thus the *abx* enhancer of *Ubx*^{195} recognizes and selects its own *cis* promoter, located ~30 kb away.

From our results one could also speculate that the sequences missing or inactivated in our mutations have a positive role in preventing transvection, that is, in impeding inappropriate control of one *Ubx* gene by the regulatory machine of its homolog. Since the somatic chromosomes of dipterans are intimately paired and much of gene regulation involves enhancers acting at long distances, in some instances the regulatory regions of one gene can act on the homolog, as in the transvection phenomenon. Therefore a mechanism might have developed to ensure *cis*-autonomy, so that each gene is controlled by its own *cis*-regulatory sequences. If this 'antitransvection' mechanism were to exist, it would explain why, despite intimate pairing, transvection phenomena are rare, even in *Drosophila*. Transvection is normally detected in special mutant combinations, that because of the particular gene architecture they generate, allow *trans* regulation to occur. This situation is clearly illustrated in the *yellow* gene, which usually does not transvect, but that under certain conditions can show strong transvection (Geyer *et al.*, 1990). One might speculate that a mechanism to prevent *trans* interactions may be biologically significant, and not only for dipterans, for eukaryotic chromosomes contain many DNA loops which may be so close that enhancers of one gene might act on the promoter of another. This would undesirably alter normal gene regulation and evolution may have developed a mechanism to prevent it.

Materials and methods

Fly stocks and culture conditions

Mutant alleles used in this work are referred to in the main text when appropriate. Most of them have been previously described (Lindsley and Grell, 1968; Lewis, 1978; Sánchez-Herrero *et al.*, 1985; Peifer and Bender, 1986; Casanova *et al.*, 1987; Weinzierl *et al.*, 1987; Micol and García-Bellido, 1988; Busturia *et al.*, 1990). A description of the deletions *Df(3R) P9*, *Df(3R) bxd*^{100} and *Df(3R) Ubx*^{109} can be found in Lewis (1978) and Morata *et al.* (1983). Rearranged chromosomes used to test the transvection effect are the following: *Tp(3,3) P47* [Tp(3,3) 66B; *89D*; *92A*], *R(bx*^{34e}*)* in the text; *In(3LR) P88* [Inv(3LR) 61A1–2; *89C2–4*], *R2(bx*^{34e}*)*; *T(2,3) bw*^{vDe3} [T(2,3) 59D; *81F*], *R(pbx*^{1}*)*; they have been described previously (Lindsley and Grell, 1968; Castelli-Gair, 1989).

Flies were cultured on standard media and under uncrowded conditions at 25°C, except crosses with *abx*^{2} which were done at 17°C to increase the penetrance of the *ppx* transformation (Casanova *et al.*, 1985a).

Phenotypic analysis

Many of the results presented in this paper involve comparing the phenotypes of different genetic combinations in the presence or absence of pairing. In all the cases considered the difference is qualitatively obvious, although in many of them we have quantified the transformations in order to facilitate the comparison. Nevertheless, we judge that it is not necessary to present all the numerical values found for each combination. Most of the phenotypes involve the transformation of halteres, which have no bristles, into wing, which contains long rows of bristles in the anterior (87 $\pm$ 6) and in the posterior (212 $\pm$ 21) margins. Thus the transformation towards wing can be measured as the percent of bristles in the mutant haltere with respect to the number in the wing. A similar measure can be given of the transformation of metanotum, with no bristles, into mesonotum, which contains an average of 114 $\pm$ 9 bristles.

Clonal analysis

Mutant clones for *Ubx*^{195rx1} were generated by X-irradiation (1000 rad) of second and third instar larvae of genotype *y;Dp(1;3)sc*^{J4}*Dp(3;3)146 M(3)i*^{55} *Df(3R) P115/mwh jv Ubx*^{195rx1}. In these larvae, a mitotic recombination event in the left arm of the third chromosome proximal to the *Dp (3;3) 146*, which carries a normal dose of the BX-C, results in clones of cells marked with *y*, *mwh*, *jv* and *M*$^{+}$ which are hemizygous for the *Ubx* mutation; as these cells lose the retarding *Minute* condition, they proliferate faster than the surrounding cells [see Morata *et al.* (1983) for details of this method of mitotic recombination].

For *Ubx*^{MX6}, we constructed and irradiated larvae of genotype *Ubx*^{MX6} *abd-A*^{M1}*/Ki Sb*^{63} *M(3) w*^{124} to generate *Ki*$^{+}$ *Sb*$^{+}$ *Ubx*^{MX6} *abd-A*^{M1} *M*$^{+}$ cell clones. The fact that the clones were also defective in *abd-A* function is irrelevant for their differentiation in the metathorax and first abdominal segment because *abd-A* is not expressed in those segments. Since *Sb*^{63} and *Ki* are not reliable markers for the first abdominal segment, we also X-irradiated third instar larvae of genotype *y;Dp(1;3)sc*^{J4} *Dp(3;3)146 M(3)i*^{55} *Df(3R)P115/mwh jv Ubx*^{MX6} *abd-A*^{M1} to produce clones marked with *y*, *mwh*, *jv* and *M*$^{+}$ (Morata *et al.*, 1983) which are readily scorable in the first abdominal segment.

Preparation of the larval and adult cuticle

For the larval cuticle we used the method of Van der Meer (1977), slightly modified by using Hoyer mountant diluted 1:1 with lactic acid (Wieschaus and Nüsslein-Volhard, 1986). Adult cuticle was prepared by cutting the appropriate pieces under the dissecting microscope. The internal organs were

digested with hot 10% KOH, and the cuticle was washed in alcohol and mounted in Euparal.

Antibody staining

We used the monoclonal anti-Ubx antibody developed by White and Wilcox (White and Wilcox, 1984). Antibody staining was done using the Vectastain ABC kit as previously reported (Macías *et al.*, 1990). Embryos were dehydrated in an alcohol series and mounted in Araldite.

Southern analysis

For DNA extraction, flies were homogenized in hydration buffer (0.1 M NaCl, 0.2 M sucrose, 10 mM EDTA, 30 mM Tris pH 8.0, 0.5% Triton X-100). The homogenates were filtered and centrifuged and the pellet was resuspended in lysis buffer (10 mM Tris pH 8.0, 10 mM EDTA, 0.35 NaCl, 1% sarcosyl). After adding proteinase K, samples were incubated for 2 h at 37°C and extracted three times with phenol:chloroform (1:1). The nucleic acids were precipitated twice with ethanol and resuspended in TE (10 mM Tris pH 8.0, 1 mM EDTA) with 20 μg/ml boiled RNase A.

Southern blots and synthesis of labelled DNA probes were made as described in Sambrook *et al.* (1989). Exposures were at −70°C with intensifying screens for 3 days.

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