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Function of an *Ultrabithorax* minigene in imaginal cells

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Summary

An *Ultrabithorax* (*Ubx*) minigene constructed from three key *Ubx* control regions is capable of supporting development of *Ubx* null mutants throughout larval life and beyond to pharate flies, thereby rescuing the larval lethality due to the homeotic mutation. The cuticle of these flies shows that the minigene provides at least partial *Ubx* function in each of the four compartments whose morphogenetic pathways are determined by *Ubx*. We analyse β-galactosidase patterns in imaginal discs conferred by each individual *Ubx* control region. From the comparison of these patterns with *Ubx* expression in *Cbx* mutants, we infer that long-range repressor elements in the chromosomal *Ubx* gene play an important role in the generation of *Ubx* expression patterns in imaginal discs. Expression and function of our *Ubx* minigenes indicate that *Ubx* control regions are capable of functioning properly out of context and detached from their normal chromosomal location within the homeotic gene complex.

Key words: *Drosophila* homeotic gene, imaginal discs, long-range repression.

Introduction

The *Ultrabithorax* (*Ubx*) gene belongs to the group of homeotic genes in *Drosophila* whose function it is to control the morphogenesis of various position-specific structures in the embryo, the larva and the adult (Lewis, 1963, 1978; Sánchez-Herrero et al., 1985a; Teugels and Ghysen, 1985; Hooper, 1986; Bienz and Tremml, 1988). It is one of three genes located within the bithorax complex (Sánchez-Herrero et al., 1985b) and it extends over more than 130 kb (Bender et al., 1983). *Ubx* transcripts first appear in the early embryo within a domain restricted along the anteroposterior axis (Akam and Martínez-Arias, 1985). Although the pattern of *Ubx* expression becomes modified subsequently, its limits along the axis are maintained throughout development (Akam, 1983; White and Wilcox, 1984, 1985a; Beachy et al., 1985).

The main realm of *Ubx* function comprises parasegments (ps) 5 and 6 in the epidermis (Lewis, 1963, 1978; Morata and García-Bellido, 1976; Morata and Kerridge, 1981; Struhl, 1981, 1984; Hayes et al., 1984; Sánchez-Herrero et al., 1985a; Casanova et al., 1985) and in the nervous system (Teugels and Ghysen, 1985; Weinzierl et al., 1987). Minor effects of *Ubx* mutation can be observed also in more posterior segments of the larval epidermis (Lewis, 1978; Bender et al., 1983; Struhl, 1984). Internally, *Ubx* exerts a control function in abdominal segments 1-5 in the larval somatic mesoderm (Hooper, 1986), in ps7 in the embryonic visceral mesoderm (Bienz and Tremml, 1988) and, indirectly, in the endoderm (Immerglück et al., 1990).

Absence of *Ubx* function is not lethal for individual cells, though animals lacking *Ubx* function usually die as young larvae (Lewis, 1978), perhaps due to cumulative defects in different germ layers. However, clones of homozygous Ubx^- cells survive till adulthood and form adult cuticular structures (Lewis, 1963; Morata and García-Bellido, 1976; Morata and Kerridge, 1981; Miñana and García-Bellido, 1982). From studies of these Ubx^- clones, it was concluded that, in the absence of *Ubx* function, adult cuticular structures belonging to ps5 and 6 (T2p-T3a-T3p-A1a) were transformed into those of ps4 (T1p-T2a; reviewed by Sánchez-Herrero et al., 1985a; T and A, thoracic and abdominal segments; a and p, anterior and posterior compartments). As a rule, *Ubx* function is required continuously throughout development (Morata and García-Bellido, 1976), except for T2p and, to some extent, T3p in which *Ubx* function becomes dispensable after an early embryonic stage (Morata and Kerridge, 1981; Casanova et al., 1985). This early *Ubx* requirement in T2p and T3p was termed *postprothorax* (ppx^+) function (Morata and Kerridge, 1981).

In an attempt to reconstruct the embryonic *Ubx* expression pattern, we identified three key control regions in remote areas of the *Ubx* gene which, upon combination, are capable of directing a *Ubx*-like β-galactosidase (β-gal) expression pattern in transformed embryos (Müller and Bienz, 1991). We found that these control regions, if linked to a *Ubx* cDNA in a minigene construct, confer *Ubx* function in the larval epidermis. Here, we ask whether these control regions are also capable of supporting β-gal expression in imaginal disc

cells. We test whether the same minigene can provide *Ubx* function in the adult epidermis.

Materials and methods

Plasmids

The basic β-gal and *Ubx* minigene constructs used for transformation were previously described (Bienz et al., 1988; Müller and Bienz, 1991; Müller, 1991). Details of maps are available on request.

Fly strains

cn; ry^{42} flies were used for P-element transformation, and transformant lines were obtained as described (Bienz et al., 1988). In 2 of the 11 newly isolated BPuA lines (T2 and T8), the minigene transposon is inserted in the chromosomal *Ubx* gene, disrupting endogenous *Ubx* function (to be described elsewhere). The U12 transposon is inserted near the *fushi tarazu* gene (D. Yen and J. M., unpublished results); however, none of the other transposons analysed is inserted near a homeotic gene complex (U81 maps to the third chromosome at a distance from the *Ubx* gene of at least 50 Morgans; T7 and T10 map to the second chromosome).

Recombinant chromosomes bearing a minigene transposon and the Ubx^1 mutation were constructed (using a ry^{42} Ubx^1 recombinant in which at least 3/4 of the original Ubx^1 chromosome was replaced), and balanced strains were established by standard genetic procedures. These were crossed with balanced strains containing various *Ubx* mutations (see text), and non-balancer offspring flies (*Ubx* homozygotes or transheterozygotes bearing a minigene) were analysed. Control crosses were done with a Ubx^1/TM1 strain whose original Ubx^1 chromosome was "cleaned up" by recombination (J. C.-G., unpublished). All *Ubx* mutations have been described (Lewis, 1963, 1982).

To determine the lethality of Ubx^1 mutants, crosses were done, as described above, and homozygous *Ubx* larvae, recognisable by their extra spiracles (Lewis, 1978), were counted at various stages. We could not find any of these homozygous Ubx^- larvae which survived beyond the early first instar. Homozygous *Ubx* larvae bearing a minigene can be recognised by their denticle belt pattern (Müller and Bienz, 1991).

Staining of imaginal discs and analysis of adult cuticle

Inverted anterior halves of third instar larvae (typically homozygous for their transposon insert) were prepared in Ringer's solution, rinsed in phosphate-buffered saline (PBS) and fixed for 2 minutes in 1% glutaraldehyde in PBS. After thorough rinsing in PBS, they were incubated in β-gal staining solution (Bienz et al., 1988) for two hours. Stained discs were separated from the larval remains, dehydrated, passed through methylsalicylate and mounted as described for embryos (Bienz et al., 1988). The location of β-gal staining in posterior parts of discs was confirmed by β-gal staining of larvae obtained from a cross between ABP transformants with an *engrailed*/β-gal transformant line (in the latter, β-gal staining is restricted to posterior compartments; Hama et al., 1990). Staining with a monoclonal antibody against *Ubx* protein (White and Wilcox, 1984) was done as described (Castelli-Gair et al., 1990). Analysis of adult cuticle was done by following standard procedures (Morata and García-Bellido, 1976).

Results

In this study, we use the same *Ubx* fragments from the PBX, ABX and BXD control regions whose maps and regulatory properties we have described (Müller and Bienz, 1991). Briefly, the PBX and BXD fragments are derived from remote regions upstream, the ABX fragment from an intronic region downstream of the *Ubx* transcription start site, as sketched out in Fig. 1. Of these fragments, PBX and ABX direct an early pattern of β-gal expression which is restricted to the *Ubx* domain along the anteroposterior axis of the embryo and strongest anteriorly within ps6 and ps5, respectively. The BXD fragment is activated at a later embryonic stage to direct a pattern extending from

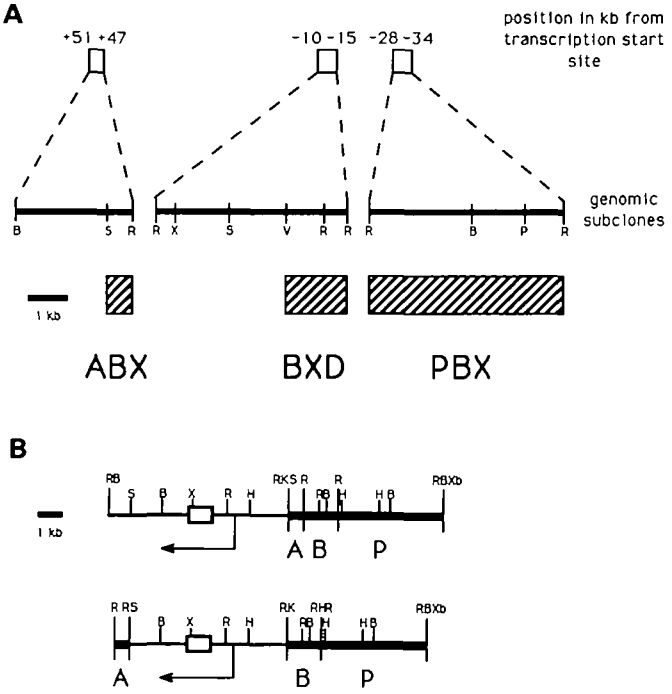

Fig. 1. Map of *Ubx* control regions and plasmids. (A) Maps of PBX, ABX and BXD fragments (genomic subclones; B, *Bam*HI; P, *Pst*I; R, *Eco*RI; S, *Sal*I; V, *Eco*RV; X, *Xho*I; all sites shown in the case of B, R, S) and their position with respect to the *Ubx* transcription start site (transcription from right to left). Fragments used in our constructs are hatched; in the case of ABX and BXD, these correspond to the minimal fragments conferring embryonic expression (Müller and Bienz, 1991). The imaginal PBX pattern is due to sequences downstream of the *Bam*HI site within the PBX fragment (the 5′ end of the genomic subclone 3104; see text), whereas sequences upstream of this *Bam*HI site do not direct any expression in imaginal discs (although they contain the minimal PBX fragment conferring embryonic expression; Müller and Bienz, 1991). (B) Maps of the two types of minigenes (ABP and, underneath, BPuA; Xb, *Xba*I; K, *Kpn*I; other restriction sites as in Fig. 1A; all sites shown except for *Xho*I). Open boxes, *Ubx* coding region; thick lines, *Ubx* control regions (see Fig. 1A); thin lines, *Ubx* sequences flanking the *Ubx* coding region (transcription start site and direction of transcription indicated by arrow). Orientation of fragments same as in *Ubx* gene.

head to tail which resembles the *Ubx* expression pattern in abdominal segments.

Most transformants used for this analysis have been isolated previously (including the minigene-bearing transformant lines U12 and U81; Müller and Bienz, 1991). In addition, we constructed a second type of minigene in which the ABX fragment is placed downstream of the coding region (BPuA; Fig. 1). We isolated 11 individual lines of which 5 showed a strong Cbx^1-like phenotype (Fig. 3D), like the U12 and U81 lines, a phenotype that we presumed to be caused by minigene-derived ectopic Ubx^+ activity (Müller and Bienz, 1991). We took this phenotype as an indication of high minigene efficacy and chose two of these lines (T7 and T10) for further analysis.

Imaginal disc patterns

We stained imaginal discs from late third instar larvae of PBX, ABX and BXD transformants for β-gal activity. We found that neither the *Ubx* proximal promoter by itself (not shown) nor the BXD fragment linked to the *Ubx* proximal promoter (Fig. 2A,B) is capable of directing any substantial β-gal expression in imaginal discs. In contrast, both the ABX (Fig. 2C-F) and the PBX fragment (Fig. 2G-K) linked to the *Ubx* proximal promoter confer patterns of strong β-gal expression in individual imaginal discs as follows.

β-gal expression in ABX transformants is restricted to the dorsal discs, the wing (Fig. 2C) and the haltere disc (Fig. 2D); neither of the leg discs (Fig. 2E,F) nor any other disc show β-gal staining. Staining in the wing and the haltere disc is very strong in their centers, their "pouch" regions, which give rise to the most distal derivatives of these discs, the wing blade and the haltere capitellum (reviewed by Bryant, 1978). In some but not all transformant lines, we also see strong β-gal expression in the stalk region of the wing disc (Fig. 2C) and, very occasionally, a strong β-gal spot in the stalk region of the haltere disc, the regions giving rise to the meso- and metanotum. Expression in the "pouch" regions of the two discs is regularly observed and equally strong in virtually all ABX lines, although the extent of the "pouch" expression varies slightly (in some lines restricted to the most central regions; cf. Fig. 3A). We shall refer to this expression in the wing and haltere "pouches" as the imaginal ABX pattern. We observe it independently of the type of ABX construct, i.e. whether the latter contains the large genomic 3.0 kb or the minimal 0.6 kb ABX fragment, and whether the minimal fragment is located upstream or downstream of the fusion gene (BβA transformants; cf. Müller and Bienz, 1991).

The imaginal ABX pattern shows very little resemblance to *Ubx* protein expression in imaginal discs (White and Wilcox, 1984, 1985a). However, the staining in the wing "pouch" is reminiscent of the ectopic *Ubx* expression pattern in wing discs of *Hm* and Cbx^{M1}mutants (Fig. 3A,E; Cabrera et al., 1985; González-Gaitán et al., 1990). In *Hm* (Bender et al., 1983) and probably in Cbx^{M1} mutants (González-Gaitán et al., 1990), most of the upstream flanking region of the *Ubx* gene is lacking and, thus, the mutant *Ubx* expression patterns may reveal the intrinsic activity of control regions downstream of the promoter (e.g. of the ABX fragment), a notion strongly supported by our imaginal ABX pattern.

We initially obtained only one transformant line with the large genomic PBX fragment. Two further lines were generated by "jump-starting" (Robertson et al., 1988); however, one of these lines showed β-gal staining in all discs, whereas the other showed none. We shall refer to the β-gal staining pattern in the initial line as the imaginal PBX pattern since we observe virtually the same pattern in other transformants carrying part or the whole of the large PBX fragment (see below). The imaginal PBX pattern consists of strong β-gal expression in the wing and in the haltere discs (Fig. 2G,H) and of a comparatively low but significant level of β-gal expression, evenly distributed, in all three types of leg discs (Fig. 2H-K). There is also β-gal expression in the eye and in the humeral disc (not shown). β-gal staining in the haltere disc is usually observed throughout the disc (Fig. 2H), with occasional white patches in the anterior part. In the wing disc, β-gal staining is confined mostly to the posterior part, with an anterior expression boundary forming a striking line across the disc (Fig. 2G), a line reminiscent of the anteroposterior compartment boundary (Brower, 1986). The β-gal pattern varies somewhat between individuals as we frequently observe β-gal patches in the anterior part (Fig. 3B). On occasions, the whole disc is stained except for a white line approximately following the anteroposterior compartment boundary (Fig. 3C). Surprisingly, we do not see the imaginal PBX pattern in transformants containing the minimal PBX control region (between the *Bam*HI and the *Pst*I site; see Fig. 1) which is sufficient to confer the embryonic PBX pattern (Müller and Bienz, 1991), but we find a very similar pattern in imaginal discs of transformants containing a more proximal genomic fragment (3104; see Fig. 1) which partly overlaps the large PBX fragment. Thus, the regulatory sequences conferring the imaginal PBX pattern are closely linked to, but separable from (and downstream of) the sequences conferring the embryonic PBX pattern.

The imaginal PBX pattern resembles the *Ubx* protein pattern in Cbx^1 mutants, particularly within the wing disc (Fig. 3; White and Akam, 1985; Cabrera et al., 1985; see also Castelli-Gair et al., 1990). Variable patterns of *Ubx* protein expression are observed between individual Cbx^1 mutants (Fig. 3F,G), very similar to the variable β-gal patterns of PBX transformant individuals (Fig. 3B,C). The Cbx^1 mutant chromosome bears a duplicated copy of an upstream genomic fragment at an intronic location closer to and downstream of the *Ubx* transcription start site (~+13 kb; Bender et al., 1983). The transposed piece of genomic DNA contains our large PBX fragment. Evidently, the pattern of ectopic *Ubx* protein expression in Cbx^1 wing discs reflects the intrinsic capability of the PBX control region to direct this particular pattern of expression.

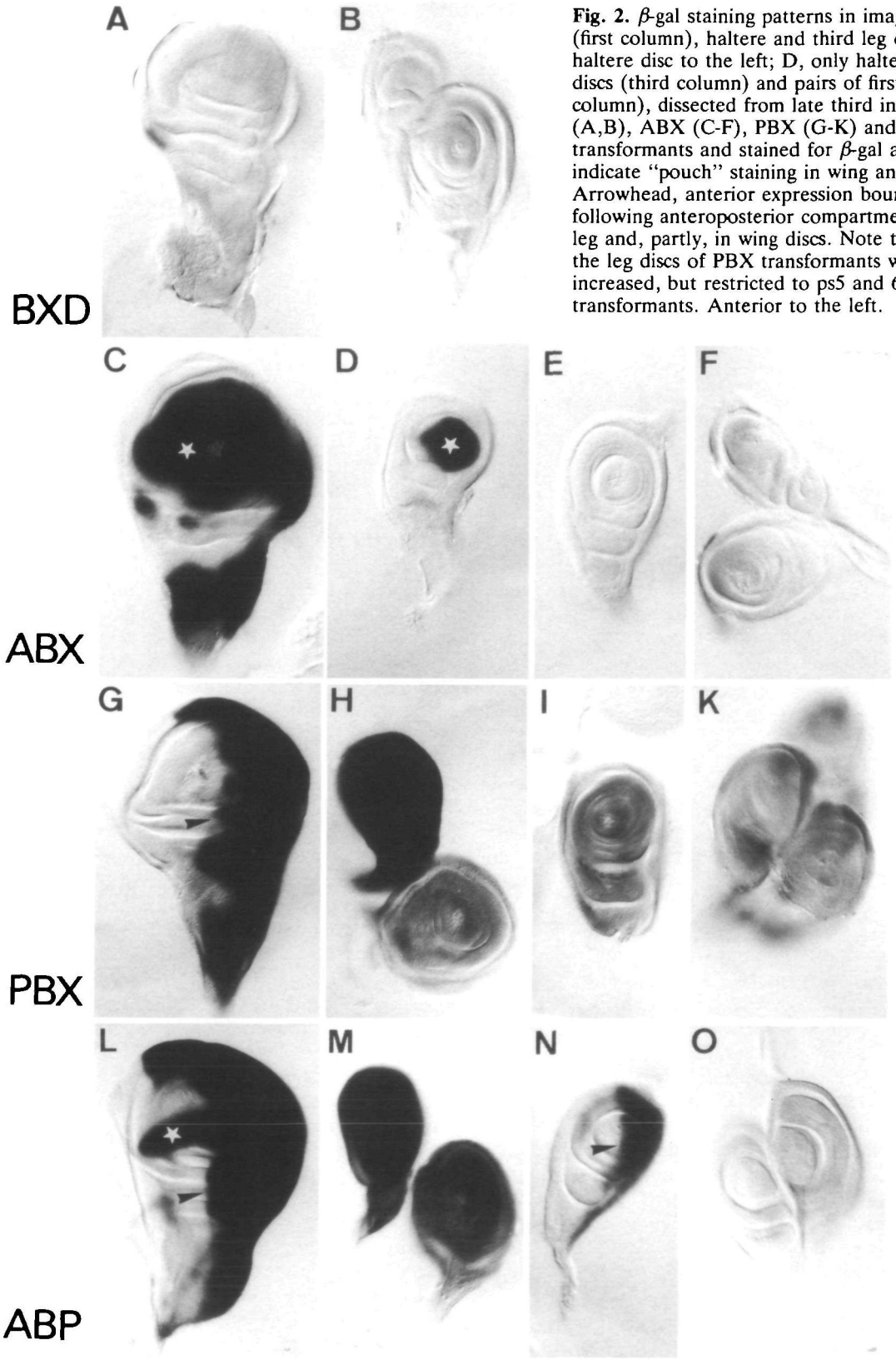

Fig. 2. β-gal staining patterns in imaginal discs. Wing discs (first column), haltere and third leg discs (second column, haltere disc to the left; D, only haltere disc), second leg discs (third column) and pairs of first leg discs (fourth column), dissected from late third instar larvae of BXD (A,B), ABX (C-F), PBX (G-K) and ABP (L-O) transformants and stained for β-gal acticvity. White asterisks indicate "pouch" staining in wing and haltere discs. Arrowhead, anterior expression boundaries, apparently following anteroposterior compartment boundaries in second leg and, partly, in wing discs. Note the β-gal staining in all the leg discs of PBX transformants which is slightly increased, but restricted to ps5 and 6 in leg discs of ABP transformants. Anterior to the left.

Finally, we examined transformants of a triple combination construct, bearing all three control regions upstream of the *Ubx* proximal promoter (ABP; Müller and Bienz, 1991). The majority of these ABP lines show a β-gal-staining pattern in the dorsal imaginal discs which corresponds to a PBX pattern superimposed on an ABX pattern (Fig. 2L,M), best visible in the wing disc where β-gal staining in the "pouch" protrudes

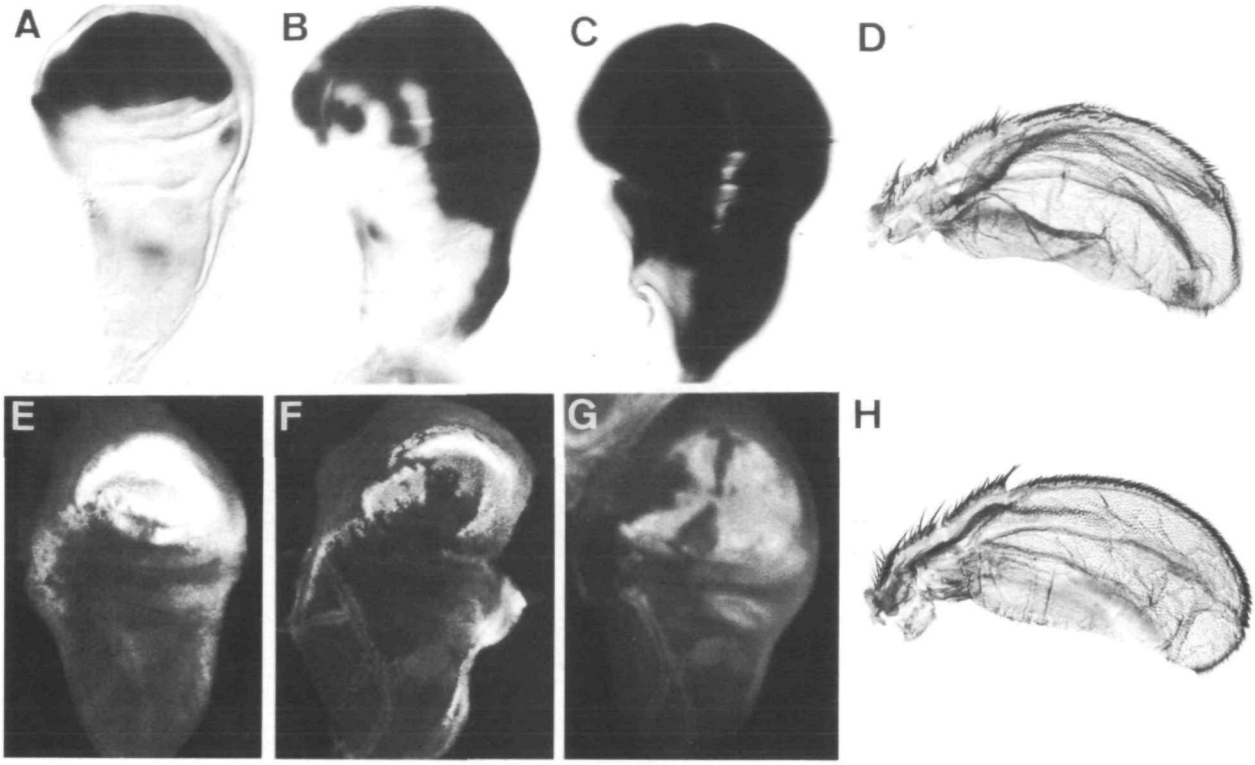

Fig. 3. *Cbx* expression patterns and phenotypes. Similarities between expression patterns in the wing disc from an ABX transformant (A; a different line from the one in Fig. 2 is shown in which β-gal staining is restricted to the center of the "pouch" and absent from the stalk region; see text) and from *Hm*/+ mutants (E; disc stained with *Ubx* antibody) as well as between the variable PBX patterns (B,C; β-gal staining) and the variable *Ubx* protein expression patterns in Cbx^1/+ mutant individuals (F,G). Wing phenotypes observed in U12 transformants (D; virtually the same phenotype is also apparent in U81, T7 and T10 transformants) and in Cbx^1/+ mutants (H).

across the PBX expression boundary into the anterior part of the disc. Compared to the wing disc pattern in PBX transformants which extends variably into the anterior compartment, we note that β-gal staining in ABP transformants is more regularly restricted to the posterior part of the disc, reflecting some degree of non-additivity of patterns. More striking non-additivity is observed in the leg discs: the first leg discs in ABP transformants do not stain at all (Fig. 2O), and β-gal staining in the second leg discs is restricted to the posterior half (Fig. 2N). There is moderately strong β-gal staining in the whole of the third leg discs (Fig. 2M). As in the wing disc, the β-gal expression boundary in the second leg disc apparently follows the anteroposterior compartment boundary (cf. Steiner, 1976). There is virtually no β-gal expression anterior to the anteroposterior compartment boundary in T2 discs. This boundary corresponds to the anterior boundary of ps5 and thus to the anterior limit of *Ubx* expression.

The imaginal ABP pattern closely resembles the pattern of *Ubx* protein expression in imaginal discs (White and Wilcox, 1984, 1985b), although it differs from the latter in two main respects. Firstly, there is ectopic β-gal expression in the wing disc which probably causes the Cbx^1-like phenotype of our minigene transformants. Secondly, the levels of β-gal staining in the leg discs are somewhat low, suggesting that the ABP construct lacks enhancer sequences required for efficient expression in ventral discs.

Minigene function in individual imaginal compartments

Since an ABP minigene (all three fragments linked to a *Ubx* cDNA; Müller and Bienz, 1991) is capable of conferring a Cbx^1 phenotype through its activity in the wing disc (Fig. 3D), we wondered whether this minigene might be sufficiently active in other imaginal disc cells to rescue aspects of the adult *Ubx* phenotype. For this, we established balanced strains containing a minigene transposon (U12, U81, T7 or T10) and the Ubx^1 mutation (an antibody-negative null mutation; Weinzierl et al., 1987; see Material and Methods). We first tested the function of these minigenes in *abx*, *bx*, *pbx* and *bxd* mutants. Flies carrying either of these mutations in combination with Ubx^1 are viable; however, they show transformations of individual compartments in their epidermis: T3a is transformed into T2a in *abx*/*Ubx* or in *bx*/*Ubx* mutants, T3p is transformed into T2p in *pbx*/*Ubx* mutants, whereas both T3p and A1a are transformed into T2p and T3a in *bxd*/*Ubx* mutants (reviewed by Sánchez-Herrero et al., 1985a; the T2p>T1p transformation caused by abx^1/Ubx^1 or

bx^3/Ubx^1 mutation cannot be discerned in the above allelic combinations).

Homeotic transformations in the haltere disc derivatives (dorsal T3a or T3p) which are caused by abx^1bx^3 (Fig. 4A) or by pbx^1 mutation (Fig. 4C) are mostly and, in the case of pbx^1 mutation, virtually fully rescued by one copy of either of the minigenes (Fig. 4B,D; U12 and U81 minigenes show slightly better rescue activity than T7 and T10 minigenes). The rescue activity is particularly strong in the distal region of T3, the haltere, but there is also some rescue activity in the proximal region, the notum. This activity is particularly striking in the case of the four-winged triple mutant $abx^1\ bx^3\ pbx^1$ (Fig. 4E) whose second pair of wings are reverted towards normal halteres and whose duplicated mesonotum is partly suppressed, due to minigene function (Fig. 4F). However, these flies have two pairs of T2 legs (not shown) and, thus, we do not observe full rescue function of the minigene in the third legs. This is somewhat surprising, given that there is β-gal ex-

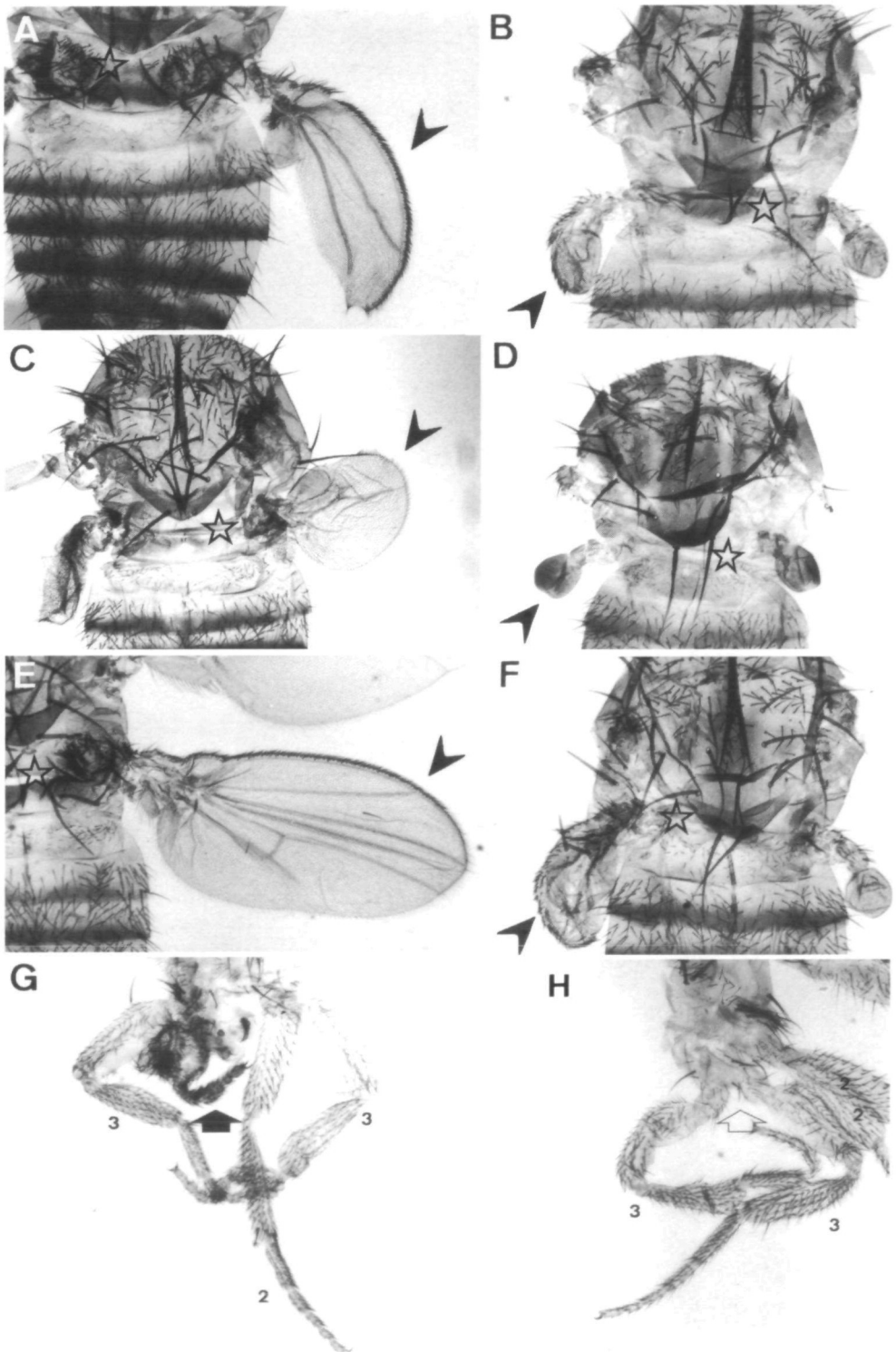

Fig. 4. Minigene function in individual adult compartments. Adult phenotypes of different mutant alleles (A,B, abx^1bx^3/Ubx^1; C,D, pbx^1/Ubx^1; E,F, $abx^1\ bx^3\ pbx^1$; G,H, bxd^1/Ubx^1) in the absence (left column) or presence (right column) of a U12 minigene. (B) Partial rescue by the minigene of the T3a>T2a transformation, resulting in a haltere-like structure (lacking most wing tissue landmarks, except for the triple row chaetae indicated by arrowhead) instead of a wing (arrowhead in A) and in the reduction of extra mesonotal tissue (marked by asterisks). (D) Virtually complete rescue of the T3p>T2p transformation in the haltere (arrowhead indicates almost normal haltere; compare to wing-like structure in C, arrowhead), but very little if any rescue of this transformation in the posterior notum (asterisks). (F) High rescue activity of the minigene, as visualised by an almost complete reversal of the haltere to wing transformation (arrowheads in E and F) as well as by the reduction of mesonotum tissue (asterisks in E and F). (H) Complete suppression of the extra pair of A1 legs (marked by black arrow in G; white arrow in H marks the position where the A1 legs normally arise in the mutants). Numbers refer to segmental identities of legs. The extent of minigene-dependent rescue varies between flies, although the A1 leg suppression is observed in all individuals. For technical reasons, the dorsal appendages of T2 were removed in all cases except E.

pression in the third leg discs of ABP transformants (Fig. 2M). It is possible that the level of expression in the third leg discs is too low, due to lack of enhancer sequences required for high levels of ventral disc expression.

In addition to affecting the haltere disc derivatives (T3p>T2p), bxd^1/Ubx^1 mutant flies show an A1a>T3a transformation, having an additional pair of legs (Fig. 4G), and lacking an A1a tergite. If these flies carry one of our *Ubx* minigenes, their T3p>T2p transformation is rescued (not shown) and their extra pair of legs is completely suppressed (Fig. 4H). However, they still lack the A1a tergite and, thus, the minigenes apparently lack sequences mediating *Ubx* expression necessary for tergite development.

Rescue of Ubx *null mutation*

The recombinant strains containing both a Ubx^1 mutation and a *Ubx* minigene allowed us to test the rescue activity of this gene in Ubx^1 homozygotes. Homozygous Ubx^1 larvae, obtained from a control cross, died shortly after eclosion from the embryonic membranes (see Materials and methods; cf. also Lewis, 1978; Frayne and Sato, 1991). However, we find that one copy of a *Ubx* minigene can support development of Ubx^1 homozygotes throughout larval life: a relatively low, but significant fraction of these homozygotes (more individuals in the case of T7 and T10, compared to U12 and U81) survive to the end of pupation and secrete adult epidermis which is fully differentiated. The morphological features of these pharate adults can be examined. Rescue of the larval lethality of *Ubx* null mutation demonstrates that our *Ubx* minigenes are capable of providing all *Ubx* function required for larval and pupal viability.

We peeled from their pupal case Ubx^1 homozygous or Ubx^1/Ubx^{195} pharate flies containing one of our minigenes and prepared their cuticle for microscopic analysis (Fig. 5; there was no difference in rescue activity in the two cases of allelic *Ubx* combinations). In agreement with our previous results, the T3>T2 transformations of the haltere derivatives are partially rescued by the minigenes (Fig. 5A,C,E), particularly in the distal region, and the fourth pair of legs is completely suppressed (Fig. 5D,F). Again, the T3>T2 transformation in the legs is not rescued (Fig. 5D,F), and the A1a tergite is also lacking (Fig. 5C,E). However, we find that the *ppx* phenotype (the T2p>T1p transformation) in the legs is fully rescued (Fig. 5B,D,F), showing that our minigenes provide ppx^+ function.

Discussion

We have analysed expression and function of two types of *Ubx* minigenes in imaginal disc cells. These minigenes contain the same three *Ubx* control fragments which, upon combination, confer a *Ubx*-like expression pattern in the embryo as well as *Ubx* function in the larval epidermis (Müller and Bienz, 1991). We now find that two of the three fragments also direct distinct patterns of expression in imaginal discs, and that their combination results in a pattern resembling *Ubx* expression in these discs. Both types of *Ubx* minigenes are capable of providing all *Ubx* function needed for larval viablity and pupal development as well as partial *Ubx* function in the adult epidermis throughout the *Ubx* domain. Certain control elements required for adult *Ubx* function are still missing from these minigenes, which is not surprising as the initial screen for control sequences was based entirely on their activity in the embryo. Nevertheless, at least some of the control sequences conferring adult *Ubx* function are evidently closely linked in the chromosomal *Ubx* gene to those conferring larval *Ubx* function.

Evidence for long-range repressor elements

It has been proposed that the gain-of-function phenotype of the Cbx^1 mutation is due to ectopic *Ubx* expression in the wing disc (Lewis, 1982), a prediction borne out by *Ubx* antibody staining results (White and Akam, 1985; Cabrera et al., 1985; González-Gaitán et al., 1990). There remained the question as to why, upon transposition, an upstream *cis*-regulatory element expected to be active in ps6 and conferring *pbx* function in T3p (Bender et al., 1983) should be activated ectopically in ps5 in Cbx^1 mutants. To explain this, Peifer et al. (1987) proposed in their "open for business" model the existence of parasegment-specific chromosomal domains which need to be "opened up" in a parasegment-specific way for enhancers located within these domains to become active. According to this model, enhancers from the *pbx* region are active ectopically in ps5 in Cbx^1 mutants, because of their new location within a chromosomal domain which is "opened up" in ps5.

Our results seem to argue against this view. The *Ubx* expression pattern in Cbx^1 mutant imaginal discs closely resembles the β-gal staining pattern conferred by the PBX fragment (which is contained within the transposed DNA in Cbx^1 mutants) and thus apparently reflects intrinsic PBX enhancer activity. This activity is overt in all our PBX-bearing transformants and therefore does not seem to require any particular chromosomal context (nor does it depend on the presence of the ABX control region). In the chromosomal *Ubx* gene, and also in a *Ubx*/β-gal construct containing 35 kb of *Ubx* upstream flanking sequence (Irvine et al., 1991), the intrinsic PBX activity in ps4 and ps5 is evidently suppressed. We suggest that there is an upstream repressor element in the *Ubx* gene, located between our PBX fragment and the *Ubx* promoter, which acts to suppress the PBX enhancer activity in ps4 and ps5. Whether this activity is suppressible by the upstream repressor may be determined by the position of the two regulatory elements with respect to each other and to the *Ubx* promoter, e.g. whether they are located on the same or opposite sides of the promoter, or perhaps by their relative distance from the promoter (for a discussion of distance affecting competition for promoter interaction between remote transcription factors,

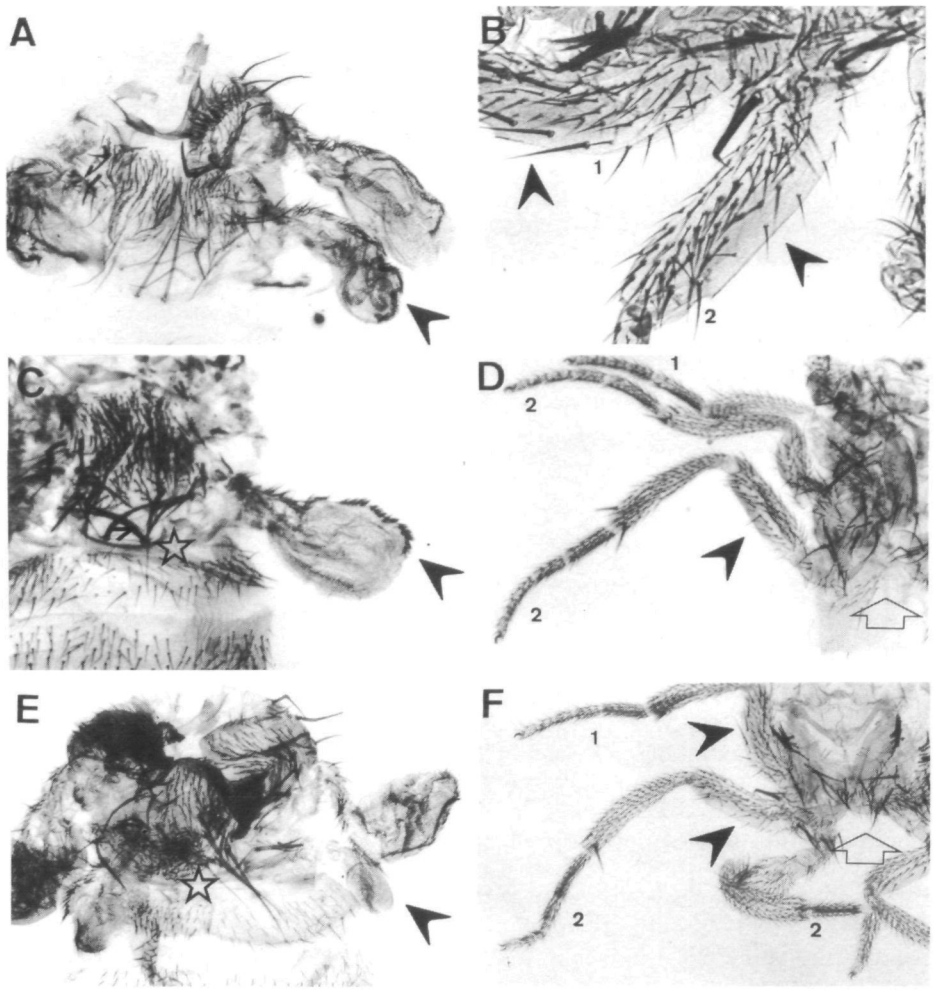

Fig. 5. Minigene rescue activity in *Ubx* mutations. Phenotypes of homozygous Ubx^{-} pharate adults (A-D, Ubx^{1} homozygotes; E,F, Ubx^{1}/Ubx^{195}) in the presence of a U12 (A,B) or T7 (C-F) minigene. Dorsal (left column) and ventral aspects (right column) of the same flies are shown. Highest rescue activity of the minigene is visible in the halteres (arrowheads) and in the complete suppression of extra A1 legs (white arrow in D and F). Full rescue of the *ppx* phenotype in the legs (B,D,F), apparent by the lack in T2p of long bristles typical for T1p (arrowheads in B). Numbers refer to segmental identities of legs. The lack of an A1a tergite (asterisks in C and E) is not rescued by the minigene. Dorsal T2 appendages (showing the Cbx^{1}-like phenotype; cf. Fig. 3) were not removed in A and E.

see Hanscombe et al., 1991). We previously found a similar repressor element within the PBX fragment itself (mediating long-range repression anterior to ps6; Müller and Bienz, 1991), but the latter is evidently only effective in the embryo and not in imaginal cells.

Hm and Cbx^{M1} mutants show ectopic *Ubx* protein expression in the wing disc "pouch" (White and Akam, 1985; Cabrera et al., 1985; González-Gaitán et al., 1990) which resembles the β-gal expression pattern conferred by the ABX fragment. It is likely that the mutant ectopic *Ubx* expression reflects the intrinsic enhancer activity of the ABX fragment, and that this intrinsic activity is normally suppressed by an upstream repressor element (which is uncoupled from the *Ubx* promoter in the mutants). This repressor element may be the same as the one mentioned above that suppresses the PBX enhancer activity in ps4 and ps5.

We found that the imaginal ABP pattern does not correspond to a simple superimposition of imaginal ABX and PBX patterns: the anterior ABP expression limit to a large extent follows the anterior boundary of ps5, although the PBX fragment on its own mediates expression anteriorly to this limit, especially in the leg discs. It thus appears that the ABX fragment contains a long-range repressor element (suppressing PBX enhancer activity in the leg discs and perhaps in the wing discs anteriorly to ps5). Again, we found that the ABX fragment does contain such a repressor element which, in the embryo, mediates an anterior expression boundary between ps4 and ps5 (Müller and Bienz, 1991). Although the imaginal disc repressors may be different from the embryonic repressors (one of which is the *hunchback* protein; Zhang et al., 1991; Zhang and M.B., unpublished), their action may depend, directly or indirectly, on the preceding action of embryonic repressors.

Ubx *minigene function affecting imaginal disc primordia*

Two features of the adult *Ubx* phenotype are apparently fully rescued by our *Ubx* minigenes: the *ppx* phenotype (the T2p>T1p transformation) and the formation of a fourth pair of legs. We propose, for the following reasons, that rescue activity in these cases reflects minigene-dependent deployment of *Ubx* in the embryo, either within imaginal disc primordia or suppressing the formation of these.

The ppx^+ function is a subfunction of *Ubx* which is required exclusively early in development to control the morphogenesis of T2p and in T3p in the adult (Morata and Kerridge, 1981; Casanova et al., 1985). In the absence of ppx^+ function, i.e. if *Ubx* product is eliminated within the first 6 hours of embryonic development, these two posterior compartments are transformed into T1p, a transformation rescued by our *Ubx* minigenes. We note that the imaginal domains within which ppx^+ function is required correspond to the embryonic domains within which ABX- and PBX-mediated expression is highest (anterior part of ps5 and ps6, corresponding to T2p and T3p; Müller and Bienz, 1991). Our evidence strongly suggests that this expression anteriorly within ps5 and ps6 is due to direct activation of the ABX and PBX control regions by the segmentation products *even-skipped* (*eve*) and *fushi tarazu* (*ftz*), respectively (Müller, 1991; Müller and Bienz, in preparation). It therefore appears likely that *eve*- and *ftz*-mediated *Ubx* expression in the early embryo confers ppx^+ function. Among the cells that we expect to be supplied with this pulse of *eve*- and *ftz*-mediated *Ubx* expression are those forming the prospective posterior compartments of the T2 and T3 imaginal disc primordia as the latter become visible at this time, straddling parasegment boundaries (Cohen, 1990; Cohen et al., 1991). This early *Ubx* protein may fade later to become virtually undetectable in the wing discs (White and Wilcox, 1984, 1985b) as these are the most actively proliferating discs (Bryant, 1978).

A fourth pair of legs is formed in *bxd* mutants (Lewis, 1963), apparently reflecting establishment of an additional pair of disc primordia in the embryo (Cohen et al., 1991). This extra pair of legs is completely suppressed by our *Ubx* minigenes. The minigenes also suppress the extra pair of Keilin's organs (corresponding to rudimentary larval legs) due to *Ubx* mutation, an activity which we ascribe to the BXD control element (Müller, 1991). It therefore appears that BXD-mediated *Ubx* expression may suppress the establishment of larval and adult leg primordia in the embryo. The latter do not seem to be suppressed by PBX- and ABX-mediated *Ubx* expression (see above); however, the PBX and ABX control regions are active at different times and levels as well as in different cells of the embryo than the BXD control region (Müller and Bienz, 1991).

Our *Ubx* minigenes are not capable of supporting development of A1a tergites which are missing in *Ubx* mutants or in *bxd* mutants lacking *Ubx* activity in ps6. These mutants lack abdominal histoblasts, the tergite precursor cells, in A1 at young larval stages; instead, they show buds of dorsal and ventral imaginal discs (Kerridge and Sang, 1981; Frayne and Sato, 1991). Although the *Ubx* minigenes apparently suppress the disc primordia in A1 (see above), they obviously do not supply sufficient *Ubx* function to support development of abdominal histoblasts in A1. It is conceivable that primordial histoblasts never form in minigene transformant embryos. We think it more likely that these form, but that they cannot develop and proliferate (cf. Frayne and Sato, 1991), due to insufficient minigene function at later stages.

Conclusion

Homeotic gene complexes are strikingly conserved between flies and mammals (Duboule and Dolle, 1989; Graham et al., 1989). It has been speculated that this conservation reflects functional significance (cf. Akam, 1989), though in the case of the bithorax complex, integrity of the complex is not necessary for bithorax gene function (Struhl, 1984). Functional importance has also been attributed to the size of homeotic genes in *Drosophila* which are unusually large, apparently due to the complexity and redundancy of *cis*-regulatory elements (Bender et al., 1985; Simon et al., 1990; Irvine et al., 1991). The latter may be needed to generate the complex pattern of *Ubx* expression, some aspects of which however are functionally insignificant (González-Reyes and Morata, 1990).

Our results seem to indicate that there is no absolute requirement for homeotic genes to be located within homeotic gene complexes: *Ubx* minigenes are capable of functioning, out of context, in a number of different chromosomal locations to provide all *Ubx* functions needed for embryonic and larval life as well as partial *Ubx* function for adult morphogenesis. The minigenes function to a perhaps surprising level, despite containing few and comparatively short control regions. Their functional shortcomings in certain imaginal cells (in the legs and the A1a tergite) are probably due to lack of the corresponding control elements which might be identifyable by appropriate searching. Finally, our minigenes do not function reliably from individual to individual, as judged by the varying rescue activities observed among individuals in any one transformed line. Thus, large control regions and/or redundancy of control elements may be required in some way to ensure reliability of the control process.

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