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Negative autoregulation by *Ultrabithorax* controls the level and pattern of its expression

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SUMMARY

The *Drosophila* homeotic gene *Ultrabithorax* (*Ubx*) encodes transcriptional regulatory proteins (UBX) that specify thoracic and abdominal segmental identities. *Ubx* autoregulation was examined by manipulating UBX levels, both genetically and with an inducible transgene, and monitoring the effect of these manipulations on the expression of *Ubx* and *Ubx-lacZ* reporter genes. Positive autoregulation by *Ubx* is restricted to the visceral mesoderm, while in other tissues *Ubx* negatively autoregulates. In some cases, negative autoregulation stabilizes UBX levels, while in others it modulates the spatial and temporal patterns of UBX expression. This modulation of UBX expression may enable *Ubx* to specify distinct identities in different segments. The upstream control region of *Ubx* contains multiple autoregulatory elements for both positive and negative autoregulation.

Key words: *Drosophila* development, bithorax complex, homeotic gene

INTRODUCTION

Segmental identities in *Drosophila* are specified by the homeotic selector genes of the bithorax and Antennapedia complexes (reviewed in Duncan, 1987 and in Kaufman et al., 1990, respectively). These genes encode transcriptional regulatory proteins that select developmental fates by regulating the expression of downstream target genes (reviewed in García-Bellido, 1975; Scott et al., 1989; Andrew and Scott, 1992). One complexity in the assignment of segmental identity is the ability of a single homeotic gene to specify the identities of multiple segments. To some extent, unique segmental identities may be specified in these circumstances by combinations of homeotic genes acting in concert (Lewis, 1978; Struhl, 1982; Carroll et al., 1988). Additionally, a single homeotic gene may contribute to the establishment of different identities by differences in the pattern or level of its expression (Peifer et al., 1987; Akam et al., 1988; Smolik-Utlaut, 1990); this requires tight and complex control over homeotic gene expression. Here, we focus on the control of expression of the homeotic gene *Ultrabithorax* (*Ubx*) by autoregulation.

Genetic analysis has demonstrated that *Ubx* has an essential role in specifying the identities of parasegments 5 and 6 (PS5 and 6) in both the larval and adult epidermis (Lewis, 1978; reviewed in Duncan, 1987). PS5 and 6 comprise the posterior compartment of the second thoracic segment (T2p), the third thoracic segment (T3) and the anterior compartment of the first abdominal segment (A1a; Martinez-Arias and Lawrence, 1985). *Ubx* null mutations transform the identity of these parasegments to that of PS4 (T1p + T2a). *Ubx* is also required in other tissues and in other metameres of the fly besides the epidermis of PS5 and 6. *Ubx* normally prevents the formation of thoracic sensory structures in the larval abdomen (Lewis, 1978), is required in the embryonic visceral mesoderm in PS7 for proper gut morphogenesis (Bienz and Tremml, 1988) and is required in the larval somatic mesoderm in abdominal segments (Hooper, 1986). Metameric requirements for *Ubx* function in the nervous system parallel those in the epidermis (Teugels and Ghysen, 1985).

A family of *Ubx* proteins (UBX) is produced by alternative splicing of the 77 kb *Ubx* transcription unit (O'Connor et al., 1988; Kornfeld et al., 1989; Gavis and Hogness, 1991). Biochemical analysis of these UBX proteins has shown that they are DNA-binding transcriptional regulators (Beachy et al., 1988; Krasnow et al., 1989; Johnson and Krasnow, 1990). The different UBX proteins are closely related and each includes the same DNA-binding homeodomain, but they differ in their tissue and temporal distributions (Lopez and Hogness, 1991). As expected,

UBX proteins are expressed where genetic analysis has revealed that *Ubx* function is required (White and Wilcox, 1984, 1985a; Beachy et al., 1985). Notably, UBX is expressed at high levels throughout most of PS6 but is expressed at lower levels and in fewer cells of PS5.

As might be expected from the diverse yet specific spatial and temporal requirements for *Ubx* function during development, this gene has large and complex transcriptional regulatory regions. These have been identified both by molecular mapping of regulatory mutations in these regions and by analysis of *Ubx-lacZ* fusion genes containing sequences from them. A 35-40 kb upstream control region (UCR) is defined by the *bithoraxoid* (*bxd*) and *postbithorax* (*pbx*) mutations (Fig. 1; Bender et al., 1983, 1985). These mutations decrease UBX expression in PS6 and consequently transform it toward PS5 (Beachy et al., 1985; Cabrera et al., 1985; Hogness et al., 1985; White and Wilcox, 1985b; Irvine et al., 1991). A downstream control region (DCR) is defined by the *anterobithorax* (*abx*) and *bithorax* (*bx*) mutations (Fig. 1; Bender et al., 1983; Peifer and Bender, 1986); these mutations decrease UBX expression in PS5 and consequently transform it toward PS4 (Cabrera et al., 1985; White and Wilcox, 1985b; Botas et al., 1988; Little et al., 1990). A *Ubx-lacZ* fusion gene (*35UZ*) including the *Ubx* 5′ untranslated leader and 35 kb of DNA directly upstream of the transcription start site expresses β-galactosidase throughout development in a pattern like that for UBX in DCR mutants (Irvine et al., 1991); this region thus appears to contain all of the UCR sequences necessary to generate the correct pattern of *Ubx* expression. *Ubx-lacZ* fusions containing sequences from the DCR also exhibit some aspects of UBX expression (Simon et al., 1990; Müller and Bienz, 1991; Qian et al., 1991). Interestingly, the characterization of β-galactosidase expression patterns generated by *Ubx-lacZ* fusion genes, as well as UBX expression patterns detected in *Ubx* control region mutants, indicates that partial redundancy between the UCR and the DCR exists, as some features of *Ubx* expression can be directed by either control region.

Homeotic gene expression is established in the early embryo, primarily or exclusively by the action of gap and pair-rule segmentation genes (reviewed in Akam, 1987; Ingham, 1988). However, as the segmental expression of these segmentation genes disappears after early embryogenesis, a different mechanism must be employed to maintain homeotic gene expression, which is required at least through the end of larval development (Lewis, 1964; Morata and García-Bellido, 1976; Struhl, 1982). One attractive hypothesis, first suggested by García-Bellido and Capdevila (1978), postulates that homeotic genes may act in some way to maintain their own expression. The observation that the homeotic genes *Deformed* and *labial* positively autoregulate in some ectodermal cells lends support to this hypothesis (Kuziora and McGinnis, 1988; Bergson and McGinnis, 1990; Chouinard and Kaufman, 1991). However, positive autoregulation by *Ubx* has been observed only in the embryonic visceral mesoderm (Bienz and Tremml, 1988). In this work, we show that in other tissues *Ubx* actually negatively autoregulates, that modulation of both the level and pattern of UBX expression result from diverse responses of the *Ubx* promoter to UBX, and that negative autoregulation thereby contributes to the specification of segmental identities by *Ubx*.

MATERIALS AND METHODS

Drosophila strains

Descriptions of the *Ubx* control region mutations employed in this work, abx^1, abx^2, bx^1, bx^3, $bx^{34e-prv}$, bx^{83ka}, bx^{83kb}, bxd^{100}, bxd^{106}, pbx^1, and pbx^2, can be found in Bender et al. (1983) and Peifer and Bender (1986). Descriptions of relevant *Ubx* mutations can be found in Lewis (1978) and Weinzierl et al. (1987). Unless otherwise specified, *Ubx* and *Ubx* control region mutations were examined as transheterozygotes over *DfUbx109*, which deletes *Ubx* and the neighboring *abdA* gene. Where flies are described as Ubx^- or *Ubx* mutants, both $Ubx^{9.22}$ and Ubx^{130} hemizygotes were examined. $Ubx^{9.22}$ is a genetic null pseudo-point mutation that differs from a *Ubx* deficiency in certain heteroallelic combinations with regulatory mutations because of transvection (Weinzierl et al., 1987). Ubx^{130} is a null mutation caused by a chromosome rearrangement that breaks in the *Ubx* transcription unit; this allele does not produce detectable protein. The Ubx^+ duplications *Dp(3;1)68* and *Dp(3;3)P5* are described in Lewis (1978) and Lewis (1985). *Dp(DpP10)* is a tandem duplication of the Ubx^+ duplication *Dp(3;2)P10* referred to as *DpP10*(5) in Smolik-Utlaut (1990). The *Ubx-lacZ* fusion genes *35UZ*, *22UZ* and *5UZ* have been previously described (Irvine et al., 1991), *E1HZ* will be described elsewhere (S. J. and D. S. H., unpublished data). The experiments described in this work were repeated with at least two independent insertions of each reporter gene. The *hsp70-Ubx* fusion genes *HS:UbxIa*, *HS:UbxIVa* and *HS:UbxIVaFS* are described in Mann and Hogness (1990). Embryos were staged according to Campos-Ortega and Hartenstein (1985). Mutant embryos were identified by their altered patterns of expression or by using balancer chromosomes marked with *lacZ* fusion genes. We found the TM3*Sb hb-lacZ* chromosome to be the most useful (gift of M. van den Heuvel, constructed by G. Struhl). $Ubx^{9.22}$ embryos could be identified with UBX antibodies because the truncated UBX protein is detected in the cytoplasm. Mutant larvae were identified by using the third chromosome balancer TM6b marked with *Tb*. A reciprocal translocation between SM5 and TM6b (L14, gift of P. Ripoll) effectively enabled us to use *Tb* as a second chromosome marker.

Heat-shock induction

Embryos and larvae were heat shocked as described previously (Mann and Hogness, 1990).

Determination of expression patterns

UBX and β-galactosidase expression patterns were visualized as described previously (Irvine et al., 1991). RNA in situs were performed using ^{35}S-labelled RNA probes transcribed with SP6 polymerase from linearized templates. A mixed probe with homology to sequences in both 5′ and 3′ untranslated regions of *Ubx* mRNAs that are not included in the *HS:Ubx* genes was used. Hand-dissected imaginal discs were used for whole-mount in situ hybridization as described in Jorgensen and Garber (1987).

β-galactosidase-specific activity assays

The haltere and third leg discs of seven larvae were dissected out in BSB (40 mM NaCl, 55 mM KCl, 10 mM $MgSO_4$, 1 mM $CaCl_2$, 10 mM tricine, 20 mM glucose, 50 mM sucrose, pH 7.0) that had been chilled to 4°C, rinsed twice in 4°C Z buffer (0.1 M phos-

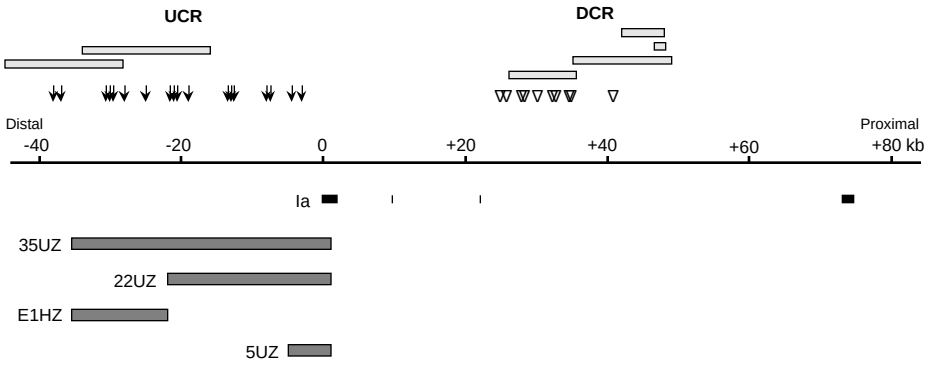

Fig. 1. Map of *Ubx*. The coordinates in kilobasepairs (kb) relative to the transcription start site (Kornfeld et al., 1989) and chromosomal orientation are marked. The locations of mutations in the UCR and DCR are shown above the coordinate line: lightly shaded bars indicate deletions, vertical arrows indicate chromosome rearrangement breakpoints, and arrowheads indicate transposable element insertions. For further details see Bender et al. (1983, 1985) and Peifer and Bender (1986).The black bars immediately below the coordinate line indicate the exons of UBX form Ia; form IVa lacks the two central microexons (Kornfeld et al., 1989).The darkly shaded bars at the bottom of the figure represent the *Ubx* DNA included in *Ubx-lacZ* fusion genes. *35UZ*, *22UZ*, and *5UZ* are described in Irvine et al. (1991). In *E1HZ* (S. J. and D. S. H., unpublished) the 13 kb *Stu*I-*Kpn*I fragment that extends from the distal end of *22UZ* to the distal end of *35UZ* is fused to *lacZ* via a minimal *hsp70* promoter that does not respond to heat shock (Hiromi and Gehring, 1987).

phate buffer, 10 mM KCl, 1 mM $MgSO_4$, 50 mM β-mercaptoethanol, pH 7.0) by transfer with a micropipet to fresh solution, and then transferred to 100 μL Z Buffer in a 1.5 mL plastic tube. Disc cells were then immediately lysed by sonication for 30 seconds in a cup sonicator and cell debris was pelleted by centrifugation for 2 minutes in a microfuge. β-galactosidase activity and protein concentration assays on the supernatant were performed as described previously (Irvine et al., 1991), with 60% of the extract used for the β-galactosidase assay and 40% for the protein assay.

RESULTS

As the positive autoregulation by *Ubx* that occurs in the embryonic visceral mesoderm has been previously described (Bienz and Tremml, 1988), we focus here on *Ubx* autoregulation in other tissues of the embryo and larva. These studies utilized two sets of transgenic fly lines. The first set harbor P element constructs in which *Ubx* regulatory sequences are fused to the *E. coli lacZ* gene. These express β-galactosidase in patterns similar to UBX. The *Ubx-lacZ* fusion gene *35UZ*, which includes the *Ubx* leader and 35 kb of DNA directly upstream of the *Ubx* transcription start site (Fig. 1), contains sequences sufficient to generate the pattern of expression regulated by the UCR throughout development (Irvine et al., 1991); β-galactosidase expression in *35UZ* flies is detected where UBX is expressed in wild type except in the thoracic region of the central nervous system and in PS5 of the imaginal discs. The second set of transgenic fly lines harbor constructs in which particular *Ubx* cDNAs are cloned downstream of a heat-shock promoter. These *HS:Ubx* fusion genes can be induced to express uniform and high levels of UBX throughout the fly; this expression transforms the identity of parasegments anterior to PS6 toward PS6 (Gonzalez et al., 1990; Mann and Hogness, 1990). The *HS:Ubx-Ia* and *HS:Ubx-IVa* lines express the form Ia and form IVa UBX proteins, respectively (Kornfeld et al., 1989; Mann and Hogness, 1990; Fig. 1). In parallel with experiments in which UBX was produced from the heat-shock promoter, a control line was induced to express a nonfunctional UBX truncated by a frame shift mutation (*HS:Ubx-IVaFS*; Mann and Hogness, 1990).

Ubx is not generally required to maintain its expression

Autoregulation can be examined using mutant alleles which make detectable but nonfunctional products. For example, the mutation $Ubx^{9.22}$ generates a truncated nonfunctional protein lacking the homeodomain, but is detectable with antibodies that recognize epitopes in the amino terminus of UBX (Weinzierl et al., 1987; Bienz and Tremml, 1988). The detection of this nonfunctional UBX protein in $Ubx^{9.22}$ mutant embryos suggests that UBX functions are not required to maintain *Ubx* expression. However, the situation is complicated because, while UBX is present in $Ubx^{9.22}$ embryos, it is detected at a lower level than in wild-type embryos. This could either reflect some positive autoregulation or result from a decreased stability of the truncated protein. Additionally, the lethality of *Ubx* mutations had precluded attempts to examine autoregulation late in development, and it was possible that a dependence of *Ubx* expression on UBX would not be apparent during embryogenesis.

To bypass the potential problem of instability of products generated from mutant alleles, we examined the requirements of UBX for *Ubx* expression by monitoring β-galactosidase expression from *35UZ* in *Ubx* mutant embryos. While β-galactosidase expression decreased in the visceral mesoderm, in all other tissues the level of expression actually increased (data not shown). This reporter gene was also used to examine autoregulation in the larval imaginal discs by using UCR mutations (pbx^1, pbx^2 and bxd^{100}) that eliminate detectable UBX in the posterior of T3 discs (where β-galactosidase is expressed in *35UZ*) but are viable at least through the end of pupal development. Again, no decrease in β-galactosidase expression was observed and instead an increase was detected (Fig. 2B cf. 2A). Thus, no evidence for positive autoregulation by *Ubx* outside the visceral mesoderm was obtained. Instead, *Ubx* appears to autoregulate negatively.

Overexpression of UBX from a heat-shock promoter represses *Ubx* expression

To examine further the effect of UBX on *Ubx* expression, *HS:Ubx-Ia* and *HS:Ubx-IVaFS* flies (Mann and Hogness,

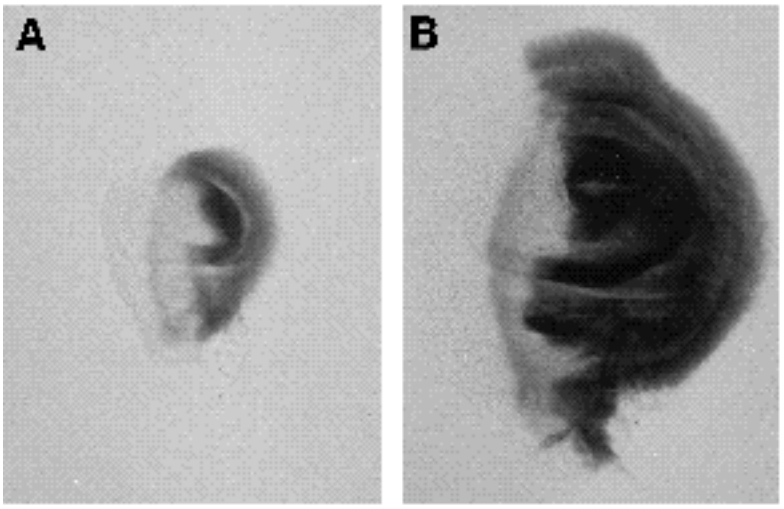

Fig. 2. *35UZ* expression in wild-type and *bxd* mutant haltere discs. Posterior is to the right. The levels of expression between these discs can be compared directly, as they were isolated from sibling progeny of a cross of *35UZ-3*; bxd^{100}/TM6b to *35UZ-3*; Ubx^{130}/TM6b that were dissected side by side. The discs were then fixed and stained with a β-galactosidase activity stain together on the same slide. (A) Haltere disc from bxd^{100} or Ubx^{130} heterozygous larva. (B) Haltere disc from bxd^{100}/Ubx^{130} mutant larva. β-galactosidase expression is increased relative to that in A. This mutation eliminates detectable UBX in all cells in the posterior of the haltere except those of the peripodial membrane (Botas et al., 1988). The increase in the size of the disc relative to A is largely a consequence of its transformation towards the wing disc. A similar increase in β-galactosidase expression was detected in pbx^1 and pbx^2 mutant T3 discs.

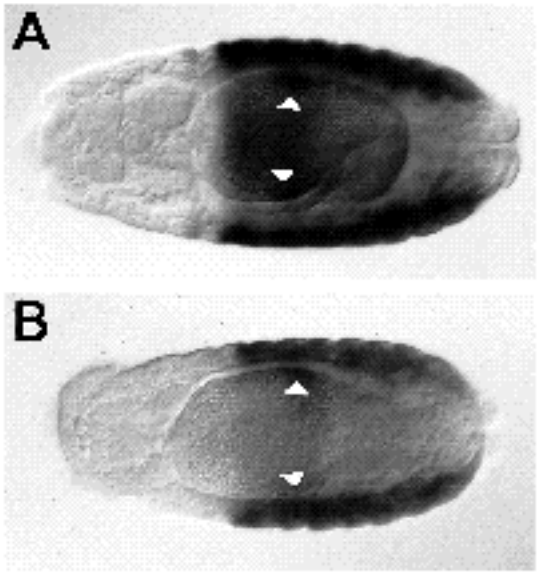

Fig. 3. Expression of *35UZ* in heat-shock-induced *HS:Ubx-Ia* and *HS:Ubx-IVaFS* embryos. Horizontal optical sections with anterior to the left and PS7 visceral mesoderm expression marked by the white arrowhead. These embryos were heat shocked for 40 minutes at 38°C when 3-6 hours old and fixed and stained side by side 9 hours later. (A) *35UZ-3*; *HS:Ubx-IVaFS* control embryo. The presence of the *HS:Ubx-IVaFS* gene does not affect *35UZ* expression (not shown). (B) *35UZ-3*; *HS:Ubx-Ia* embryo with expression repressed relative to that in A. Visceral mesoderm expression has not expanded outside of PS7 (visceral mesoderm expression in the lower half of the figure is partially out of the plane of focus).

1990), expressing the form Ia and truncated control proteins, respectively, were crossed to *35UZ* flies. Significant reduction of β-galactosidase expression was generated by heat shock of *HS:Ubx-Ia* flies compared to *HS:Ubx-IVaFS* flies processed in parallel. This decrease was detected in all embryonic tissues (Fig. 3B, cf. 3A) and in the third instar larval imaginal discs (Fig. 4B, cf. 4A). To confirm that the reporter gene was accurately reflecting the behavior of the endogenous *Ubx* gene, the expression of *Ubx* mRNA was monitored in similar experiments. A probe was constructed for in situ hybridization that would specifically detect the mRNA produced from the endogenous *Ubx* gene, but not the mRNA produced from the *HS:Ubx* genes, by virtue of its hybridization to sequences in the untranslated regions of *Ubx* mRNAs. A dramatic decrease in the expression of *Ubx* in imaginal discs was detected with this probe after heat shock of *HS:Ubx-Ia* larvae (Fig. 4D, cf. 4C). This repression of *Ubx* expression by UBX is consistent with the weak *Ubx* phenotypes occasionally detected in the halteres of *HS:Ubx-Ia* flies subjected to brief heat shocks during larval development (Fig. 4E; Mann and Hogness, 1990).

Interestingly, despite the requirement for UBX to maintain *Ubx* expression in PS7 of the visceral mesoderm, β-galactosidase expression was not induced in other parasegments of the visceral mesoderm in *35UZ*; *HS:Ubx-Ia* embryos (Fig. 3B). This could simply reflect the inability to produce UBX at the appropriate time or level to induce such expression. Alternatively, it could indicate that additional spatially restricted factors regulate *Ubx* visceral mesoderm expression, either by preventing *Ubx* expression outside of PS7 or by promoting it within PS7. In this case, the loss of β-galactosidase expression from *35UZ* in the PS7 visceral mesoderm of *Ubx* mutant embryos should be rescued by expression of UBX from the heat-shock promoter. However, heat shock of *35UZ*; *HS:Ubx-Ia* Ubx^- embryos did not restore visceral mesoderm expression (not shown); this presumably indicates that activation of *Ubx* expression by UBX in the visceral mesoderm is sensitive to the timing or level of UBX expression. Although a variety of heat-shock regimens were employed, it would be extremely difficult to mimic precisely the level and timing of normal *Ubx* expression with the heat-shock promoter.

To determine whether different UBX protein forms differ in their ability to autoregulate, the effect of overexpression of UBX form IVa from the *HS:Ubx-IVa* line on *35UZ* expression was examined. Form Ia is normally expressed in the epidermis and mesoderm, but not in the central nervous system, while form IVa, which lacks the two central microexons and consequently 34 amino acids from the central part of the protein, is normally expressed in the central nervous system but not in epidermal or mesodermal tissues (Lopez and Hogness, 1991). Nonetheless, in both embryos

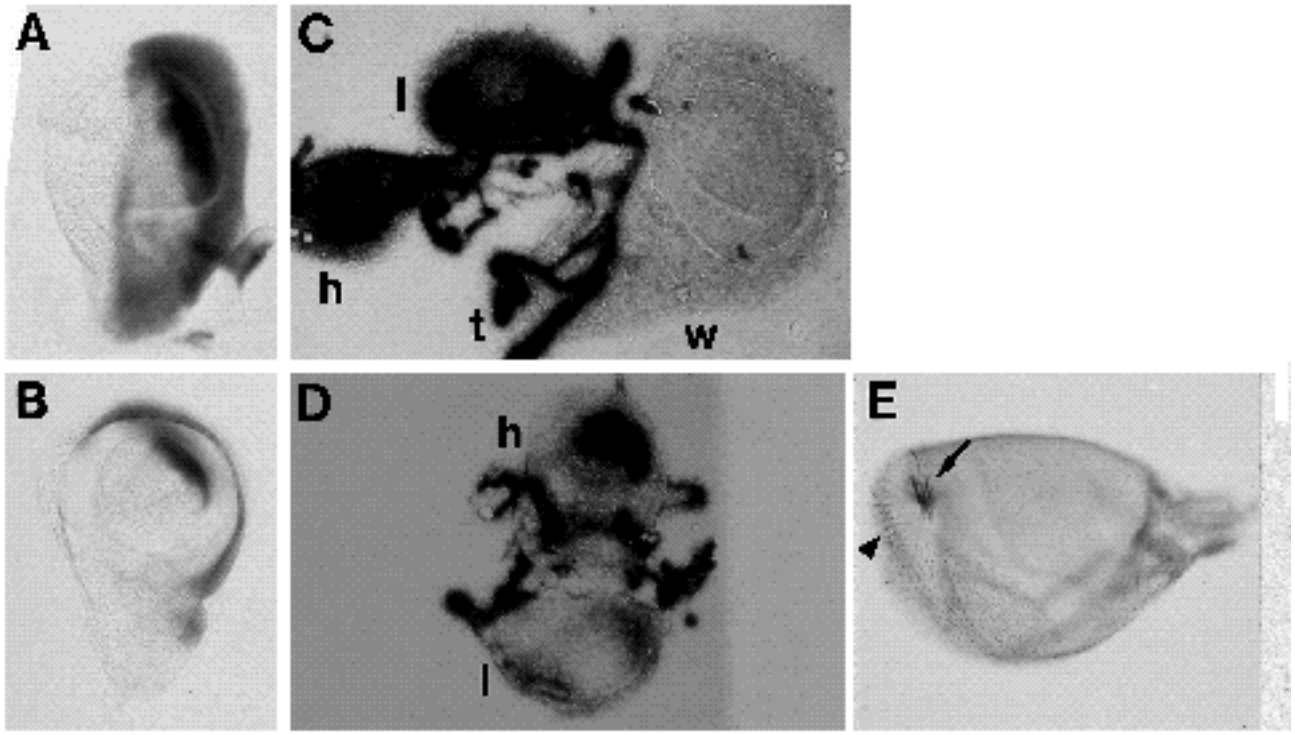

Fig. 4. Repression of *Ubx* expression in imaginal cells by induction of *HS:Ubx-Ia* expression. Control discs (A) and (C) were treated identically to experimental discs (B) and (D), respectively, by dissecting larvae that had been heat shocked for 30 minutes at 37°C 72, 48 and 24 hours previously, and then fixing and staining the dissected discs together on the same slide. (A) *35UZ-3*; *HS:Ubx-IVaFS* haltere disc with β-galactosidase expression in the posterior compartment. (B) *35UZ-3; HSUbx-Ia* haltere disc with expression repressed compared to that in A. (C) *HS:UbxIVaFS* wing (w), haltere (h), and third leg (l) discs with wild-type *Ubx* mRNA expression. The dark lines are trachea (t), to which the probe sticks non-specifically. (D) *HS:Ubx-Ia* haltere and third leg discs with expression repressed compared to that in C. (E) Haltere from *HS:Ubx-Ia* fly heat shocked for 10 minutes at 36°C during second instar. This haltere has a weak *Ubx* phenotype, manifested as an increase in size and the appearance of wing trichomes (arrowhead) and wing margin bristles (arrow). For comparison to a wild-type haltere see Fig. 5B.

and larvae, form IVa was also able to repress *35UZ* expression (data not shown).

Ubx negatively autoregulates at wild-type levels of UBX

Having demonstrated repression of the *Ubx* promoter by UBX, we sought to determine the biological relevance of this phenomenon. One particular concern was that the repression observed in *HS:Ubx-Ia* and *HS:Ubx-IVa* lines could be an artifact of abnormally high levels of UBX. In addition, while *35UZ* staining in *Ubx* mutants indicated that negative autoregulation was occurring at wild-type levels of UBX, it was difficult to quantify the magnitude of the effect with these in situ stains. Therefore, a sensitive enzymatic assay was employed to quantify changes in the level of expression from the *Ubx* promoter in response to discrete changes in the level of UBX. Flies homozygous for *35UZ* but carrying different doses of Ubx^{+}, as a consequence of chromosomal duplications, chromosomal deficiencies or *Ubx* mutations, were generated. As the copy number of *35UZ* was kept constant, and the pattern of β-galactosidase expression was unchanged (not shown), changes in β-galactosidase expression should reflect changes in the level of expression from the *Ubx* promoter. Since it was not possible in many cases to generate homogeneous populations of embryos of the desired genotypes, the analysis was restricted to imaginal tissue. An extract of third leg and haltere (T3) imaginal discs was prepared from third instar larvae of the appropriate genotype. This was assayed both for β-galactosidase activity and total protein concentration to determine the β-galactosidase-specific activity of the extract; normalizing β-galactosidase activity to protein concentration controls for differences in the number of cells. Although the *35UZ* reporter line expresses β-galactosidase only in the posterior compartment of T3, the results will likely apply to the anterior compartment as well, because *Ubx* mRNA levels were repressed similarly in both compartments by UBX (Fig. 4D).

As shown in Table 1, the level of β-galactosidase expression from the reporter increased when the dose of Ubx^{+} was decreased, whether the decrease was caused by a deletion of the entire *Ubx* gene (*DfUbx109*), a mutation that encodes a truncated protein ($Ubx^{9.22}$), or mutations in regulatory sequences that eliminate *Ubx* expression ($bx^{3}bxd^{106}$). Conversely, the level of β-galactosidase expression decreased when the dose of Ubx^{+} was increased with the duplications *DpP5* or *Dp*(*DpP10*). Two different *35UZ* lines, *35UZ-1* and *35UZ-3*, containing independent insertions of the reporter gene, were assayed to control for chromosomal position effects. While the wild-type expression levels differ between the lines, the relative changes in β-galactosidase expression with changes in

Table 1. Sensitivity of *Ubx-lacZ* expression to *Ubx*$^+$ dose

Genotype	Copies *Ubx*$^+$	β-galactosidase specific activity (units/mg)	% Wild-type expression
35UZ-3			
bx^3*bxd*^{106}/*DfUbx109*	0*	200±56.3	182
bx^3*bxd*^{106}/+	1*	150±11.8	136
DfUbx109/+	1	146±8.1	133
Ubx$^{9.22}$/+	1	156±11.9	142
+	2	110±18.2	100
DpP5/*abdA*^{M1}	3	94.5±7.3	85.9
Dp(*DpP10*)/+	4	82.4±11.5	74.9
35UZ-1			
DfUbx109/+	1	242±60.2	143
Ubx$^{9.22}$/+	1	241±25.7	143
+	2	169±24.2	100
DpP5/+	3	139±30.0	82.2
DpP5	4	110±20.9	65.1
35UZ-3; 35UZ-1			
+	2	277±40.6	99.3†

β-galactosidase-specific activities are mean values plus or minus one standard deviation and were calculated from assays of four to five independently prepared extracts.

*bx^3bxd^{106} hemizygotes have an almost but not quite complete transformation of the haltere disc to wing disc; some undetectably low level of UBX may therefore be present in the discs.

†Wild-type expression in this case is defined as the sum of the β-galactosidase-specific activities detected in the *35UZ-3* and *35UZ-1* lines in wild type.

Ubx$^+$ dose were similar. Two observations rule out the hypothetical possibility that the changes in level of expression could be caused by titration of positive regulatory factors with the change in copy number of *Ubx* regulatory sequences, rather than by autoregulation. First, *DfUbx109*, which removes the *Ubx* control regions, and *Ubx*$^{9.22}$, which does not, resulted in similar increases in β-galactosidase expression, and second, when the number of UCR copies was increased independently of *Ubx*$^+$ by generating a strain with both reporter genes (*35UZ-3*; *35UZ-1*), the expression level was the sum of that in the lines with individual reporter genes.

The detection of both repression with increased *Ubx*$^+$ dose and derepression with decreased *Ubx*$^+$ dose demonstrates that *Ubx* negatively autoregulates at wild-type levels of UBX. An important consequence of this negative autoregulation is that, like any negative feedback loop, it acts to stabilize the level of *Ubx* expression. For example, if the copy number of *Ubx*$^+$ loci is increased from two to four, the UBX protein level increases by less than two fold because of the increased repression that occurs as the amount of UBX increases. If the copy number is decreased from two to one, UBX levels decrease by less than half because of the derepression that occurs as the amount of UBX decreases. This stabilization may be an important function of negative autoregulation. Assuming that the response of *35UZ* quantitatively as well as qualitatively mimics that of *Ubx*, the relative changes in UBX levels can be calculated by factoring together the change in level of expression from the promoter and the change in the number of chromosomal copies of *Ubx*$^+$ (Fig. 5 legend).

Importantly, the slight changes in *Ubx*$^+$ dose that modify the level of expression have significant phenotypic consequences. *Ubx* is haploinsufficient (Lewis, 1963); flies with only one copy of *Ubx*$^+$ have a weak transformation of haltere toward wing, manifested as an enlargement of the haltere and the appearance of one or more bristles characteristic of the wing on the haltere (Fig. 5A). Recently, it has also been shown that increases in *Ubx*$^+$ dose cause partial transformations of thorax toward abdomen, including the

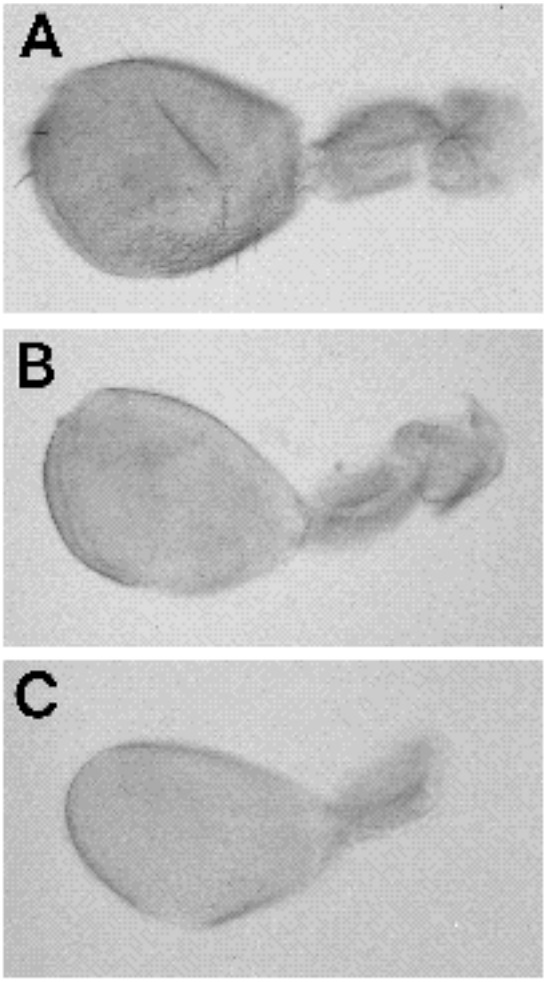

Fig. 5. Correlation of haltere size with *Ubx*$^+$ dose and estimated UBX protein levels. The genotypes, number of copies of the *Ubx*$^+$ locus, and level of UBX protein for the halteres shown in panels A, B, and C are given below.

	Genotype	Copies *Ubx*$^+$	Level UBX
(A)	*DfUbx109*/+	1	0.70
(B)	*Dp68*/+; *DfUbx109*/+	2	1.0
(C)	*Dp68*/+; *DpP5*/+	4	1.4

To minimize effects of culture conditions and genetic background these halteres were taken from sibling female offspring from an outcross of *Dp68*/FM7c; *DpP5*/*DfUbx109* to wild type. The variations in relative haltere size are highly reproducible. UBX levels were estimated by factoring together the number of copies of *Ubx*$^+$ with the mean level of expression relative to wild type from Table 1. For example, with four copies of *Ubx*$^+$ the level of expression from the promoter averaged 70% of that in wild type [(74.9% + 65.1%)/2], and the number of copies of the *Ubx* gene is twice that in wild type, so the relative level of UBX is estimated to be 0.70 × 2 = 1.4.

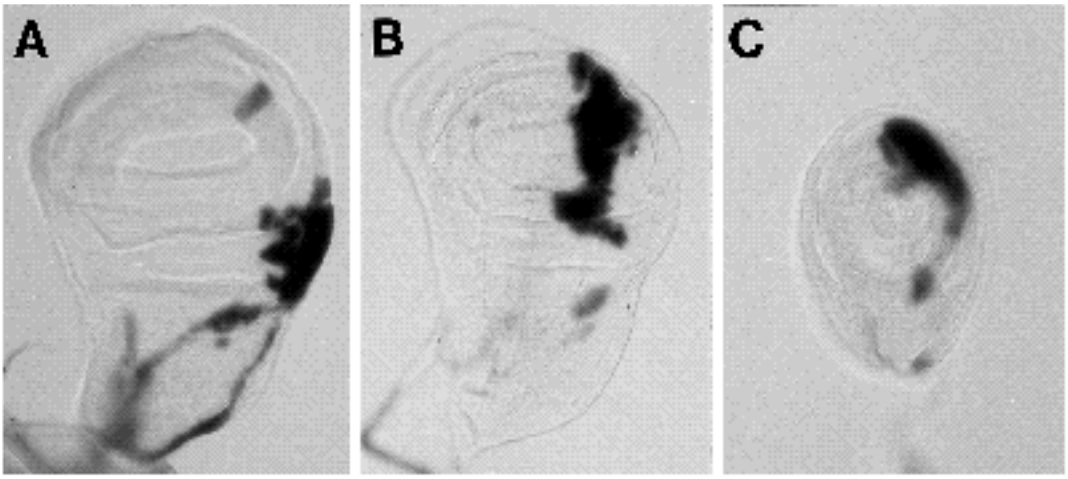

Fig. 6. Expression of *35UZ* in T2 discs in the DCR mutant abx^2. Posterior is to the right; β-galactosidase expression was detected with an activity stain. (A) Wing disc.(B) Wing disc. (C) Second leg disc. Expression is variable but restricted to the posterior compartment. β-galactosidase expression is not normally detected in *35UZ* T2 discs (Irvine et al., 1991). Similar patches of β-galactosidase expression were also detected in the DCR mutants abx^1, bx^3, bx^{83ka}, and bx^{83kb}, all of which have homeotic transformations in T2p. By contrast, no significant β-galactosidase expression was detected in bx^1 or $bx^{34e-prv}$, which lack T2p mutant phenotypes. Rare patches of β-galactosidase expression were detected in $+/Ubx^-$ discs, indicative of some haploinsufficiency for β-galactosidase repression.

transformation of larval ventral denticles and reduction or elimination of the haltere (Smolik-Utlaut, 1990). Duplications of Ubx^+ increase the level but do not affect the pattern of UBX expression (Smolik-Utlaut, 1990; data not shown). We have reproducibly observed the reduction in haltere size, using three different Ubx^+ duplications (*Dp68*, *DpP5*, $DpP10_2$), in flies with as few as four copies of Ubx^+ (Fig. 5C). Fig. 5 illustrates the correlations between haltere size and estimated UBX protein levels. Changes in size were also visible in the haltere imaginal disc (data not shown). The sensitivity of haltere phenotype to UBX protein levels suggests that negative feedback stabilization could be functionally significant.

Control of the pattern of *Ubx* expression by negative autoregulation

DCR mutations

The data presented above indicate that negative autoregulation affects the level of UBX expression and thereby the phenotype of segments whose identity *Ubx* specifies. Surprisingly, negative autoregulation by *Ubx* also controls the pattern of UBX expression. A shift in the spatial expression pattern of UBX occurs during development. In the embryo, UBX is expressed in T2p and functions there to prevent transformation of T2p toward T1p in both the larval cuticle and imaginal disc precursor cells (Lewis, 1963; Morata and Kerridge, 1981; Lewis, 1982; Miñana and García-Bellido, 1982; Hayes et al., 1984). Later in development, however, UBX expression in T2p is reduced to very weak expression in the leg disc and in the peripodial membrane of the wing disc (White and Wilcox, 1984; Beachy et al., 1985). Further, clonal analysis has indicated that *Ubx* is no longer required in T2p after mid-embryogenesis (Morata and Kerridge, 1981). Similarly, *35UZ* is expressed in epidermal cells of T2p in the embryo, but is not expressed in T2 in the imaginal discs (Irvine et al., 1991). In fact, the *Contrabithorax* (*Cbx*) mutations demonstrate that UBX expression must be repressed in T2p imaginal cells. These mutations are dominant gain-of-function *Ubx* alleles that express UBX aberrantly in T2 imaginal discs and, consequently, transform them toward T3 (Lewis, 1955, 1982; Cabrera et al., 1985; White and Akam, 1985). Thus, *Ubx* is expressed and required early in development in T2p, but later in development this expression must be repressed. We show below that at least one factor involved in this repression is UBX.

The role of UBX in repression of *Ubx* expression was indicated by the detection of β-galactosidase expression from *35UZ* in the posterior of T2 discs in DCR mutants (Fig. 6). That is, mutations in *Ubx* regulatory sequences that cause a loss of UBX expression, activate, in *trans*, expression from the *Ubx* promoter. The detection of β-galactosidase expression in DCR mutant discs was highly variable. Not all T2 discs in a population expressed β-galactosidase and in those that did the number and position of cells expressing β-galactosidase varied (Fig. 6A cf. 6B). The position and timing of UBX and β-galactosidase expression argue that the mechanism of activation of *35UZ* expression in DCR mutant discs is a partial loss of UBX expression in the early embryo and consequent inability to repress the embryonic T2p expression of *35UZ*. First, β-galactosidase expression in mutant discs was always restricted to the posterior compartment, the region of the segment in which UBX and β-galactosidase are expressed in the embryo. Second, genetic analysis of DCR mutations has indicated that most cause a variable loss of UBX during embryogenesis within the disc cells of T2p. They cause a variable and incomplete transformation of T2p toward T1p (Casanova et al., 1985; Peifer and Bender, 1986), and clonal analysis has shown that this transformation is effected during embryonic development (Casanova et al., 1985). Finally, the hypothesis that β-galactosidase expression in T2p discs of DCR mutants results from the loss of UBX

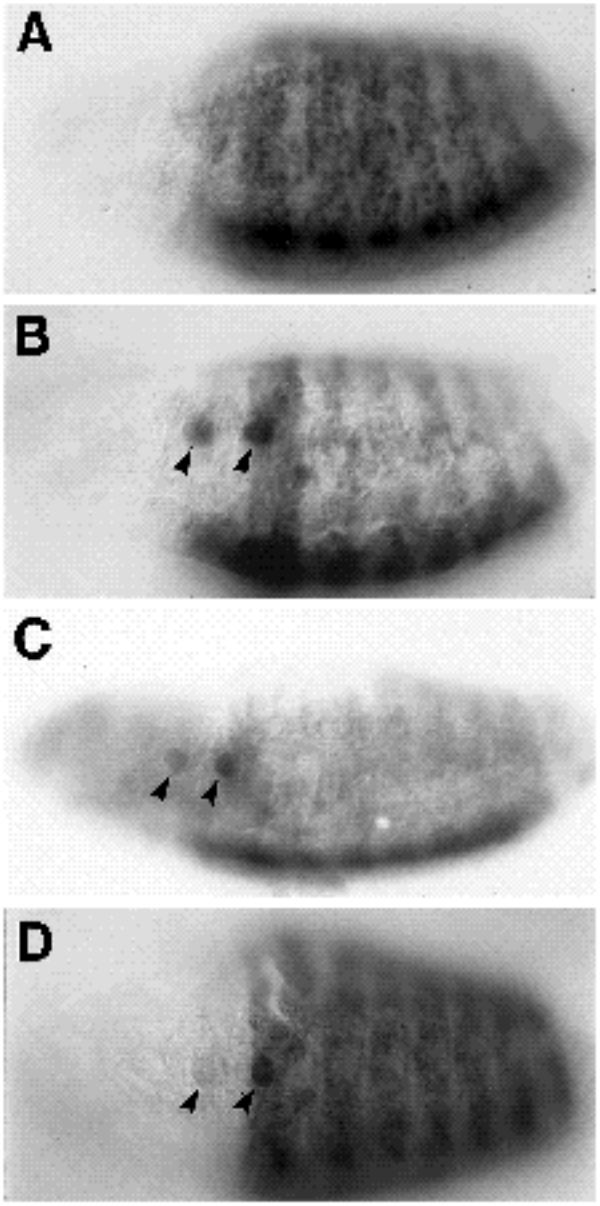

Fig. 7. Expression of UBX and β-galactosidase in stage 14-15 *Ubx* mutant embryos. Anterior is to the left and ventral is down. These are parasagittal optical sections focussed just below the embryo surface. The cells clusters identified by increased expression in *Ubx* mutants are indicated by arrowheads.(A) UBX expression in wild type. (B) UBX expression in $Ubx^{9.22}$. (C) UBX expression in Ubx^{195}. (D) β-galactosidase expression in *35UZ-3*; Ubx^{130}.

expression predicts that UBX will not be detected in these discs. This was confirmed by monitoring expression of both UBX and β-galactosidase in the same disc by immunofluorescence microscopy (data not shown). Some DCR mutations (e. g. $bx^{34e-prv}$) appear not to affect early UBX expression in T2p, as they lack a mutant phenotype in this segment (Casanova et al., 1985; Peifer and Bender, 1986). While UBX has been detected in $bx^{34e-prv}$ larval T2 discs (Little et al., 1990), β-galactosidase expression from *35UZ* was not detected in discs of this genotype, consistent with the lack of phenotypic effect. Although the reason for the particular effects of this allele on *Ubx* expression is uncertain, it is noteworthy that Little et al. (1990) suggested that the detection of UBX in $bx^{34e-prv}$ T2 discs could be an indication of UBX-dependent repression of *Ubx* expression in T2.

Ubx null mutations

While the activation of β-galactosidase expression in DCR mutations indicated that negative autoregulation was controlling the pattern of *Ubx* expression, we sought to strengthen this conclusion by examining *Ubx* null mutations. Interestingly, close examination of late $Ubx^{9.22}$ embryos revealed clusters of cells in PS5 and 6 that actually had increased UBX expression (Fig. 7B cf. 7A). This strong expression contrasts with that in the rest of the embryo, where UBX expression appeared to be lower than in wild-type, presumably due to instability of the truncated protein encoded by $Ubx^{9.22}$. The cell clusters with increased UBX expression could first be detected in $Ubx^{9.22}$ embryos soon after germ band retraction (stage 13; data not shown). Identical cell clusters were also detected in Ubx^{195} (Fig. 7C), which in the epidermis also produces a truncated protein (Weinzierl et al., 1987; Lopez and Hogness, 1991). Finally, β-galactosidase expression from *35UZ* was detected in these cell clusters in both $Ubx^{9.22}$ and Ubx^{130} mutant embryos (Fig. 7D). The observation that the ratio of expression in the PS5 cluster to that in the PS6 cluster in *Ubx* mutants was noticeably less for *35UZ* than for $Ubx^{9.22}$ and Ubx^{195} (Fig. 7D cf. 7B and C) may reflect the absence of the DCR from *35UZ* versus its presence in both *Ubx* mutations.

The strong expression detected in these cells in *Ubx* mutants indicates that during wild-type development they require UBX to repress *Ubx* transcription. Although *Ubx* expression is also repressed in other embryonic cells by UBX, the effect appears to be much greater in the prominent cell clusters. By differentially repressing *Ubx* expression in different cells, UBX effectively alters the pattern of its expression. The cell clusters are also transformed to a more anterior identity as a result of *Ubx* mutation. Their location within the segment and association with the tracheal trunk, together with the distinctive morphology of the cells within the cluster, indicate that they are ectopic anterior spiracles (Campos-Ortega and Hartenstein, 1985; Bate and Martinez-Arias, 1991). The anterior spiracles normally form only in PS4.However, in *Ubx* mutant larvae, two additional pairs of spiracles are formed in PS5 and 6 (Lewis, 1978; Bate and Martinez-Arias, 1991). The identification of the cell clusters as ectopic anterior spiracles suggests that in wild-type embryos these cells would form part of the tracheal system. It is also possible that the cell clusters include some imaginal cells, as the humeral (dorsal T1) disc develops in close association with the anterior spiracle (Madhavan and Schneiderman, 1977), and early clones of Ubx^{-} cells in the wing (dorsal T2) and haltere (dorsal T3) discs are partially transformed to humerus (Lewis, 1963, 1982; Miñana and García-Bellido, 1982).

Duplication of autoregulatory elements within the UCR

Fusion genes with smaller fragments of the UCR fused to

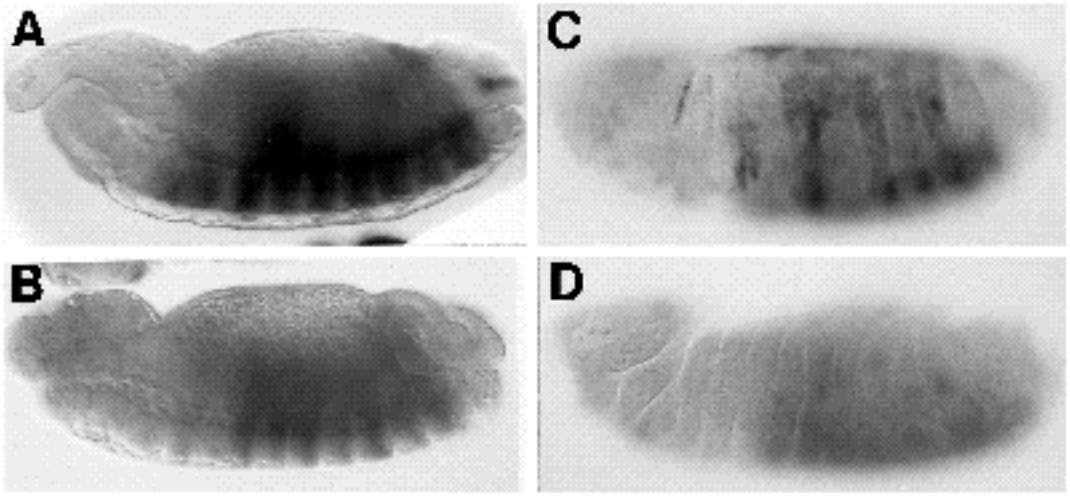

Fig. 8. Expression of *22UZ* and *E1HZ* in heat-shock-induced *HS:Ubx-Ia* and *HS:Ubx-IVaFS* embryos. Anterior is to the left and ventral is down. (A,B) Sagittal optical sections; (C,D) Focussed on the lateral surface of the embryo. These embryos were heat shocked for 40 minutes at 38°C when 3-6 hours old and fixed and stained side-by-side 7 hours later. (A) *22UZ-3*; *HS:Ubx-IVaFS* control embryo. (B) *22UZ-3*; *HS:Ubx-Ia* embryo with expression repressed relative to that in A. (C) *E1HZ-1*; *HS:Ubx-IVaFS* control embryo. (D) *E1HZ-1*; *HS:Ubx-Ia* embryo with expression repressed relative to that in C.

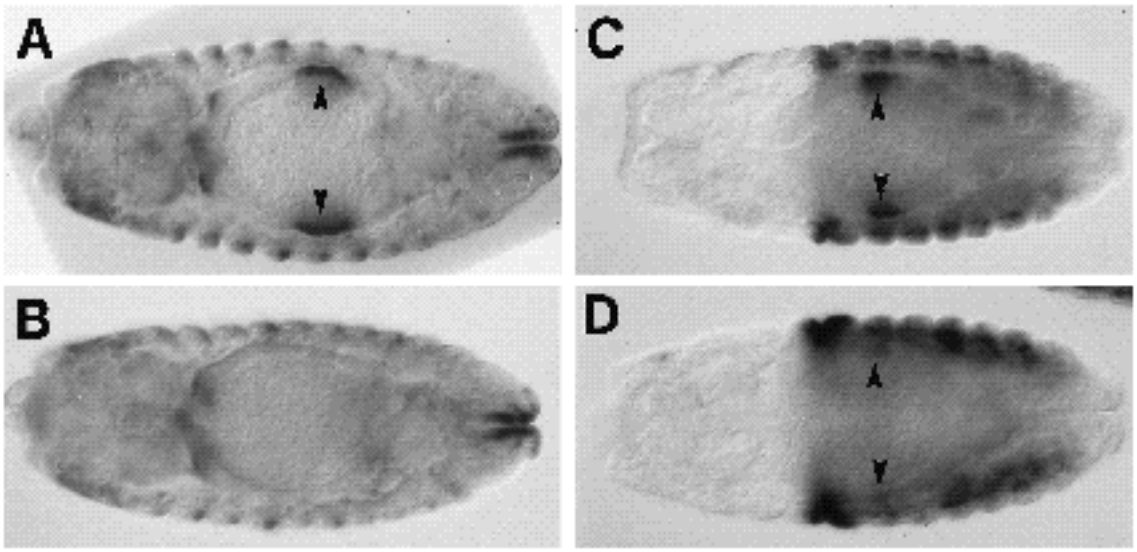

Fig. 9. Expression of *5UZ* and *E1HZ* in wild-type and *Ubx* mutant embryos. These are horizontal optical sections with anterior to the left. β-galactosidase expression in the visceral mesoderm is indicated by arrowheads. (A) *5UZ-5* embryo. (B) *5UZ-5*; Ubx^{130} embryo; no visceral mesoderm expression can be detected. (C) *E1HZ-1* embryo. (D) *E1HZ-1*; Ubx^{130} embryo.Visceral mesoderm expression is much reduced relative to that in C, while expression in other tissues is increased.

lacZ confer some of the aspects of *Ubx* expression detected in *35UZ* (Bienz et al., 1988; Irvine et al., 1991; Müller and Bienz, 1991; S. J. and D. S. H., unpublished data). Two such *Ubx-lacZ* fusion genes were used to localize regulatory sequences within the UCR that can confer UBX-dependent repression: *22UZ*, which contains the proximal 22 kb of the UCR and the *Ubx* leader, and *E1HZ*, which contains the complementary distal 13 kb of the UCR DNA included in *35UZ* (Fig. 1). β-galactosidase expression from both of these genes was, like *35UZ* expression, repressed by heat-shock-induced expression of UBX in *HS:Ubx-Ia* embryos (Fig. 8B cf. A; 8D cf. C). Additionally, derepression of β-galactosidase expression from both of these *Ubx-lacZ* genes was detected when expression in wild-type embryos was compared to that in Ubx^{-} embryos (Fig. 9D cf. C; data not shown). Although future work will be required to narrow down the repression elements in these constructs, these results establish that there are at least two distinct regulatory elements within the UCR that can confer UBX-dependent repression.

Similar attempts were made to localize sequence elements through which the positive autoregulation in the visceral mesoderm occurs. The expression of each of the *Ubx-lacZ* fusion genes depicted in Fig. 1 was examined in *Ubx* mutant embryos; in each case β-galactosidase expression decreased dramatically in the visceral mesoderm. DNA

sequences between 1.78 and 3.15 kb upstream of the *Ubx* transcription start site confer UBX-dependent visceral mesoderm expression on a heterologous promoter (Müller et al., 1989).This region presumably accounts for the UBX-dependent visceral mesoderm expression of *5UZ* (Fig. 9B cf. A). In addition, however, another UBX-dependent visceral mesoderm element was detected within the upstream sequences included in *E1HZ* (Fig. 9D cf. C). Thus there are also at least two distinct sequence elements within the UCR that can confer UBX-dependent activation in the visceral mesoderm. Further dissection of these elements should reveal whether the positive and negative autoregulatory sequence elements coincide or are distinct.

Interestingly, although the visceral mesoderm expression of *5UZ* was absolutely dependent on UBX (Fig. 9B), some faint visceral mesoderm expression could usually be detected in *35UZ* and *E1HZ Ubx* mutant embryos (Fig. 9D; data not shown). This is consistent with the observation that weak visceral mesoderm expression of UBX was detected in $Ubx^{9.22}$ and Ubx^{195} embryos (Bienz and Tremml, 1988; data not shown). This weak visceral mesoderm expression appeared to be stronger in embryos that have just completed germ band retraction than in older embryos. This suggests that the distal sequences of the UCR contain a UBX-independent regulatory element that could initiate *Ubx* visceral mesoderm expression, while both promoter proximal and distal sequences of the UCR contain UBX positive autoregulatory elements that enhance and maintain this expression.

DISCUSSION

Ubx negatively autoregulates

Previously described examples of autoregulation by developmental control genes in *Drosophila* have generally involved positive autoregulation (Hiromi and Gehring, 1987; Bienz and Tremml, 1988; Frasch et al., 1988; Kuziora and McGinnis, 1988; Heemskerk et al., 1991), although *labial* not only positively autoregulates, but also appears to negatively autoregulate in some imaginal cells (Chouinard and Kaufman, 1991). Positive autoregulation can maintain the expression of a gene, allowing continued expression to become independent of those factors required for the initiation of expression. Additionally, if a threshold protein level for autoregulation exists, it can transform graded levels of expression to discrete on-off states, and may participate in this way in sharpening the stripes of expression of the segmentation genes *even-skipped* and *fushi tarazu* (Frasch et al., 1988; Lawrence and Johnston, 1989). Although *Ubx* does positively autoregulate in the visceral mesoderm, only negative autoregulation was detected in other tissues. Importantly, negative autoregulation was detected whether UBX levels were increased above or decreased below the wild-type level, demonstrating that it occurs at wild-type levels of UBX. Such negative autoregulation can stabilize expression levels by counteracting increases or decreases in expression; indeed, we think it likely that this is a function of *Ubx* negative autoregulation. Alternatively, negative autoregulation can decrease or eliminate expression, as occurs in the tracheal and imaginal cells that require UBX to repress *Ubx* transcription. These different consequences of autoregulation presumably result from differences among the *Ubx*-regulatory factors present in different cell types. The large size of the *Ubx* transcription unit (Kornfeld et al., 1989) may also be important in enabling UBX to eliminate its own expression in some cells, as this affords a one hour lag between an effect on transcription initiation and a change in UBX protein levels (Kornfeld et al., 1989; Irvine et al., 1991). We consider below further aspects of the function and mechanism of *Ubx* negative autoregulation.

PS 6 cells maintain a memory of their metameric position

The absence of positive autoregulation by *Ubx* has profound implications for the maintenance of *Ubx* expression, and consequently, of segmental identity. Although *Ubx* is the only homeotic selector gene that specifies PS6 identity and is required for all known aspects of PS6 development, Fig. 2B shows that PS6-specific expression of the *Ubx* promoter does not require Ubx^+ function. Thus, in this respect PS6 cells maintain a functional memory of their metameric position that is independent of their developmental fate.

Ubx expression is initiated in the embryo by the action of segmentation genes that are only transiently expressed (reviewed in Akam, 1987; Ingham, 1988). What then maintains *Ubx* expression? The positive (*Regulator of bithorax* or *trithorax*) and negative (*Polycomb*) classes of global homeotic regulatory genes (Duncan and Lewis, 1982; reviewed in Paro, 1990) are known to be required for the maintenance, but not for the initiation, of correct homeotic gene expression patterns (Struhl and Akam, 1985). The demonstration that *Ubx* does not positively autoregulate outside of the visceral mesoderm suggests that these genes are not only necessary to maintain homeotic gene expression, but that they may be sufficient for it. These genes are thought to act as a cellular memory by maintaining patterns of gene expression through some influence on chromosome structure (Paro, 1990; Paro and Hogness, 1991).

Negative autoregulation and homeotic gene expression levels

Negative autoregulation occurs at wild-type levels of UBX (Table 1). Consequently, it acts to stabilize UBX levels. Two observations, the patterned and reproducible heterogeneity between UBX levels of different cells, and the sensitivity of phenotypes to Ubx^+ dose, argue that levels of UBX protein are critical during development. Indeed, the initial observations of heterogeneity in UBX levels between different cells, both within and between parasegments, led immediately to the suggestion that different levels of expression could specify different fates (White and Wilcox, 1985a). The larval ventral denticle and adult haltere phenotypes that are observed with changes in Ubx^+ dose confirm this suggestion (Lewis, 1963; Smolik-Utlaut, 1990). Notably, the detection of these phenotypes despite the counteracting effect of negative autoregulation suggests that they are even more sensitive to UBX levels than was formerly realized. This leads us to argue that a mechanism to stabi-

lize UBX levels against any perturbations that might occur during development could be functionally important.

Interestingly, other homeotic and homeobox-containing genes also use differences in levels of expression to specify different fates. Perhaps the best example is the homeobox-containing gene *bicoid*. *bicoid* protein, which has been described as a classic morphogen, forms a concentration gradient along the anterior-posterior axis of the early *Drosophila* embryo, and can activate expression of different target genes at different positions along this axis depending upon their *cis* regulatory sequences (Driever et al., 1989; Struhl et al., 1989). Other homeotic selector genes besides *Ubx* also have heterogeneous levels of expression and are dosage sensitive, and so apparently specify different fates with different levels of expression. *Abdominal B* (*AbdB*) and *Sex combs reduced* have haploinsufficient epidermal identity transformations (Kaufman et al., 1980; Sánchez-Herrero et al., 1985), while *abdominal A* has haploinsufficient CNS identity transformations (Ghysen et al., 1985). Further, the gradient of *AbdB* expression from PS10 to 13 (Celniker et al., 1989), together with the correlating adult phenotypes and effects on *AbdB* expression of *cis* regulatory (*iab*) mutations (Celniker et al., 1990; Boulet et al., 1991; Sánchez-Herrero, 1991), suggest that the mechanism by which *AbdB* specifies unique identities for each of these parasegments is through differences in the level of its expression. By analogy to *Ubx* then, we suggest that other homeotic genes might employ a negative autoregulation mechanism to stabilize levels of expression.

Temporal patterning of *Ubx* expression by negative autoregulation

Two cases in which negative autoregulation modulates the pattern of UBX expression were observed. By altering patterns of UBX expression, negative autoregulation contributes to the ability of *Ubx* to participate in specifying distinct segmental identities. First, β-galactosidase expression was detected from *35UZ* in DCR mutant T2p disc cells, revealing that UBX normally represses expression from the *Ubx* promoter in these cells (Fig. 6). Early UBX expression in T2p is required to prevent transformation toward T1p, however, the *Cbx* alleles demonstrate that later UBX expression must be repressed to prevent transformation of T2p toward T3p. The unique T2 identity thus requires a particular temporal pattern of UBX expression, a pattern that is is effected by negative autoregulation. Once repressed, the absence of UBX expression in T2p could be maintained by other *trans* regulatory factors. Partial loss of *Polycomb* activity, for example, allows some UBX expression in the wing disc, and consequently transforms it towards haltere (Duncan and Lewis, 1982; Cabrera et al., 1985). Intriguingly, *labial* also negatively regulates its own expression in some imaginal cells (Chouinard and Kaufman, 1991), suggesting that negative autoregulation may be a common mechanism for the modulation of homeotic gene expression patterns.

In the second case, strong expression of both UBX and β-galactosidase was detected in cell clusters in PS5 and 6 of *Ubx* mutant embryos (Fig. 7). The loss of Ubx^+ function has two distinct effects on these cells. First, expression from the *Ubx* promoter is not repressed. Second, the cells are transformed into ectopic anterior spiracles. This latter effect implies that in wild-type embryos the cells of the clusters would contribute to tracheal structures. By analogy to the modulation of UBX expression in T2p imaginal cells, we suggest that development of segmentally appropriate tracheal structures in PS5 and 6 requires a particular temporal profile of UBX expression in these cells. That is, after UBX performs early functions in tracheal development (repression of anterior spiracle formation), its expression must be repressed. In the thorax, but not in the abdomen, the tracheal system must make attachments to imaginal discs (Madhavan and Schneiderman, 1977), and a number of other segment-specific tracheal structures have also been described (Rühle, 1932). However, the actual consequences of failure to repress expression of a functional UBX protein in the cell clusters remain to be determined.

Is *Ubx* autoregulation direct or indirect?

UBX is a DNA-binding transcriptional regulatory protein, and so could autoregulate directly, by binding to *Ubx* control regions. Alternatively, autoregulation could be indirect, mediated by the regulation of intermediate genes that in turn regulate *Ubx* expression. Previous studies identified UBX-binding sites near the *Ubx* transcription start site (Beachy et al., 1988) and further studies using an immunoprecipitation assay have identified other regions of *Ubx* DNA that are bound with high affinity by UBX (S. J., J. B. and D. S. H., unpublished data), including fragments within the regions of the UCR that confer autoregulation. UBX may also bind to *Ubx* DNA in vivo. When UBX is expressed from the heat-shock promoter in the larval salivary glands, it binds to a number of polytene chromosome bands, including 89E (J. B. and D. S. H., unpublished data), the cytological location of *Ubx* (Lewis, 1963). While the identification of UBX-binding sites within *Ubx* DNA must be interpreted with caution, as other homeodomain proteins could also bind to these sites (reviewed in Scott et al., 1989), their existence nonetheless argues in favor of the possibility of direct autoregulation. Furthermore, the identification of these sites provides the means for testing that argument.

Although UBX acts as a positive transcription factor in vitro (Johnson and Krasnow, 1990), it could still act directly as a repressor of *Ubx* transcription in vivo, by, for example, competing for DNA-binding with a more potent transcriptional activator, or by its association with or modification by other regulatory factors. Indeed, UBX has been observed to have either positive or negative effects on the transcription of two different promoters in a cultured *Drosophila* cell line, and both of these effects may be direct (Krasnow et al., 1989). In these experiments using cultured cells, UBX positively stimulated a *Ubx* promoter fragment via UBX DNA-binding sites. However, because of the small fraction of *Ubx cis* regulatory sequences that were present in these experiments, and the distinct cell type, they do not address the question of whether direct autoregulation by *Ubx* in the fly would be positive or negative.

Conclusion

Ubx autoregulation is quite complex: positive autoregulation in the embryonic visceral mesoderm maintains *Ubx*

expression, negative autoregulation in certain imaginal and tracheal cells shuts off *Ubx* expression, and negative autoregulation in other cells stabilizes the level of *Ubx* expression. While these roles may seem contradictory, UBX is only one of many factors regulating *Ubx* expression, which has over 60 kb of *cis* regulatory sequences. Further, *Ubx* is undoubtedly only one of many downstream target genes whose expression is regulated (directly or indirectly) by UBX. Elucidation of the regulatory interactions that allow the diverse autoregulatory responses of *Ubx* should provide further insights into both the activity and the regulation of the *Ubx* gene.

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REFERENCES

Akam, M. (1987). The molecular basis for metameric pattern in the *Drosophila* embryo. *Development* **101**, 1-22.

Akam, M., Dawson, I. and Tear, G. (1988). Homeotic genes and the control of segment diversity. *Development* **104 Supplement**, 123-133.

Andrew, D. J. and Scott, M. P. (1992). Downstream of the homeotic genes. *New Biol.* **4**, 5-15.

Bate, M. and Martinez-Arias, A. (1991). The embryonic origin of imaginal discs in *Drosophila*. *Development* **112**, 755-761.

Beachy, P. A., Helfand, S. L. and Hogness, D. S. (1985). Segmental distribution of bithorax complex proteins during *Drosophila* development. *Nature* **313**, 545-51.

Beachy, P. A., Krasnow, M. A., Gavis, E. R. and Hogness, D. S. (1988). An *Ultrabithorax* protein binds sequences near its own and the *Antennapedia* P1 promoters. *Cell* **55**, 1069-81.

Bender, W., Akam, M., Karch, F., Beachy, P. A., Peifer, M., Spierer, P., Lewis, E. B. and Hogness, D. S. (1983). Molecular genetics of the bithorax complex in *Drosophila melanogaster*. *Science* **221**, 23-29.

Bender, W., Weiffenbach, B., Karch, F. and Peifer, M. (1985). Domains of cis-interaction in the bithorax complex. *Cold Spring Harbor Symp. Quant. Biol.* **50**, 173-180.

Bergson, C. and McGinnis, W. (1990). An autoregulatory enhancer element of the *Drosophila* homeotic gene *Deformed*. *EMBO J.* **9**, 4287-97.

Bienz, M., Saari, G., Tremml, G., Müller, J., Zust, B. and Lawrence, P. A. (1988). Differential regulation of *Ultrabithorax* in two germ layers of *Drosophila*. *Cell* **53**, 567-76.

Bienz, M. and Tremml, G. (1988). Domain of *Ultrabithorax* expression in *Drosophila* visceral mesoderm from autoregulation and exclusion. *Nature* **333**, 576-8.

Botas, J., Cabrera, C. V. and García-Bellido, A. (1988). The reinforcement-extinction process of selector gene activity: a positive feed-back loop and cell-cell interactions in *Ultrabithorax* patterning. *Roux's Arch. Dev. Biol.* **197**, 424-434.

Boulet, A. M., Lloyd, A. and Sakonju, S. (1991). Molecular definition of the morphogenetic and regulatory functions and the *cis*-regulatory elements of the *Drosophila Abd-B* homeotic gene. *Development* **111**, 393-405.

Cabrera, C. V., Botas, J. and García-Bellido, A. (1985). Distribution of *Ultrabithorax* proteins in mutants of *Drosophila* bithorax complex and its transregulatory genes. *Nature* **318**, 569-571.

Campos-Ortega, J. A. and Hartenstein, V. (1985). *The Embryonic Development of Drosophila melanogaster.* Berlin: Springer-Verlag.

Carroll, S. B., DiNardo, S., O'Farrell, P. H., White, R. A. and Scott, M. P. (1988). Temporal and spatial relationships between segmentation and homeotic gene expression in *Drosophila* embryos: distributions of the *fushi tarazu*, *engrailed*, *Sex combs reduced*, *Antennapedia*, and *Ultrabithorax* proteins. *Genes Dev.* **2**, 350-60.

Casanova, J., Sanchez, H. E. and Morata, G. (1985). Prothoracic transformation and functional structure of the *Ultrabithorax* gene of Drosophila. *Cell* **42**, 663-9.

Celniker, S. E., Keelan, D. J. and Lewis, E. B. (1989). The molecular genetics of the bithorax complex of *Drosophila:* characterization of the products of the *Abdominal-B* domain. *Genes Dev.* **3**, 1424-36.

Celniker, S. E., Sharma, S., Keelan, D. J. and Lewis, E. B. (1990). The molecular genetics of the bithorax complex of *Drosophila*: *cis*-regulation in the *Abdominal-B* domain. *EMBO J.* **9**, 4277-4286.

Chouinard, S. and Kaufman, T. C. (1991). Control of expression of the homeotic *labial* (*lab*) locus of *Drosophila melanogaster*: evidence for both positive and negative autogenous regulation. *Development* **113**, 1267-1280.

Driever, W., Thoma, G. and Nusslein, V. C. (1989). Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the *bicoid* morphogen. *Nature* **340**, 363-7.

Duncan, I. (1987). The bithorax complex. *Annu. Rev. Genet.* **21**, 285-319.

Duncan, I. and Lewis, E. B. (1982). Genetic control of body segment differentiation in *Drosophila.* In *Developmental Order: Its Origin and Regulation*, (eds. S. Subteleny and P. B. Green),pp. 533-554. New York: Alan R. Liss.

Frasch, M., Warrior, R., Tugwood, J. and Levine, M. (1988). Molecular analysis of *even-skipped* mutants in *Drosophila* development. *Genes Dev.* **2**, 1824-1838.

García-Bellido, A. (1975). Genetic control of wing disc development in *Drosophila*. *Ciba Found. Symp.* **29**, 161-82.

García-Bellido, A. and Capdevila, M. P. (1978). The initiation and maintenance of gene activity in a developmental pathway of *Drosophila*. In *The Clonal Basis of Development, 36th Symp. Soc. Dev. Biol*., (eds. S. Subtelny and I. M. Sussex),pp. 3-21. New York: Academic Press.

Gavis, E. R. and Hogness, D. S. (1991). Phosphorylation, expression, and function of the *Ultrabithorax* protein family in *Drosophila melanogaster*. *Development* **112**, 1077-1093.

Ghysen, A., Jan, L. Y. and Jan, Y. N. (1985). Segmental determination in Drosophila central nervous system. *Cell* **40**, 943-8.

Gonzalez, R. A., Urquia, N., Gehring, W. J., Struhl, G. and Morata, G. (1990). Are cross-regulatory interactions between homoeotic genes functionally significant? *Nature* **344**, 78-80.

Hayes, P. H., Sato, T. and Denell, R. E. (1984). Homoeosis in *Drosophila*: the *Ultrabithorax* larval syndrome. *Proc. Natl. Acad. Sci. USA* **81**, 545-9.

Heemskerk, J., DiNardo, S., Kostriken, R. and O'Farrell, P. H. (1991). Multiple modes of *engrailed* regulation in the progression towards cell fate determination. *Nature* **352**, 404-410.

Hiromi, Y. and Gehring, W. J. (1987). Regulation and function of the Drosophila segmentation gene *fushi tarazu*. *Cell* **50**, 963-74.

Hogness, D. S., Lipshitz, H. D., Beachy, P. A., Peattie, D. A., Saint, R. B., Goldschmidt, C. M., Harte, P. J., Gavis, E. R. and Helfand, S. L. (1985). Regulation and products of the *Ubx* domain of the bithorax complex. *Cold Spring Harb. Symp. Quant. Biol.* **50**, 181-194.

Hooper, J. E. (1986). Homeotic gene function in the muscles of *Drosophila* larvae. *EMBO J.* **5**, 2321-2329.

Ingham, P. W. (1988). The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature* **335**, 25-34.

Irvine, K. D., Helfand, S. L. and Hogness, D. S. (1991). The large upstream control region of the *Drosophila* homeotic gene *Ultrabithorax*. *Development* **111**, 407-424.

Johnson, F. B. and Krasnow, M. A. (1990). Stimulation of transcription by an *Ultrabithorax* protein *in vitro*. *Genes Dev.* **4**, 1044-1052.

Jorgensen, E. M. and Garber, R. L. (1987). Function and misfunction of the two promoters of the *Drosophila Antennapedia* gene. *Genes Dev.* **1**, 544-555.

Kaufman, T. C., Lewis, R. and Wakimoto, B. (1980). Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: The homeotic gene complex in polytene chromosome interval 84A-B. *Genetics* **94**, 115-133.

Kaufman, T., Seeger, M. A. and Olsen, G. (1990). Molecular and genetic organization of the Antennapedia gene complex of *Drosophila*

melanogaster. In *Advances in Genetics*, (ed. T. R. F. Wright), pp. 309-362. San Diego: Academic Press.

Kornfeld, K., Saint, R. B., Beachy, P. A., Harte, P. J., Peattie, D. A. and Hogness, D. S. (1989). Structure and expression of a family of *Ultrabithorax* mRNAs generated by alternative splicing and polyadenylation in *Drosophila*. *Genes Dev* **3**, 243-58.

Krasnow, M. A., Saffman, E. E., Kornfeld, K. and Hogness, D. S. (1989). Transcriptional activation and repression by *Ultrabithorax* proteins in cultured Drosophila cells. *Cell* **57**, 1031-43.

Kuziora, M. A. and McGinnis, W. (1988). Autoregulation of a Drosophila homeotic selector gene. *Cell* **55**, 477-485.

Lawrence, P. A. and Johnston, P. (1989). Pattern formation in the *Drosophila* embryo: allocation of cells to parasegments by *even-skipped* and *fushi tarazu*. *Development* **105**, 761-7.

Lewis, E. B. (1955). Some aspects of position pseudoallelism. *Amer. Nat.* **89**, 73-89.

Lewis, E. B. (1963). Genes and developmental pathways. *Am. Zool.* **3**, 33-56.

Lewis, E. B. (1964). Genetic control and regulation of developmental pathways. In *The Role of Chromosomes in Development*, (ed. M. Locke), pp. 231-252. New York: Academic Press.

Lewis, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565-70.

Lewis, E. B. (1982). Control of body segment differentiation in *Drosophila* by the bithorax gene complex. *Prog. Clin. Biol. Res.* **85**, 269-288.

Lewis, E. B. (1985). Regulation of the genes of the bithorax complex in *Drosophila*. *Cold Spring Harb. Symp. Quant. Biol.* **50**, 155-164.

Little, J. W., Byrd, C. A. and Brower, D. L. (1990). Effect of *abx*, *bx* and *pbx* mutations on expression of homeotic genes in *Drosophila* larvae. *Genetics* **124**, 899-908.

Lopez, A. J. and Hogness, D. S. (1991). Immunochemical dissection of the *Ultrabithorax* homeoprotein family in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **88**, 9924-9928.

Madhavan, M. M. and Schneiderman, H. A. (1977). Histological analysis of the dynamics of growth of imaginal discs and histoblast nests during the larval development of *Drosophila melanogaster*. *Wilhelm Roux's Arch.Dev. Biol.* **183**, 269-305.

Mann, R. S. and Hogness, D. S. (1990). Functional dissection of *Ultrabithorax* proteins in D. melanogaster. *Cell* **60**, 597-610.

Martinez-Arias, A. and Lawrence, P. A. (1985). Parasegments and compartments in the *Drosophila* embryo. *Nature* **313**, 639-42.

Miñana, F. J. and García-Bellido, A. (1982). Preblastoderm mosaics of mutants of the bithorax-complex. *Roux's Arch. Dev. Biol.* **191**, 331-334.

Morata, G. and García-Bellido, A. (1976). Developmental analysis of some mutants of the bithorax system of *Drosophila*. *Wilhelm Roux's Arch.Dev. Biol.* **179**, 125-143.

Morata, G. and Kerridge, S. (1981). Sequential functions of the bithorax complex of *Drosophila*. *Nature* **290**, 778-81.

Müller, J. and Bienz, M. (1991). Long range repression conferring boundaries of *Ultrabithorax* expression in the *Drosophila* embryo. *EMBO J.* **10**, 3147-3155.

Müller, J., Thuringer, F., Biggin, M., Zust, B. and Bienz, M. (1989). Coordinate action of a proximal homeoprotein binding site and a distal sequence confers the *Ultrabithorax* expression pattern in the visceral mesoderm. *EMBO J.* **8**, 4143-51.

O'Connor, M. B., Binari, R., Perkins, L. A. and Bender, W. (1988). Alternative RNA products from the *Ultrabithorax* domain of the bithorax complex. *EMBO J.* **7**, 435-45.

Paro, R. (1990). Imprinting a determined state into the chromatin of *Drosophila*. *Trends Genet.* **6**, 416-421.

Paro, R. and Hogness, D. S. (1991). The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of *Drosophila*. *Proc. Natl. Acad. Sci. USA* **88**, 263-267.

Peifer, M. and Bender, W. (1986). The *anterobithorax* and *bithorax* mutations of the bithorax complex. *EMBO J.* **5**, 2293-303.

Peifer, M., Karch, F. and Bender, W. (1987). The bithorax complex: control of segmental identity. *Genes Dev* **1**, 891-898.

Qian, S., Capovilla, M. and Pirrotta, V. (1991). The *bx* region enhancer, a distant *cis*-control element of the *Drosophila Ubx* gene and its regulation by *hunchback* and other segmentation genes. *EMBO J.* **10**, 1415-1425.

Rühle, H. (1932). Das larvale tracheensystem von *Drosophila melanogaster* Meigen und seine variabilität. *Z. wiss. Zoologie* **141**, 159-245.

Sánchez-Herrero, E. (1991). Control of the expression of the bithorax complex genes *abdominal-A* and *Abdominal-B* by *cis*-regulatory regions in *Drosophila* embryos. *Development* **111**, 437-449.

Sánchez-Herrero, E., Vernos, I., Marco, R. and Morata, G. (1985). Genetic organization of *Drosophila* bithorax complex. *Nature* **313**, 108-13.

Scott, M. P., Tamkun, J. W. and Hartzell, G. W. (1989). The structure and function of the homeodomain. *Biochim. Biophys. Acta* **989**, 25-48.

Simon, J., Peifer, M., Bender, W. and O'Connor, M. (1990). Regulatory elements of the bithorax complex that control expression along the anterior-posterior axis. *EMBO J.* **9**, 3945-3956.

Smolik-Utlaut, S. M. (1990). Dosage requirements of *Ultrabithorax* and *bithoraxoid* in the determination of segment identity in *Drosophila melanogaster*. *Genetics* **124**, 357-366.

Struhl, G. (1982). Genes controlling segmental specification in the *Drosophila* thorax. *Proc. Natl. Acad. Sci. USA* **79**, 7380-7384.

Struhl, G. and Akam, M. (1985). Altered distributions of *Ultrabithorax* transcripts in *extra sex combs* mutant embryos of *Drosophila*. *EMBO J.* **4**, 3259-64.

Struhl, G., Struhl, K. and Macdonald, P. M. (1989). The gradient morphogen *bicoid* is a concentration-dependent transcriptional activator. *Cell* **57**, 1259-73.

Teugels, E. and Ghysen, A. (1985). Domains of action of *bithorax* genes in *Drosophila* central nervous system. *Nature* **314**, 558-561.

Weinzierl, R., Axton, J. M., Ghysen, A. and Akam, M. (1987). *Ultrabithorax* mutations in constant and variable regions of the protein coding sequence. *Genes Dev.* **1**, 386-397.

White, R. A. H. and Wilcox, M. (1984). Protein products of the bithorax complex in Drosophila. *Cell* **39**, 163-171.

White, R. A. H. and Akam, M. E. (1985). *Contrabithorax* mutations cause inappropriate expression of *Ultrabithorax* products in *Drosophila*. *Nature* **318**, 567-569.

White, R. A. H. and Wilcox, M. (1985a). Distribution of *Ultrabithorax* proteins in *Drosophila*. *EMBO J.* **4**, 2035-2043.

White, R. A. H. and Wilcox, M. (1985b). Regulation of the distribution of *Ultrabithorax* proteins in *Drosophila*. *Nature* **318**, 563-567.

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