Development 111, 437–449 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

Control of the expression of the bithorax complex genes abdominal-A and Abdominal-B by *cis*-regulatory regions in *Drosophila* embryos

ERNESTO SÁNCHEZ-HERRERO

Centro de Biología Molecular, Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain

Summary

The abdominal-A (*abd-A*) and Abdominal-B (*Abd-B*) genes of the bithorax complex (BX-C) specify the identity of most of the *Drosophila* abdomen. Six different classes of infraabdominal (*iab*) mutations within the BX-C transform a subset of the parasegments affected by the lack of these two genes. It is thought that these mutations define parasegmental *cis*-regulatory regions that control the expression of *abd-A* and *Abd-B*. By staining embryos mutant for different *iab* mutations with anti-*abd-A* and anti-*Abd-B* antibodies I show here that the expression of *Abd-B* (and probably also *abd-A*) exhibit a parasegmental regulation. I have also studied the significance of the chromosomal order of parasegmental *iab* regulatory sequences, and the possible presence of chromosomal 'boundaries' between them, by looking at the expression of *abd-A* and *Abd-B* in embryos carrying the Uab^1 and *Mcp* mutations. These data are discussed in the light of models of parasegmental-specific regulatory regions within the BX-C.

Key words: bithorax complex, parasegments, *cis*-regulation, homeotic.

Introduction

The identity of segments in *Drosophila* is determined by a group of genes called homeotic genes, mutations in which transform a segment or a group of segments into another (Gehring and Hiromi, 1986; Mahaffey and Kaufman, 1988). These genes share a number of developmental and molecular characteristics, including the presence of a 180 bp coding sequence, the homeobox, which is present in many other species (McGinnis *et al.* 1984; Scott and Weiner, 1984; reviewed in Scott *et al.* 1989). It was this conservation of the homeobox that led to the identification in other organisms of genes homologous to those described as homeotic in the fruit fly (reviewed in Scott *et al.* 1989).

In *Drosophila* most of these genes are clustered in two complexes: Antennapedia (ANT-C) (Kaufman *et al.* 1980; Kaufman, 1983) and Bithorax (BX-C) (Lewis, 1978; Sánchez-Herrero *et al.* 1985). A main feature of both complexes is that the order of the genes (and in the BX-C the regulatory sequences) on the chromosome correlates with their spatial expression and requirement in the anteroposterior axis of the animal: more proximal genes are expressed (and required) more anteriorly than more distal ones (Lewis, 1978; Kaufman, 1983). This relationship also occurs in vertebrates, revealing a surprising evolutionary conservation (Gaunt *et al.* 1988; Graham *et al.* 1989; Duboule and Dollé, 1989; Akam, 1989).

This correlation was first discovered by the phenotypic study of mutations in the BX-C (Lewis, 1978). This complex is composed of three genes: Ultrabithorax (*Ubx*), abdominal-A (*abd-A*) and Abdominal-B (*Abd-B*) (Sánchez-Herrero *et al.* 1985; Tiong *et al.* 1985), each with a homeobox (Regulski *et al.* 1985). Their concerted action is responsible for the specification of part of the thoracic and all of the abdominal (A) segments, although each gene has its proper realm of action. This is defined by the analysis of patterns of gene expression and by mutational studies, which also show that these genes act on units called parasegments (PS; Martínez-Arias and Lawrence, 1985), rather than segments: mutations in the *Ubx* gene transform PS5 and PS6 (and to a lesser extent PS7–12), in *abd-A* PS7–13 and in *Abd-B* PS10–14 (or PS10–15) (Sánchez-Herrero *et al.* 1985; Tiong *et al.* 1985; Casanova *et al.* 1987; reviewed in Duncan, 1987).

Many of the mutations in the BX-C, however, do not completely inactivate any of these three genes. Their phenotype is part of that produced by *Ubx*, *abd-A* or *Abd-B* mutations, which they fail to complement. Molecular analyses have shown that they map within introns or outside protein-coding transcription units (Bender *et al.* 1983; Hogness *et al.* 1985; Karch *et al.* 1985; Celniker *et al.* 1989; Zavortink and Sakonju, 1989; Karch *et al.* 1990), suggesting that their effect could be mainly, or exclusively, regulatory.

The existence of mutations in regulatory regions that transform certain parasegments and their particular order on the chromosome prompted a model, based on the original of Lewis (1978), of how the BX-C could be activated (Peifer *et al.* 1987). This process would be

accomplished in two steps: first, segmentation genes would activate (open) the different regulatory regions in certain parasegments, making them accessible to *trans*-regulatory factors. The spatially restricted transcriptional activity in these regulatory domains (Lipshitz *et al.* 1987; Sánchez-Herrero and Akam, 1989; Cumberledge *et al.* 1990) is possibly a result of this activation. In a second step, the regulatory regions, which are active from a precise anterior parasegmental boundary, would control the expression of *Ubx*, *abd-A* and *Abd-B*.

The parasegmental regulation has been carefully studied in the Ultrabithorax domain. *Ubx* mutations transform PS5 and PS6 (and to a lesser extent PS7–12; Lewis, 1978). *abx* (or *bx*) mutations, which map to an intron of the *Ubx* transcription unit (Bender *et al.* 1983; Peifer and Bender, 1986), affect PS5 in the adult, while *bxd* mutations, which map upstream of the coding unit (Bender *et al.* 1983; Hogness *et al.* 1985), transform PS6 (and to a lesser extent PS7–12; Lewis, 1978; Struhl, 1984; Hayes *et al.* 1984; Casanova *et al.* 1985; Peifer and Bender, 1986). The study of the distribution of *Ubx* proteins in *abx* and *bxd* mutants has revealed that the *abx* region is responsible for characteristic levels of *Ubx* expression in PS5–13 while the *bxd* region promotes higher levels in PS6–13 (Cabrera *et al.* 1985; White and Wilcox, 1985; Beachy *et al.* 1985). Thus, *abx* and *bxd* regions recognize precise anterior parasegmental boundaries.

In the abdominal region of the BX-C a similar relationship may apply. The *iab* mutations (*iab-2* to *iab-7*) transform a subset of the A2-A7 segments (or PS7–12) in the abdominal region (Lewis, 1978; Kuhn *et al.* 1981; Karch *et al.* 1985; Duncan, 1987). Except for the *iab-2* class, these mutations map between the *abd-A* and *Abd-B* transcription units and they are ordered on the chromosome colinearly with the more anterior parasegment transformed (Lewis, 1978; see Fig. 1). It has been proposed that the *iab* regions (delimited by the mutations) regulate the expression of *abd-A* and *Abd-B* products in a parasegmental manner (Karch *et al.* 1985; Casanova *et al.* 1987; Peifer *et al.* 1987; Tiong *et al.* 1987; Sánchez-Herrero *et al.* 1988).

I have studied this issue by looking at the distribution of *abd-A* and *Abd-B* products in different mutations from the abdominal region of the BX-C. The results obtained, together with previous phenotypic analyses of the mutations, strongly suggest that the *iab* mutations affect parasegmental, *cis*-regulatory regions that control the expression of *abd-A* and *Abd-B*. The interactions between these two genes, and their restricted spatial expression, directed by these *iab* regulatory regions, establish the identity of the abdominal segments.

Materials and methods

Drosophila *strains*

The BX-C mutations studied have all been described (Lewis, 1978; Kuhn *et al.* 1981; Karch *et al.* 1985; Sánchez-Herrero *et al.* 1985; Casanova *et al.* 1986; Celniker and Lewis, 1987). The nomenclature of Duncan 1987 was followed for the *iab* mutations. Balancer chromosomes and other mutations are described in Lindsley and Zinn (1985, 1989).

To identify embryos homozygous for certain mutations these were balanced over a *TM3* balancer chromosome carrying an insertion of the *β-gal* coding region under the control of a *hb* promoter. This balancer chromosome was generously provided by G. Struhl. Embryos homozygous or heterozygous for the balancer chromosome show *β-gal* expression mostly in the head region, and this can be detected with an anti-*β gal* antibody at least until stage 16 of embryonic development (Campos-Ortega and Hartenstein, 1985). By double staining with anti-*β-gal* (Cappel) and either anti-*abd-A* or anti-*Abd-B* antibodies, the distribution of *abd-A* or *Abd-B* products could be easily observed in embryos homozygous for the mutation, as these embryos would lack the *hb* expression pattern. In other cases, the identification of the mutant embryos was straightforward and did not require the use of this balancer chromosome.

Antibody staining

This was performed basically as described by MacDonald and Struhl, 1986. Overnight collections of embryos were dechorionated with bleach, washed with water and fixed for 20 to 30 min in 4 % paraformaldehyde in PEM (0.1 M Pipes, pH 6.9, 1 mM $MgSO_4$, 2 mM EGTA)/heptane (1:1); the vitelline membrane was removed by replacing the paraformaldehyde solution with methanol and shaking the embryos for 30 s. After several washes in methanol and PTX (PBS with 0.1 % Triton X-100), the embryos were preincubated for 2 h at 4 °C in PBTX (PBS with 0.1 % Triton X-100, 0.1 % BSA, 10 mM sodium azide), and incubated with the antibodies overnight at 4 °C. Embryos were later washed at room temperature with PBTX several times for a total time of 3 h, incubated with biotinilated secondary antibodies (Amersham) 1:400 in PBTX 1.5 h at 4 °C and washed four times for 30 min with PT (PBS with 0.1 % Tween-20) at room temperature. The avidin–biotin complex (Vector Laboratories) was then added to the embryos and incubated for 30 min at room temperature. After five washes in PT for a total of 30 min the following mixture was added to the embryos: 0.5 mg DAB, 0.01 % hydrogen peroxide, 0.6 % nickel sulphate (or 0.2 % nickel sulphate and 0.2 % cobalt chloride) and PT to 500 μl. The colour reaction was stopped with several washes of PT. Embryos were then dehydrated in 50 %, 70 %, 95 %, 100 % ethanol and isopropanol (5 min each), cleared in xylene and mounted in DPX mountant. For double stainings, after stopping the color reaction with PT, embryos were washed with PBTX and then the process continued with the second antibody as described above. For the experiments, either the first reaction was done with metal compounds added and the second without them or this order was reversed.

The antibodies were diluted in PBTX at the following concentrations: anti-*abd-A* (Macías *et al.* 1990), 1:5000; anti-*Abd-B* (Celniker *et al.* 1989), 1:4; anti-*β gal* (Cappel), 1:2000. The anti-*abd-A* and anti-*Abd-B* antibodies were generously provided by J. Casanova and S. Celniker, respectively.

Results

Complementation studies demonstrate that mutations of the *iab-2*, *iab-3* and *iab-4* classes fail to complement *abd-A* mutations, while *iab-5*, *iab-6* and *iab-7* mutations do not complement *Abd-B* mutations (Karch *et al.* 1985; Duncan, 1987). I have looked at the distribution of

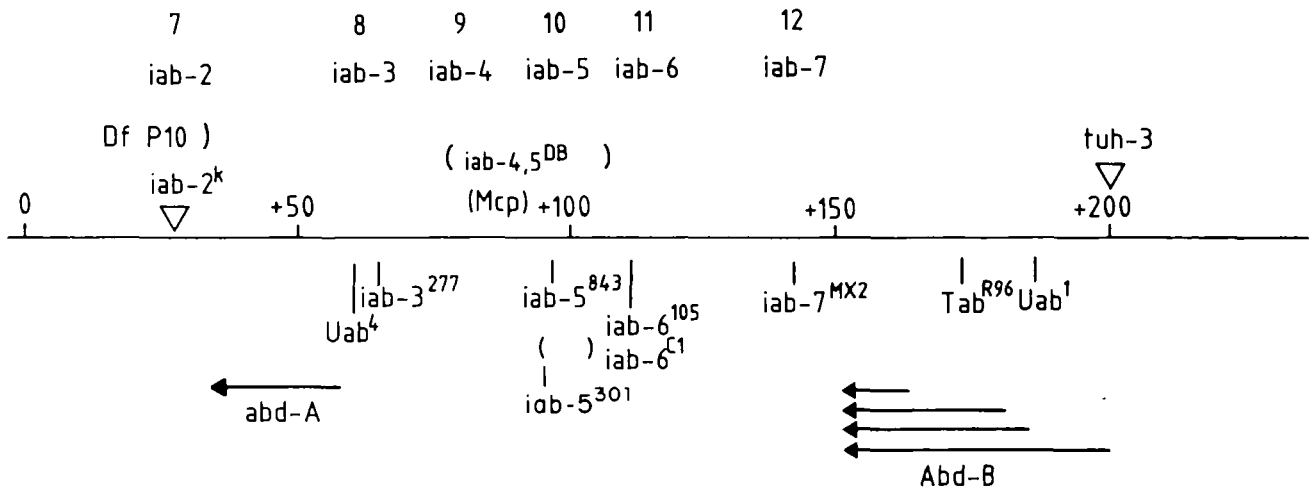


Fig. 1. DNA map of the abdominal region of the BX-C showing the location of some of the mutations used in this study. Numbers on the DNA refer to kilobases in the BX-C walk (Bender *et al.* 1983). Horizontal arrows indicate *abd-A* and *Abd-B* transcription units. Brackets show the extent of deficiencies and vertical lines indicate breakpoints. On top the approximate extent of the *iab* regulatory regions and the more anterior parasegment in which each regulatory region is active. Data taken from Karch *et al.* 1985; Celniker and Lewis, 1987; Zavortink and Sakonju, 1989, and Karch *et al.* 1990).

either *abd-A* or *Abd-B* proteins (depending on which is expected to be altered) in one or two *iab* alleles representative of each class and in some characteristic dominant mutations. The study has concentrated on the distribution of these proteins in the epidermis and ventral nerve cord. Although mutations in the BX-C also affect other tissues (Hooper, 1986; Tremml and Bienz, 1989), the study of the former two allows a simple correspondence with the described phenotypic effect of *iab* mutations.

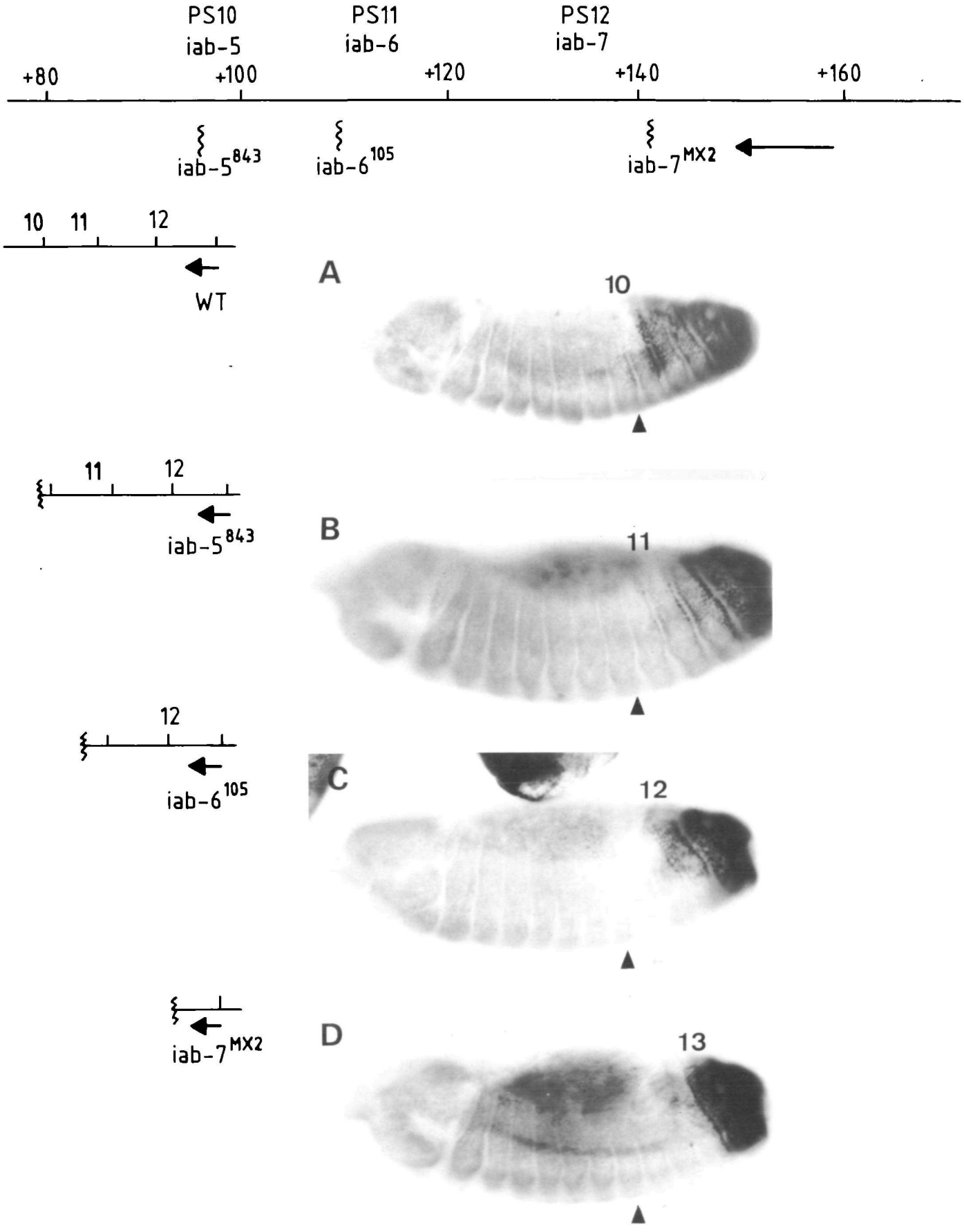
Although each of the *iab* mutation classes is correlated (and named) with respect to a certain metamere, many of them affect more than one parasegment. There are two reasons for this. (1) Some mutations are breakpoints separating several regions from the *abd-A* and *Abd-B* transcription units. These breakpoints, therefore, transform more metameres than those expected to be affected by small deficiencies in the region where the break lies. For example, *iab-7* breakpoints separate not only the *iab-7* regulatory region from the *Abd-B* transcription unit, but the *iab-5* and *iab-6* ones as well (see Fig. 2). (2) A certain regulatory region is activated in a certain parasegment and more posterior ones. Therefore, a mutation in this region may affect elements of the pattern in all these metameres. Transformations due to *iab* mutations are probably parasegmental both in embryos (Duncan, 1987) and adults. However, since this is not proven for the adult cuticle except in one case (Busturia *et al.* 1989), I refer to transformations as both segmental or parasegmental (each segment is equated to the parasegment containing its anterior compartment).

The detection of subtle changes in *abd-A* or *Abd-B* expression due to certain mutations was possible because homozygous embryos could be identified. This was done in some cases by the use of a particular *TM3* balancer chromosome (*TM3, hb-βgal*, see Material and methods). This method was applied to the $iab\text{-}2^{K}$, $iab\text{-}3^{277}$, $iab\text{-}4,5^{DB}$, $iab\text{-}5^{301}$, $iab\text{-}6^{105}$ and *tuh-3* mutations. It could not be used with other mutations ($iab\text{-}5^{843}$, Uab^{1}, Tab^{R96}) since the flies carrying these mutations balanced with this *TM3* chromosome proved to be sterile or poorly fertile. Fig. 1 shows the location on the chromosome of the mutations used in this study and their position relative to *abd-A* and *Abd-B* transcription units.

The iab-5, iab-6 *and* iab-7 *mutations change* Abd-B *expression in specific parasegments*

The *Abd-B* gene is required for the development of PS10–14 (or PS10–15) (Sánchez-Herrero *et al.* 1985; Tiong *et al.* 1985; Karch *et al.* 1985; Casanova *et al.* 1986; Whittle *et al.* 1986; Sato and Denell, 1986). Genetic studies have identified mutations that define two genetic elements within this gene, called *m* and *r*, required in PS10–13 and PS14 (and maybe PS15), respectively (Casanova *et al.* 1986). *In situ* hybridizations to embryonic tissues with *Abd-B* probes and antibody stainings with anti-*Abd-B* antibodies have shown there are two different sets of transcripts and proteins that correspond to the two genetic elements (*m* and *r*) and that they are expressed in either PS10–13 or PS14–15 respectively (Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988; DeLorenzi *et al.* 1988; Celniker *et al.* 1989; Zavortink and Sakonju, 1989; DeLorenzi and Bienz, 1990).

Only the *m* protein(s) is detected, after germ band extension, in PS10–12, while *r* protein(s) remains in PS14–15 throughout development (Celniker *et al.* 1989; DeLorenzi and Bienz, 1990). Mutations of the *iab-5*, *iab-6* and *iab-7* classes fail to complement *Abd-B* (*m*) mutations (but complement *r* mutations) and affect precisely those parasegments that show late *Abd-B* expression (PS10 to 12). The three classes map 3′ to the *Abd-B* transcription unit (Karch *et al.* 1985; see Figs 1


PS10
iab-5
PS11
iab-6
PS12
iab-7
+80
+100
+120
+140
+160
iab-5843
iab-6105
iab-7MX2
10 11 12
WT
A
10
11 12
iab-5843
B
11
12
iab-6105
C
12
iab-7MX2
D
13

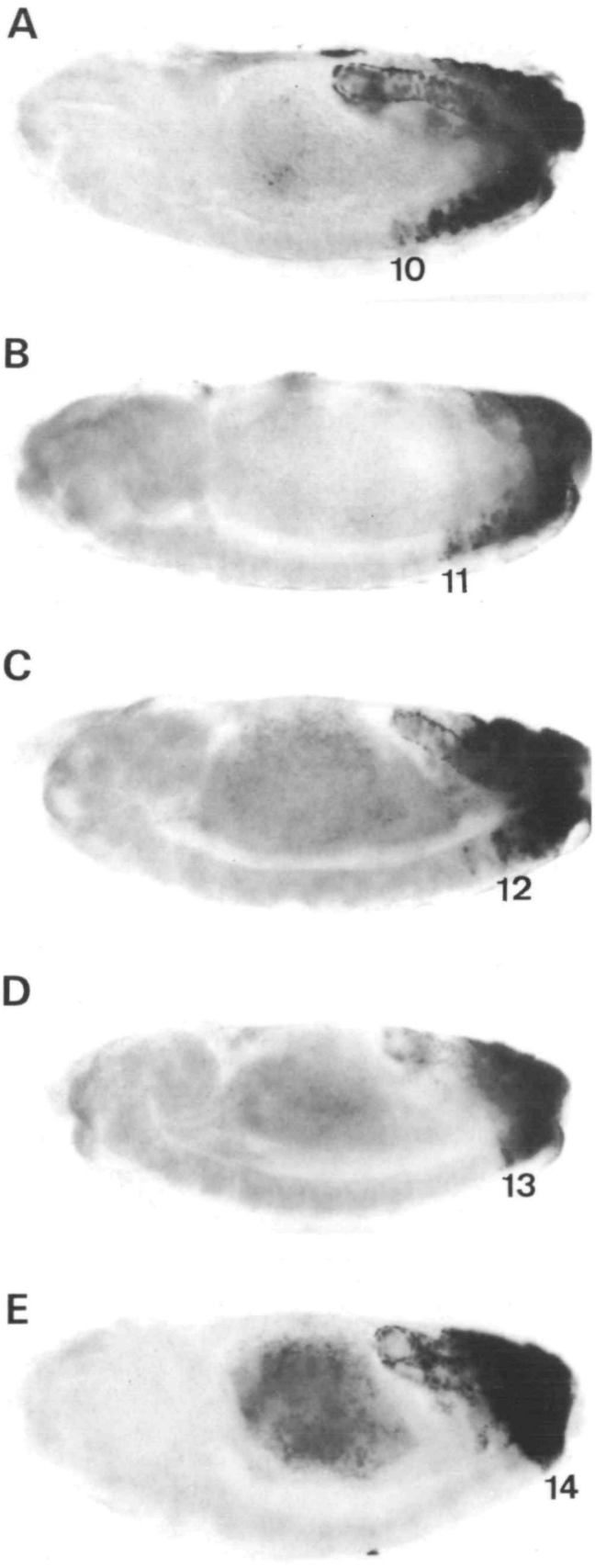


Fig. 2. Effect of *iab-5*, *iab-6* and *iab-7* mutations on *Abd-B* expression patterns. At the top of the Figure, there is a map for the BX-C region where these mutations map. Numbers on the DNA refer to kilobases and breakpoints are indicated by wavy lines. The arrow represents the *Abd-B* (*m*) transcription unit. The approximate location of *iab-5*, *iab-6* and *iab-7* regulatory regions is shown, and each corresponds with the more anterior parasegment where they are activated (PS10–12). To the left a schematic representation of the regions remaining *in cis* with the *Abd-B* transcription unit in wild-type or mutant chromosomes. (A–D) *Abd-B* patterns in the epidermis of wild-type and different mutant embryos. In each embryo the more anterior parasegment presenting label is numbered. Arrowheads indicate the position of the posterior compartment of PS10 (A4p), where the more anterior *Abd-B* expression is observed in wild-type embryos (Celniker *et al.* 1989; DeLorenzi and Bienz, 1990). In this and following Figures anterior is to the left and dorsal is up. (A) Wild-type *Abd-B* expression. (B) $iab\text{-}5^{843}/DfP9$ embryo. (C) Embryo of the genotype $iab\text{-}6^{105}/DfP9$. (D) $iab\text{-}7^{MX2}/DfP9$ embryo. The parasegmental domains that remain *in cis* correspond with the parasegments that show *Abd-B* protein (compare drawing to the left with A–D). The parasegments without *Abd-B* signal in each *iab* mutation coincide with those transformed in the adult (Karch *et al.* 1985; Sánchez-Herrero and Akam, 1989 and unpublished observations).

and 2). In what follows, I describe the effect of mutations of each group on *Abd-B* expression.

iab-5

Mutations of this class transform A5 into A4 (or PS10 into PS9) (Karch *et al.* 1985; Duncan, 1987). Some embryos from the cross of $iab\text{-}5^{843}/TM1$ males to *Sb DpP5/DfP9* females (*DfP9* is a deficiency for the whole BX-C) lack *Abd-B* expression in PS10, both in the epidermis (Fig. 2B) and ventral nerve cord. To confirm that the embryos without *Abd-B* staining in PS10 are the mutant ones, and since it was not possible to balance this mutation over the *TM3, hb-β gal* chromosome, another *iab-5* mutation ($iab\text{-}5^{301}$) was balanced over this chromosome. In all the embryos homozygous for the mutation, *Abd-B* expression was greatly reduced or absent, both in the epidermis and ventral nerve cord. Almost all of the rest of the embryos from the balanced stock presented staining in PS10 in the epidermis, central nervous system or both. A similar result was obtained with the mutation $iab\text{-}4,5^{DB}$ (Fig. 3B), although this mutation transforms also the fourth and (partially) the sixth abdominal segments.

Fig. 3. Effect of *iab-5*, *iab-6*, *iab-7* and *Abd-B* (*m*) mutations on *Abd-B* expression in the ventral nerve cord. The more anterior parasegment presenting *Abd-B* expression is indicated. (A) Wild-type. (B) $iab\text{-}4,5^{DB}$ homozygous embryo. (C) $iab\text{-}6^{CI}$ homozygous embryo. Note there are some cells labelled in PS11. (D) $iab\text{-}7^{MX2}/DfP9$ embryo. (E) $iab\text{-}7^{D14}$ homozygous embryo. This *m* mutation is a small deficiency within the *Abd-B* transcription unit that affects only *m* transcripts (Karch *et al.* 1985; Zavortink and Sakonju, 1989).

iab-6

This region is defined by *iab-6* breakpoints, which, when hemizygous, transform A5 and A6 into A4 (or PS10 and PS11 into PS9) (Karch *et al.* 1985; Duncan, 1987). Some embryos from the cross of *iab-6^{105}/TM2* males to *Sb DpP5/DfP9* females lack *Abd-B* expression in PS10 and PS11 in the epidermis (Fig. 2C). In the ventral nerve cord, no label is observed in PS10 and only a few nuclei in the anterior region of PS11 show signal. Except for these nuclei, the boundary of *Abd-B* expression is a sharp one and coincides with the anterior border of PS12, as confirmed by double staining of these embryos with anti-*Abd-B* and anti-*engrailed* antibodies. That the embryos are of the *iab-6^{105}/DfP9* genotype is corroborated by studying embryos from the *iab-6^{105}/TM3, hb-β gal* stock. Only those embryos without signal due to the *β gal* expression (those homozygous for the *iab-6* mutation) presented the *Abd-B* pattern described above. A similar pattern was obtained with some embryos (probably homozygous for the mutation) from the *iab-6^{C1}/TM1* stock (Fig. 3C). The *iab-6^{C1}* breakpoint maps in the same 3 kb restriction fragment as the *iab-6^{105}* mutation (Karch *et al.* 1985).

iab-7

iab-7 mutations transform A5–A7 into A4 (or PS10–12 into PS9) (Karch *et al.* 1985; Duncan, 1987). *Abd-B* expression in embryos homozygous or hemizygous for the mutation *iab-7^{MX2}* show an evident anterior parasegmental boundary, that corresponds to that of PS13, both in the epidermis (Fig. 2D) and ventral cord (Fig. 3D), as confirmed with double stainings with anti-*Abd-B* and anti-engrailed antibodies. There is no *Abd-B* signal in PS10–12. This restricted expression in *iab-7^{MX2}* embryos has been previously described for the distribution of the *Abd-B* transcripts (Sánchez-Herrero and Akam, 1989). In the homozygous embryos, there is also ectopic *Abd-B* expression in the ventral cord (ectopic *Abd-B* expression is also observed in some embryos from the *iab-6^{105} /TM3* stock).

It may be relevant to point out that *iab-6* and *iab-7* mutations are breakpoints that separate either *iab-6* and *iab-5*, or *iab-7*, *iab-6* and *iab-5* regulatory regions from the *Abd-B* transcription unit (Fig. 2). The effect of deficiencies for just *iab-6* or *iab-7* regions is predicted to be a transformation of PS11 expression into the wild-type *Abd-B* pattern of PS10 (for *iab-6* deficiencies) or of PS12 into that of PS11 (for *iab-7* deficiencies). However, no such deficiencies are avalaible to allow testing of this prediction.

Effects of the iab-2, iab-3 *and* iab-4 *mutations on the* abd-A *expression pattern*

The distribution of *abd-A* protein in wild-type embryos has been recently reported (Karch *et al.* 1990; Macías *et al.* 1990) and is not described here. *abd-A* products are present in the epidermis and ventral nerve cord of PS7–13 (see Fig. 4A), the same parasegments affected in the cuticle by *abd-A* mutations (Sánchez-Herrero *et*

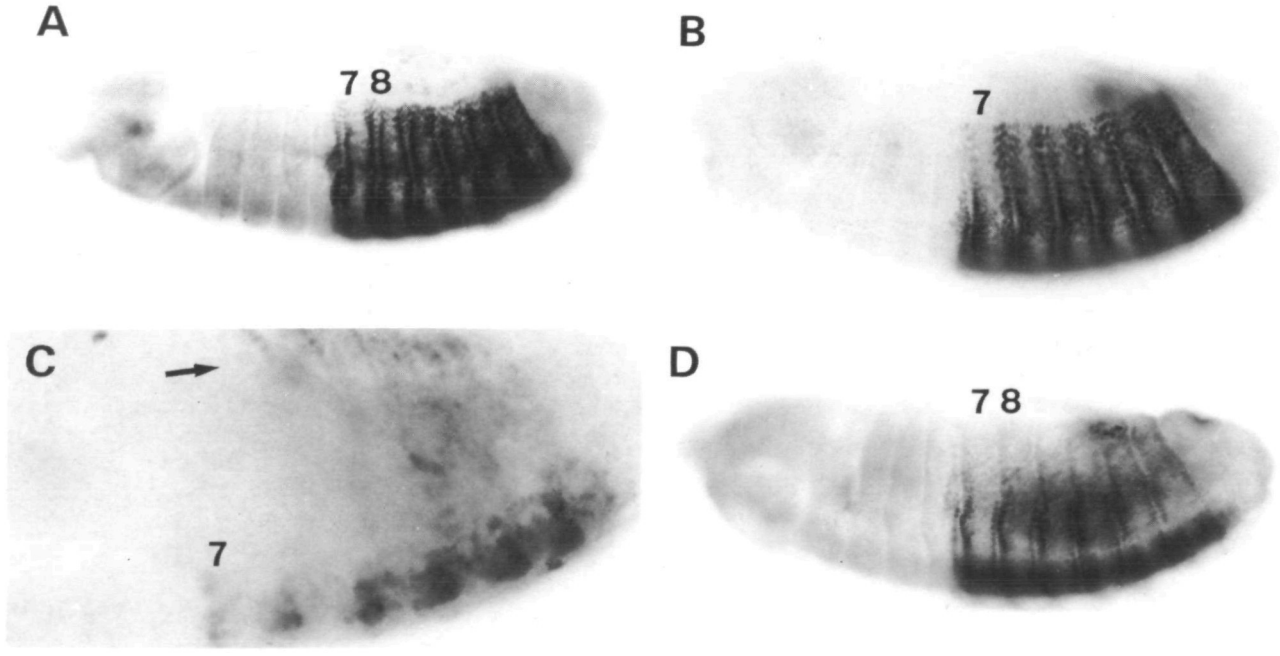


Fig. 4. Patterns of *abd-A* expression in embryos homozygous for some *iab* mutations. (A) *abd-A* expression in a stage 14 embryo (stages according to Campos-Ortega and Hartenstein, 1985). (B) *iab-2^{k}* homozygous embryo. No major change with respect to wild-type expression is observed. (C) Detail of a *DfP10* homozygous embryo. Note the apparent lack of expression in the epidermis of PS7 (arrow) and the presence of signal in the ventral nerve cord. The gradient of expression in the epidermis is also visible. (D) *iab-3^{277}* homozygous embryo. The expression in PS8 (and posterior parasegments) resembles that of PS7 (Compare with A).

al. 1985; Tiong *et al.* 1985). Three different classes of *iab* mutations fail to complement *abd-A* mutations: *iab-2*, *iab-3* and *iab-4* (Karch *et al.* 1985).

iab-2

iab-2 mutations transform in adults A2 into A1 and define a regulatory region 3′ to the transcription unit (Kuhn *et al.* 1981; Karch *et al.* 1985; Busturia *et al.* 1989). Embryos homozygous for the $iab\text{-}2^{k}$ or Uab^{1} (a weak *iab-2*) mutations do not exhibit any major change in the *abd-A* staining pattern with respect to wild-type embryos (Fig. 4B).

DfP10 is a deficiency that removes the *iab-2* regulatory region as well as part of the 3′ from the *abd-A* transcription unit (Karch *et al.* 1985, 1990). Embryos homozygous for this deficiency transform PS7–13 into PS6, the transformation being much stronger in more anterior metameres (Morata *et al.* 1983). *DfP10* homozygous embryos present a gradient of expression of *abd-A* products in the epidermis and (less evident) in the ventral nerve cord (Fig. 4C): There is no staining (or is barely detectable) in the epidermis of PS7, it is weakly present in PS8 and increases gradually in more posterior parasegments. This gradient of *abd-A* expression directly correlates with the cuticular phenotype of *DfP10* homozygous embryos (see Morata *et al.* 1983) and might be an exaggerated manifestation of a more shallow gradient observed in wild-type embryos (Macías *et al.* 1990; Karch *et al.* 1990) due to the effect of the deficiency on *abd-A* transcripts or protein (Karch *et al.* 1990). In the ventral cord, the staining is stronger than in the epidermis, and readily detected in PS7. This difference in *abd-A* expression between epidermis and ventral cord in PS7 suggests that regulatory elements for the two tissues are not located in the same DNA region.

iab-3

Two mutations are described that define the *iab-3* regulatory region, which is located 5′ to the *abd-A* transcription unit: $iab\text{-}3^{277}$ and $iab\text{-}3^{Uab4}$. Both transform in hemizygous condition segments A3–A7 towards A2 (or PS8–12 into PS7) (Karch *et al.* 1985; Duncan, 1987; Busturia *et al.* 1989). $iab\text{-}3^{277}$ homozygous embryos show a reduction in staining in PS8–13, particularly in nuclei located in a dorsolateral position of the epidermis (Fig. 4D). In PS7 of wild-type embryos, and when compared with the staining of more posterior parasegments, this region presents a weaker *abd-A* signal (Fig. 4A). Therefore, *abd-A* expresion in mutant embryos suggests a transformation of PS8 and more posterior parasegments towards PS7, in accordance with the described adult phenotype (Busturia *et al.* 1989). Some embryos from the $iab\text{-}3^{Uab4}/TM6B$ stock (probably homozygous for the mutation), exhibit the same pattern described above.

iab-4

iab-4 mutations transform A4 (and partially A5) into A3 (Karch *et al.* 1985) and delimit the *iab-4* regulatory region, located 5′ to the *abd-A* transcription unit and more distally than the *iab-3* domain (see Fig. 1). In embryos homozygous for the $iab\text{-}4,5^{DB}$ deficiency, I cannot detect any major change in *abd-A* staining pattern. This is not surprising since both wild-type *abd-A* expression and cuticular phenotype in these two parasegments are almost identical.

Dominant phenotypes are commonly associated with *iab-2*, *iab-3* and *iab-4* mutations and most of them are revealed by a transformation of A1 to a more posterior segment (Lewis, 1978; Karch *et al.* 1985; Lewis, 1985; Busturia *et al.* 1989). In accordance with this phenotype, there is ectopic *abd-A* expression in parasegments anterior to PS7 in many of these *iab* mutations. Examples of this are shown on Fig. 5. There is some specificity as to this ectopic expression. In $iab\text{-}3^{Uab4}$ embryos, *abd-A* expression is turned on in some cells of the lateral epidermis of PS6 (Fig. 5C) and more rarely, in PS6 of the ventral nerve cord. By contrast, *Hab* embryos show ectopic signal preferentially on the ventral cord (Fig. 5D; *Hab* mutations transform the third thoracic and the first abdominal segment into the second abdominal one, Lewis, 1978). Ectopic expression is less common in $iab\text{-}3^{277}$ embryos.

A special case of ectopic *abd-A* expression is observed in $su\text{-}Hw^{2}$ $iab\text{-}2^{K}$ homozygous embryos. The $iab\text{-}2^{k}$ mutation is due to the insertion of a *gypsy* transposon (Karch *et al.* 1985), and its phenotypic effect is, therefore, suppressed by mutations in the *su-Hw* locus (Modolell *et al.* 1983). Thus, $su\text{-}Hw^{2}$ $iab\text{-}2^{K}$ homozygous flies are wild-type in the second abdominal segment. In addition, these flies transform the first abdominal segment into a posterior one (Karch *et al.* 1985; Busturia *et al.* 1989). Embryos with this genotype present ectopic *abd-A* expression in some cells of the lateral epidermis of PS4–6 and in the ventral nerve cord (Fig. 5A). One possible explanation for the two phenotypes (dominant and recessive) associated with this insertion is that the breakpoint provokes an activation (or lack of repression) of *iab-2* regulatory sequences in more anterior parasegments. This does not entail ectopic expression of *abd-A* (except in a few cells and with low frequency) because of the presence of the $su\text{-}Hw^{2}$ protein, which would prevent interaction of the *iab-2* regulatory sequences with the *abd-A* promoter (see Harrison *et al.* 1989). The lack of this interaction would also explain the recessive phenotype in A2. In the absence of the $su\text{-}Hw^{2}$ protein, however, this interaction is possible and provokes the suppression of the recessive phenotype and the ectopic *abd-A* expression and its resulting phenotype.

Uab^{1} *and* Mcp

The expression of *abd-A* and *Abd-B* in these two mutations are described separately because they present special features relevant to two important issues in the BX-C: the existence of boundaries between regulatory regions and the importance of the alignment of these regions on the chromosome.

Uab^{1} is a small inversion with breakpoints in the *bxd* and *Abd-B (r)* transcription units (Karch *et al.* 1985; DeLorenzi *et al.* 1988; Kuziora and McGinnis, 1988). As

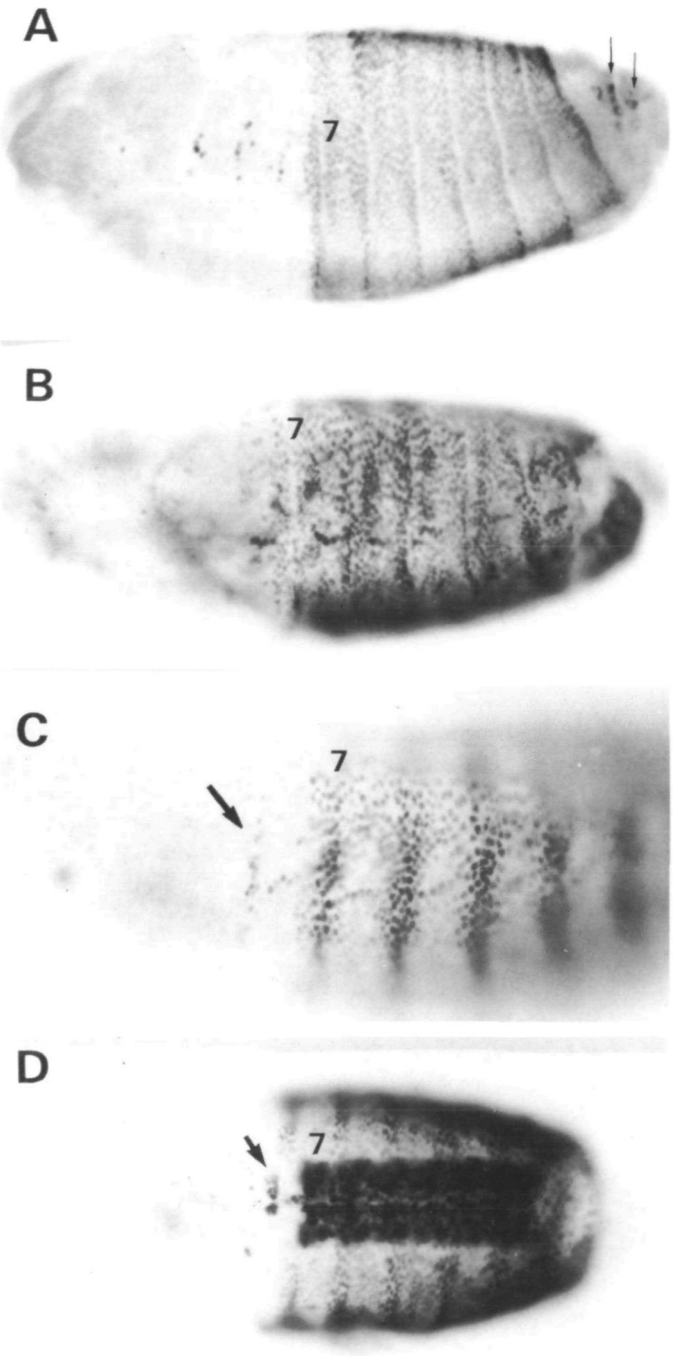


Fig. 5. *abd-A* expression in some dominant mutations. Numbers refer to parasegments. (A) *su-Hw*^{2} *iab-2*^{k} *tuh-3* homozygous embryo. There is ectopic expression anteriorly to the normal *abd-A* domain and also posteriorly, in PS14 and PS15 (arrows), probably due to homozygosis for the *tuh-3* mutation. (B) Embryo homozygous or heterozygous for the *Uab*^{1} mutation. *abd-A* is now expressed anteriorly to PS7. (C) Detail of an embryo of genotype *iab-3*^{Uab4}/ *TM6 B*. There is also expression anterior to PS7 (arrow). (D) Embryo homozygous or heterozygous for the *Hab* mutation. Note ectopic expression in the anterior region of PS6 in the ventral cord (arrow). See also the lack of staining in the PS6 cells adjacent to PS7.

a result, the order of *iab-2–iab-7* regulatory regions, *abd-A* and *Abd-B* (*m*) transcription units is inverted with respect to the *Ubx* region of the complex. Apart from being mutant for *bxd* and *r* functions, in *Uab*^{1} hemizygous adults, there is a partial transformation of A2 to A1 (Busturia *et al.* 1989). Adults heterozygous for *Uab*^{1} also show a transformation of A1 to A2 (Lewis, 1978) and present patches of genital and posterior abdominal tissue in the anterior abdominal segments (Duncan, 1987; Kuhn and Packert, 1988).

The pattern of *abd-A* in embryos from the *Uab*^{1}/ *TM6B* stock present several alterations. First, there is some label in the anterior compartment of the first abdominal segment, mostly in cells near the anterior border of PS7 *abd-A* expression (Fig. 5B). As a consequence, the anterior limit of *abd-A* expression is frequently not sharp, as in wild-type embryos. Second, there is a general reduction in *abd-A* label and an ectopic expression in PS14 and PS15 described below.

The pattern of *Abd-B* also differs from the wild-type. Many of the embryos present single cells or small patches of cells with strong label in the epidermis and, less frequently, the ventral cord, of abdominal segments A1-A4. These groups of cells with strong signal are also present within the normal *Abd-B* domain of expression, being specially conspicuous in PS10 and PS11, where *Abd-B* expression is weaker in wild-type embryos (Fig. 7B).

Mcp is a small deficiency located between *iab-4* and *iab-5* regulatory regions (Karch *et al.* 1985). It transforms A4 into A5 (or PS9 into PS10) (Lewis, 1978; Fig. 6F). *Mcp* embryos exhibit in PS9 (normally without staining) a pattern of *Abd-B* expression that is normally seen in PS10, both in the epidermis and ventral nerve cord (Fig. 6B,D). It is remarkable that in this latter tissue the replication (as far as can be observed) is precise. This is especially obvious since only some cells are strongly labelled in PS10 of wild-type embryos and this pattern is reproduced in PS9 of *Mcp* mutants (Fig. 6B).

Interactions within Abd-B *and between* abd-A *and* Abd-B

As described above, the *iab* regions control the expression of *abd-A* and *Abd-B* products. A further refinement in the establishment of the abdominal pattern comes from the interactions between these two genes. Since the organization of the *Abd-B* gene is complex, and this gene comprises two genetic elements (see above; Casanova *et al.* 1986), we need to separate the contribution of each of these elements to the abd-A and Abd-B expression.

In the absence of the *m* element, PS10–13 are transformed into PS9 (Casanova *et al.* 1986). In embryos homozygous for the *m* mutation *Abd-B*^{D14}, the *Abd-B m* protein is not present in PS10–13. The *r* protein is not affected, and is observed in PS14 (Fig. 3E), as in wild-type embryos. This effect of *m* mutations on the expression of *Abd-B* has been previously reported (DeLorenzi and Bienz, 1990). Absence of *m* function also affects *abd-A* expression. In

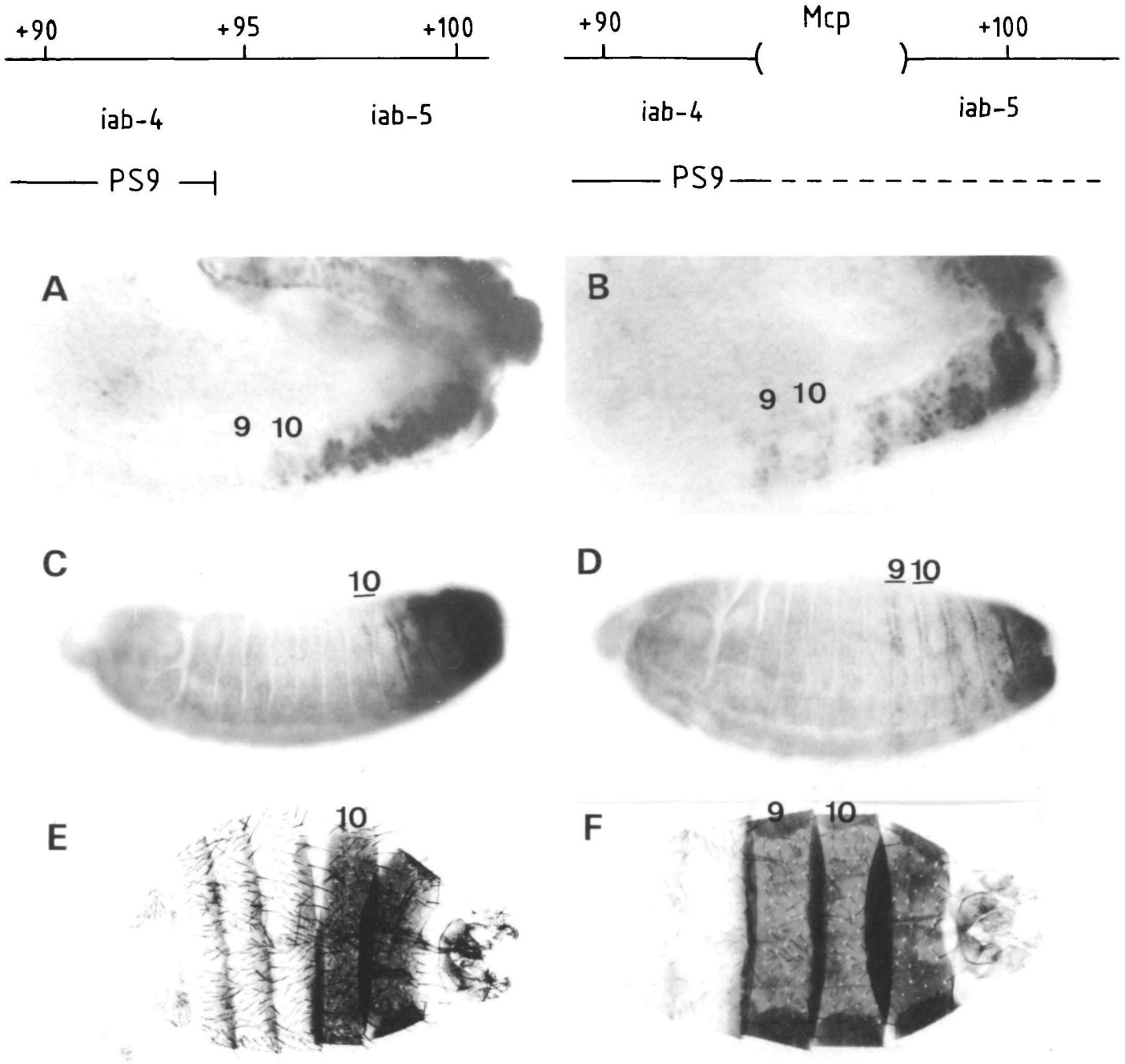


Fig. 6. Interpretation of the *Mcp* phenotype (according to Gyurkovics *et al.* 1990), comparison of *Abd-B* expression and adult cuticular patterns in wild-type and *Mcp* animals. On top, wild-type and *Mcp* DNA (*Mcp* is a deficiency whose extent is represented by brackets). Numbers refer to kilobases. In wild-type embryos, the *iab-4* regulatory sequences are activated in PS9 (and posterior parasegments), while *iab-5* sequences are activated in PS10 (and posterior parasegments). In *Mcp* embryos the 'boundary' between the two *iab* regions is eliminated and the PS9 'open' configuration 'spreads' to *iab-5* regulatory sequences (discontinuous line), that are now active in PS9, and make *Abd-B* to be expressed in this parasegment (Gyurkovics *et al.* 1990; see B and D). (A) Wild-type embryonic *Abd-B* pattern in the ventral cord, shown in detail. In wild-type embryos (A), the anterior limit of expression is PS10. There is no label in PS9. (B) In *Mcp* embryos there is now *Abd-B* expression in PS9. Note that PS9 levels of expression are a replication of those of PS10. (C) Wild-type embryonic *Abd-B* pattern in the epidermis. The anterior limit of *Abd-B* expression is PS10. (D) In *Mcp* embryos the anterior limit is PS9. (E) Wild-type abdomen of an adult male. (F) *Mcp* adult male abdomen. Note transformation of A4 (PS9), into A5 (PS10), easily detected by pigmentation patterns, as a result of the ectopic *Abd-B* expression (the transformation, however, is in part to A6, Duncan, 1987).

embryos homozygous for the *m* mutation *Abd-B*^{M5}, *abd-A* signal is observed at high levels in PS13 (not shown; Macías *et al.* 1990), while in wild-type embryos only some cells of this parasegment express *abd-A* and at lower levels (Macías *et al.* 1990; Karch *et al.* 1990).

In the absence of the *r* element, PS14 is partially transformed into PS13. This transformation is due to the expression of the *m* element of *Abd-B* in this parasegment, and suggests one of the functions of the *r* element is to suppress *m* expression in PS14 (Casanova *et al.* 1986). The expression of *Abd-B* was studied in the *r* mutations *tuh-3*, *Uab*^{1} and *Tab*^{R96}. Only with the first one, a weak *r* allele, could homozygous embryos be unambiguously identified (by the use of the *TM3, hb-β gal* chromosome). None of the embryos from the stocks carrying any of these three *r* mutations presented a major change in PS14 *Abd-B* distribution compared with wild-type embryos, as previously reported (DeLorenzi and Bienz, 1990), although in some of them the expression seemed weaker in this parasegment. That the change in PS14, if any, is small, is possibly explained because the *m* protein could be present in this parasegment in the absence of the *r* protein (DeLorenzi and Bienz, 1990). Since the antibody cannot distinguish between the two proteins, the alterations in PS14 result in an apparent lack of change.

r mutations also affect the expression of *abd-A*. In embryos homozygous for the *Uab*^{1}, *Tab*^{R96} and much more rarely, *tuh-3* mutations, there are nuclei labelled with anti-*abd-A* antibody posteriorly to PS13. The ectopic expression is in the posterior region of A8 and in some cells of the posterior region of A9 (Fig. 7A). This has also been observed in other *r* mutations and mutant combinations (Macías *et al.* 1990).

In the absence of both the *m* and *r* elements (as in embryos homozygous for the *Abd-B*^{M1} mutation), the *abd-A* pattern is the addition of those two described above: *abd-A* protein is observed in almost the whole PS13 and PS14 and in many nuclei of PS15 (not shown; Macías *et al.* 1990).

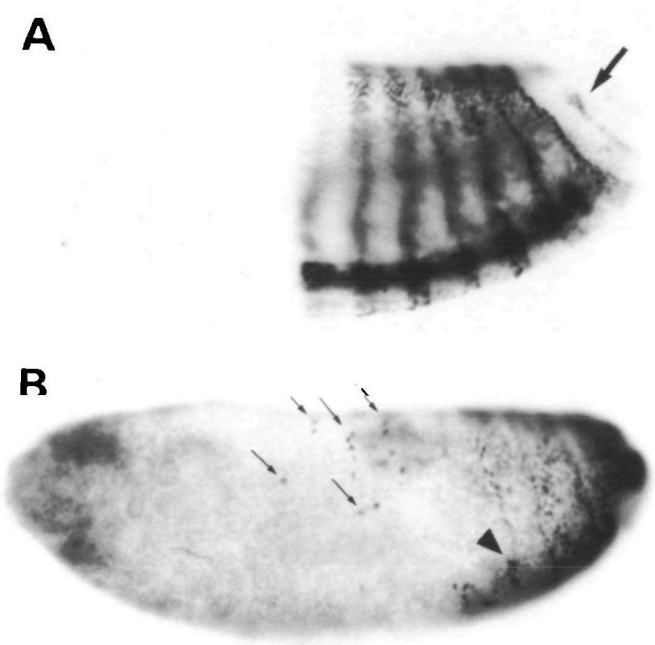


Fig. 7. *abd-A* and *Abd-B* expression in *Uab*^{1} embryos. (A) Homozygous embryo presenting *abd-A* expression in PS14 (arrow) and PS15. (B) This embryo is either homozygous or heterozygous for *Uab*^{1}. Note ectopic *Abd-B* expression (arrows), and the abnormally strong signal in some cells within an otherwise weakly labelled parasegment in the *Abd-B* domain (arrowhead).

Discussion

In the bithorax complex, there are three protein-coding transcription units and a series of regulatory regions identified by mutations that transform particular parasegments in the embryo (Duncan, 1987) and possibly also in the adult. Homeobox-containing genes homologous to those of the BX-C are also present in clusters in the mouse genome. Moreover, the order of their expression in the anteroposterior axis of this animal is colinear with the order of the genes on the chromosome, as in *Drosophila* (Lewis, 1978; Gaunt *et al.* 1988; Graham *et al.* 1989; Duboule and Dollé, 1989; Akam, 1989). It has even been suggested that *iab* regulatory regions might play the role of mammalian homeoproteins present in homologous complexes (Acampora *et al.* 1989). Therefore, the study of how BX-C genes are deployed may prove to be rewarding for elucidating development mechanisms in different species.

The model of Peifer *et al.* 1987 predicts that each regulatory region is activated from an anterior parasegmental border posteriorly and that these regulatory domains control in *cis* the expression of *Ubx*, *abd-A* and *Abd-B*. *cis*-regulatory regions regulate the expression of *Ubx* in PS5–13 and PS6–13 (Hayes *et al.* 1984; Struhl, 1984; Casanova *et al.* 1985; Cabrera *et al.* 1985; White and Wilcox, 1985; Beachy *et al.* 1985; Peifer and Bender, 1986). I have shown here that this seems also to be the case for the *Abd-B* and, probably, the *abd-A* gene. The results are more clear for the former than for the latter, although this may be due more to the particular expression of these two genes than to a functionally different organization.

The expression of Abd-B *in PS10–12 is parasegmentally regulated by* cis*-regulatory regions*

After the germ band is extended, the *m* element of *Abd-B* (first expressed in PS13) is sequentially transcribed in PS12, PS11 and PS10 (Sánchez-Herrero and Crosby, 1988; Kuziora and McGinnis, 1988; Celniker *et al.* 1989; DeLorenzi and Bienz, 1990). This succesive activation in more anterior parasegments is shown here to be dependent on the presence of the *iab-5*, *iab-6* and *iab-7* regulatory regions in *cis* with the *Abd-B* transcription unit (see also Sánchez-Herrero and Akam, 1989). This is revealed by the progressive elimination of *Abd-B* (*m*) protein in more posterior parasegments with breakpoints more proximal to the transcription unit

(Fig. 2). The *Abd-B* gene has, therefore, a 50 kb regulatory region 3′ to the transcription unit. The anterior boundary of *r* element expression is also strictly parasegmental (Casanova *et al.* 1986; Sanchez-Herrero and Crosby, 1988; DeLorenzi *et al.* 1988; Kuziora and McGinnis, 1988; DeLorenzi and Bienz, 1990). Therefore the regulation of *Abd-B* is, like that of the *Ubx* gene, parasegmental.

The abd-A *gene is also probably parasegmentally regulated by* cis*-regulatory regions*

The parasegmental control of gene expression is not so evident for the *abd-A* gene. There are two explanations for this difference. First, the positioning of regulatory sequences with respect to the transcription unit is such that only *iab-2* breakpoints would show a lack of expression in a certain parasegment. Second, the pattern of *abd-A* expression is almost identical in different parasegments (Macías *et al.* 1990; Karch *et al.* 1990), making it very hard to ascertain if a particular parasegmental transformation has occurred.

The results obtained with *iab-3* mutations suggest that the *iab-3* region controls the parasegmental expression of *abd-A* in PS8 and posterior ones. This parasegmental control is not so clear for *iab-2* and *iab-4* regions. *DfP10*, which removes the *iab-2* regulatory region, eliminates or greatly reduces *abd-A* expression in the epidermis of PS7, but not in the ventral nerve cord, making it difficult to precisely define the *iab-2* regulatory region. Although I have not detected any change in *abd-A* staining pattern in *iab-2* or *iab-4* mutations, Karch *et al.* (1990) provide evidence for such changes in the expected parasegments. Taking all the results together, and considering the parasegmental transformations of most *iab* mutations (Duncan, 1987; Busturia *et al.* 1989) and the parasegmental regulation of *Ubx* and *Abd-B*, it seems likely that the *abd-A* expression is regulated in a parasegmental manner as well. Although the dominant effects observed in the adult cuticle of heterozygotes for several *iab* mutations may suggest a different type of regulation (Busturia *et al.* 1989), these phenotypic effects do not preclude the existence of *abd-A* parasegmental regulation by *iab-2*, *iab-3* and *iab-4* sequences.

Dominant mutations and the order of regulatory regions

The model of Peifer *et al.* (1987) predicts there are multiple tissue-specific enhancers within each parasegmental DNA domain. This hypothesis may explain the differences of expression in PS11 epidermis and ventral cord of embryos homozygous for $iab\text{-}6^{105}$ or $iab\text{-}6^{C1}$ mutations (compare Figs 2C and 3C). It is possible that, in the two breakpoints, the sequences controlling *Abd-B* expression in some PS11 cells of the ventral cord remain in *cis* with the *Abd-B* transcription unit. If there are such multiple enhancers that control *abd-A* and *Abd-B* expression in different tissues, they are probably spread on the chromosome though belonging to different parasegmental domains. This correspondence with a particular metamere relates to the hypothesis of boundaries that isolate domains on the chromosome (Gyurkovics *et al.* 1990). These authors propose that there are 'borders' on the DNA separating each parasegmental region and that the *Mcp* and *Fab* mutations, which are small deficiencies, remove such boundaries, activating a regulatory region in a more anterior parasegment. In *Mcp* embryos there is indeed a perfect reproduction of *Abd-B* PS10 levels of expression in PS9 (see Fig. 6).

As pointed out by Gyurkovics *et al.* (1990), dominant mutations in which the pattern of *abd-A* or *Abd-B* is exactly reproduced in a more anterior parasegment constitute a special class and are very rare in the BX-C. The rest of the dominant mutations produce ectopic *abd-A* or *Abd-B* expression in just some cells of more anterior parasegments (Celniker and Lewis, 1987; Karch *et al.* 1990; Macías *et al.* 1990; this report; Fig. 5).

The proposed 'spreading' occurring in the *Mcp* mutation requires *iab-4* and *iab-5* regulatory regions to be contiguous. Colinearity between the order of regulatory sequences and parasegmental expression is, in fact, a main characteristic of the BX-C complex (Lewis, 1978). The study of the Uab^{1} mutation bears on this issue because it is an inversion within the complex in which the proximity of some enhancers to their promoters is changed. The ectopic *Abd-B* expression observed in mutant embryos may be due to the presence of *bxd* regulatory sequences close to the *Abd-B* promoters. This would result in the described phenotype of patches of genital and posterior abdominal tissue in segments A2–A7 (Duncan, 1987; Kuhn and Packert, 1988). Similarly, part of the changes in *abd-A* expression might be due to the proximity of *Abd-B* (*r*) regulatory sequences with respect to the *abd-A* promoter (or *iab-2* region), resulting in the complex phenotypes observed (Lewis, 1978; Karch *et al.* 1985; Busturia *et al.* 1989).

Recent models (Reuter *et al.* 1990; Gaunt and Singh, 1990) propose a mechanism for the activation or stabilization of *iab* regulatory domains that require a spreading mechanism of a multimeric protein complex on the chromosome. This complex would 'close' different *iab* regions in a 5′ to 3′ (distal to proximal) direction, and would stop once a certain regulatory domain is active. This activation would be determined by segmentation genes in the early embryo. The inversion in the Uab^{1} chromosome would prevent or alter such a mechanism (at least for the Ultrabithorax region), unless an independent 'spreading' is supposed for the *Ubx* gene. Independence is also required to explain the lack of phenotype of genetic combinations that split the BX-C (Struhl, 1984; Tiong *et al.* 1987).

Although the need for the alignment of regulatory sequences on the chromosome is not clear, it may have functional significance. The abundance of dominant effects associated with breakpoints in the *iab* region (Karch *et al.* 1985; Lewis, 1985; Duncan, 1987; Busturia *et al.* 1989) suggest that proper expression of the gene they control requires a close contact of regulatory sequences or a certain chromatin structure.

The interactions between abd-A *and* Abd-B

It has been shown that the absence of *Abd-B* products results in an extension of the posterior limit of *abd-A* expression (Macías *et al.* 1990; Karch *et al.* 1990; this report). This repression of a certain gene by another acting more posteriorly also occurs between other homeotic genes (Hafen *et al.* 1984; Struhl and White, 1985; Harding *et al.* 1985; Carroll *et al.* 1986; Wirz *et al.* 1986; Riley *et al.* 1987; Macías *et al.* 1990). The case of *abd-A* and *Abd-B* is unusual in that it happens posteriorly to PS12; that is, only in part of the *Abd-B* domain. This may be due to any of these reasons. (1) There are distinct *Abd-B* products in the PS10–13 region. (2) *Abd-B* protein appears too late in PS10–12 to be effective. (3) Only the high levels of *Abd-B* protein present in PS13–15 (Celniker *et al.* 1989; DeLorenzi and Bienz, 1990) are capable of repressing *abd-A*. (4) A third factor, only present in PS13–15, is required.

The apparent lack of major change in PS14 *Abd-B* expression in r^- homozygous embryos is consistent with the hypothesis that the *m* element is now expressed in PS14 (Casanova *et al.* 1986), since the antibody used cannot distinguish between the *m* and *r* proteins (Celniker *et al.* 1989). The ectopic expression of *abd-A* in these embryos is also consistent with this hypothesis. In wild-type embryos, the *r* element suppresses *abd-A* and *Abd-B* (*m*) expression in PS14 (Casanova *et al.* 1986), while the *m* element only prevents *abd-A* expression in the anterior (but not the posterior) compartment of PS13. Therefore, in r^- homozygous embryos, which have high *m* expression in PS13 and PS14, there is *abd-A* expression only in the posterior compartment of these two parasegments (and PS15 as well). More difficult to explain is why $Abd\text{-}B^{M1}$ homozygous embryos do not present any characteristics of *abd-A* pattern in the cuticle of PS14 (for example the suppression of sclerotic plates, which are suppressed by *abd-A* in PS7–13, but not PS14, of these embryos). It might be that *abd-A* expression in PS14 occurs too late to give a cuticular phenotype.

I thank G. Morata for his encouragement throughout this work and for comments on the manuscript, S. Celniker for the *Abd-B* antibody and the Tab^{R96} mutation, J. Casanova for the *abd-A* antibody and comments on the manuscript and G. Struhl for the *TM3, hb-β gal* balancer chromosome. I also thank A. Macías for communicating results before publication, help with the antibody stainings and multiple discussions, F. Karch for communicating results before publication, I. Guerrero, J. Sampedro, A. González-Reyes and M. Akam for comments on the manuscript, E. Reoyo and R. González for mounting the cuticle of adult flies and R. Sanchez and R. Uña for the photographic work. This work has been supported by the Dirección General de Investigación Científica y Técnica and by the Fundación Ramon Areces.

References

Acampora, D., D'esposito, M., Faiella, A., Pamese, M., Migliaccio, E., Morelli, F., Stornaiuolo, A., Nigru, V., Simeone, A. and Boncinelli, X. (1989). The human HOX gene family. *Nucl. Acids Res.* **17**, 10385–10402.

Akam, M. (1989). HOX and HOM: Homologous gene clusters in insects and vertebrates. *Cell* **57**, 347–349.

Beachy, P. A., Helfand, S. L. and Hogness, D. S. (1985). Segmental distribution of bithorax complex proteins during *Drosophila* development. *Nature* **313**, 545–551.

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.

Busturia, A., Casanova, J., Sánchez-Herrero, E., González, R. and Morata, G. (1989). Genetic structure of the *abd-A* gene of *Drosophila*. *Development* **107**, 575–583.

Cabrera, C., 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., Laymon, R. A., McCutcheon, M. A., Riley, P. and Scott, M. P. (1986). The localization and regulation of *Antennapedia* protein expression in *Drosophila* embryos. *Cell* **47**, 113–122.

Casanova, J., Sánchez-Herrero, E., Busturia, A. and Morata, G. (1987). Double and triple mutant combinations of the bithorax complex of *Drosophila*. *EMBO J.* **6**, 3103–3109.

Casanova, J., Sánchez-Herrero, E. and Morata, G. (1985). Prothoracic transformation and functional structure of the *Ultrabithorax* gene of *Drosophila*. *Cell* **42**, 663–669.

Casanova, J., Sánchez-Herrero, E. and Morata, G. (1986). Identification and characterization of a parasegment specific regulatory element of the *Abdominal-B* gene of *Drosophila*. *Cell* **47**, 627–636.

Celniker, S., 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 and Dev.* **3**, 1424–1436.

Celniker, S. and Lewis, E. (1987). *Transabdominal*, a dominant mutant of the bithorax complex, produces a sexually dimorphic segmental transformation in *Drosophila*. *Genes and Dev.* **1**, 111–123.

Cumberledge, S., Zaratzian, A. and Sakonju, S. (1990). Characterization of two RNAs transcribed from the *cis*-regulatory region of the *abd-A* domain within the *Drosophila* bithorax complex. *Proc. natn. Acad. Sci U.S.A.* **87**, 3259–3263.

DeLorenzi, M., Ali, N., Saari, G., Henry, C., Wilcox, M. and Bienz, M. (1988). Evidence that the *Abdominal-B r* element function is conferred by a trans-regulatory homeoprotein. *EMBO J.* **7**, 3223–3231.

DeLorenzi, M. and Bienz, M. (1990). Expression of *Abdominal-B* homeoproteins in *Drosophila* embryos. *Development* **108**, 323–329.

Duboule, D. and Dollé, P. (1989). The structural and functional organization of the murine HOX family resembles that of *Drosophila* homeotic genes. *EMBO J.* **8**, 1497–1505.

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

Gaunt, S. J., Sharpe, P. T. and Duboule, D. (1988). Spatially restricted domains of homeo-gene transcripts in mouse embryos: relation to a body plan. *Development* **104 Supplement**, 169–179.

Gaunt, S. J. and Singh, P. B. (1990). Homeogene expression patterns and chromosomal imprinting. *Trends Genet.* **6**, 208–212.

Gehring, W. and Hiromi, Y. (1986). Homeotic genes and the homeobox. *A. Rev. Genet.* **20**, 147–173.

Graham, A., Papalopulu, N. and Krumlauf, R. (1989). The murine and *Drosophila* homeobox genes complexes have common features of organization and expression. *Cell* **57**, 367–378.

Gyurkovics, H., Gausz, J., Kummer, J. and Karch, F. (1990). A new homeotic mutation in the *Drosophila* bithorax complex removes a boundary separating two domains of regulation. *EMBO J.* **9**, 2579–2586.

Hafen, E., Levine, M. and Gehring, W. J. (1984). Regulation of

Antennapedia transcript distribution by the bithorax complex in *Drosophila*. *Nature* **307**, 287–289.

HARDING, K., WEDEEN, C., MCGINNIS, W. AND LEVINE, M. (1985). Spatially regulated expression of homeotic genes in *Drosophila*. *Science* **229**, 1236–1242.

HARRISON, D. A., GEYER, P. K., SPANA, C. AND CORCES, V. G. (1989). The gypsy retrotransposon of *Drosophila melanogaster*: mechanisms of mutagenesis and interaction with the suppressor of Hairy-wing locus. *Dev. Genet.* **10**, 239–248.

HAYES, P. H., SATO, T. AND DENELL, R. (1984). Homoeosis in *Drosophila*: The *Ultrabithorax* larval syndrome. *Proc. natn. Acad. Sci. U.S.A.* **81**, 545–549.

HOGNESS, D. S., LIPSHITZ, H. D., BEACHY, P. A., PEATTIE, D. A. AND SAINT, R. B. (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.

KARCH, F., WEIFFENBACH, B. AND BENDER, W. (1990). *abdominal-A* expression in *Drosophila* embryos. *Genes and Dev.* (in press).

KARCH, F., WEIFFENBACH, B., PEIFER, M., BENDER, W., DUNCAN, I., CELNIKER, S., CROSBY, M. AND LEWIS, E. B. (1985). The abdominal region of the bithorax complex. *Cell* **43**, 81–96.

KAUFMAN, T. C. (1983). The genetic regulation of segmentation in *Drosophila melanogaster*. In *Time, Space and Pattern in Embryonic Development*, (ed. W. R. Jeffery, R. A. Raff), pp. 365–383. New York: Liss.

KAUFMAN, T. C., LEWIS, R. AND WAKIMOTO, B. (1980). Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: the homeotic gene complex in polytene chromosomal interval 84 A, B. *Genetics* **94** 115–133.

KUHN, D. T. AND PACKERT, G. (1988). Tumorous-head-type mutants of the distal bithorax complex cause dominant gain and recessive loss of function in *Drosophila melanogaster*. *Devl Biol.* **125**, 8–18.

KUHN, D. T., WOODS, D. F. AND COOK, J. L. (1981). Analysis of a new homeotic mutation (*iab-2*) within the bithorax complex in *Drosophila melanogaster*. *Molec. gen. Genet.* **181**, 82–86.

KUZIORA, M. A. AND MCGINNIS, W. (1988). Different transcripts of the *Drosophila Abd-B* gene correlate with distinct genetic sub-functions. *EMBO J.* **7**, 3233–3244.

LEWIS, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565–570.

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

LINDSLEY, D. AND ZINN, G. (1985). The genome of *Drosophila melanogaster*. *Dros. Inf. Serv.* **62.**

LINDSLEY, D. AND ZINN, G. (1989). The genome of *Drosophila melanogaster*. *Dros. Inf. Serv.* **68.**

LIPSHITZ, H. D., PEATTIE, D. A. AND HOGNESS, D. S. (1987). Novel transcripts from the *Ultrabithorax* domain of the bithorax complex. *Genes and Dev.* **1**, 307–322.

MACDONALD, P. M. AND STRUHL, G. (1986). A molecular gradient in early *Drosophila* embryos and its role in specifying the body pattern. *Nature* **324**, 537–545.

MACÍAS, A., CASANOVA, J. AND MORATA, (1990). Expression and regulation of the *abd-A* gene of *Drosophila*. *Development* (in press).

MAHAFFEY, J. W. AND KAUFMAN, T. C. (1988). The homeotic genes of the Antennapedia complex and the bithorax complex of *Drosophila*. In *Developmental Genetics of Higher Organisms*. (ed. G. M. Malacinsky), pp. 239–260. Macmillan, New York.

MARTÍNEZ-ARIAS, A. AND LAWRENCE, P. A. (1985). Parasegments and compartments in the *Drosophila* embryo. *Nature* **313**, 639–642.

MCGINNIS, W., LEVINE, M. S., HAFEN, E., KUROIWA, A. AND GEHRING, W. J. (1984). A conserved DNA sequence found in homeotic genes of the *Drosophila* Antennapedia and bithorax complexes. *Nature* **308**, 428–433.

MODOLELL, J., BENDER, W. AND MESELSON, M. (1983). *Drosophila melanogaster* mutations suppressible by the *Suppressor of Hairy wing* are insertions of a 7,3 kilobase mobile element. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1678–1682.

MORATA, G., BOTAS, J., KERRIDGE, S. AND STRUHL, G. (1983). Homeotic transformations of the abdominal segments of *Drosophila* caused by breaking or deleting a central portion of the Bithorax complex. *J. Embryol. exp. Morph.* **78**, 319–341.

PEIFER, M. AND BENDER, W. (1986). The *anterobithorax* and *bithorax* mutations of the bithorax complex. *EMBO J* **5**, 2293–2303.

PEIFER, M., KARCH, F. AND BENDER, W. (1987). The bithorax complex: control of segment identity. *Genes and Dev.* **1**, 891–898.

REGULSKI, M., HARDING, K., KOSTRIKEN, R., KARCH, F., LEVINE, M. AND MCGINNIS, W. (1985). Homeo box genes of the Antennapedia and bithorax complexes of *Drosophila*. *Cell* **43**, 71–80.

REUTER, G., GIARRE, M., FARAH, J., GAUSZ, J., SPIERER, A. AND SPIERER, P. (1990). Dependence of position-effect variegation in *Drosophila* on dose of a gene encoding an unusual zinc-finger protein. *Nature* **344**, 219–223.

RILEY, P. D., CARROLL, S. B. AND SCOTT, M. P. (1987). The expression and regulation of *Sex combs reduced* protein in *Drosophila* embryos. *Genes and Dev.* **1**, 716–730.

SÁNCHEZ-HERRERO, E. AND AKAM, M. (1989). Spatially ordered transcription of regulatory DNA in the bithorax complex of *Drosophila*. *Development* **107**, 321–329.

SÁNCHEZ-HERRERO, E., CASANOVA, J. AND MORATA, G. (1988). Genetic structure of the bithorax complex. *Bioessays* **8**, 124–128.

SÁNCHEZ-HERRERO, E. AND CROSBY, M. A. (1988). The *Abdominal-B* gene of *Drosophila melanogaster*. overlapping transcripts exhibit two different spatial distributions. *EMBO J.* **7**, 2163–2173.

SÁNCHEZ-HERRERO, E., VERNOS, I., MARCO, R. AND MORATA, G. (1985). Genetic organization of the *Drosophila* Bithorax complex. *Nature* **313**, 108–113.

SATO, T. AND DENELL, R. (1986). Segmental identity of caudal cuticular features of *Drosophila melanogaster* larvae and its control by the bithorax complex. *Devl Biol.* **116**, 78–91.

SCOTT, M. P., TAMKUN, J. W. AND HARTZELL, G. W. (1989). The structure and function of the homeodomain. *Biochim. biophys. Acta* **989**, 25–48.

SCOTT, M. P. AND WEINER, A. (1984). Structural relationships among genes that control development: sequence homology between the *Antennapedia*, *Ultrabithorax* and *fushi tarazu* loci of *Drosophila*. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4115–4119.

STRUHL, G. (1984). Splitting the bithorax complex of *Drosophila*. *Nature* **308**, 454–457.

STRUHL, G. AND WHITE, R. A. H. (1985). Regulation of the *Ultrabithorax* gene of *Drosophila* by other bithorax complex genes. *Cell* **43**, 507–519.

TIONG, S., BONE, L. M. AND WHITTLE, J. R. (1985). Recessive lethal mutations within the bithorax complex in *Drosophila*. *Molec. gen. Genet.* **200**, 335–342.

TIONG, S. Y. K., WHITTLE, J. R. S. AND GRIBBIN, M. C. (1987). Chromosomal continuity in the abdominal region of the bithorax complex of *Drosophila* is not essential for its contribution to metameric identity. *Development* **101**, 135–142.

TREMML, G. AND BIENZ, M. (1989). Homeotic gene expression in the visceral mesoderm of *Drosophila* embryos. *EMBO J.* **8**, 2677–2685.

WHITE, R. A. H. AND WILCOX, M. (1985). Regulation of the distribution of *Ultrabithorax* proteins in *Drosophila*. *Nature* **318**, 563–567.

WHITTLE, J. R. S., TIONG, S. Y. K. AND SUNKEL, C. E. (1986). The effect of lethal mutations and deletions within the bithorax complex upon the identity of caudal metameres in the *Drosophila* embryo. *J. Embryol. exp. Morph.* **93**, 153–166.

WIRZ, J., FESSLER, L. I. AND GEHRING, W. J. (1986). Localization of the *Antennapedia* protein in *Drosophila* embryos and imaginal discs. *EMBO J.* **5**, 3327–3334.

ZAVORTINK, M. AND SAKONJU, S. (1989). The morphogenetic and regulatory functions of the *Drosophila Abdominal-B* gene are encoded in overlapping RNAs transcribed from separate promoters. *Genes and Dev.* **3**, 1969–1981.

(Accepted 25 October 1990)