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A new homeotic mutation in the *Drosophila* bithorax complex removes a boundary separating two domains of regulation

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The bithorax complex specifies the identity of parasegments 5–14 of *Drosophila*. Although nine parasegment-specific functions, *abx/bx, bxd/pbx* and *iab-2* to *iab-8,9* have been identified, the whole bithorax complex appears to encode only three classes of proteins, *Ubx, abd-A* and *Abd-B*. Many observations suggest that the parasegment-specific functions act as positive *cis*-regulatory elements of *Ubx, abd-A* and *Abd-B*. We report the molecular genetics of a new gain-of-function mutation, *Fab-7*, which transforms parasegment 11 into parasegment 12. Induction of *Abd-B* mutations in *cis* (one of which removes the *Abd-B* homeobox) causes reversion of the dominant phenotype, demonstrating that *Fab-7* misregulates *Abd-B*. A 4 kb deletion, 30 kb downstream from the *Abd-B* transcription unit, is solely responsible for the *Fab-7* phenotype. We consider that the parasegment-specific functions lie in DNA domains that are sequentially and independently 'opened' along the chromosome. Once a domain is opened, the *cis*-regulatory sequences within it can carry out their function. We propose that the *Fab-7* deletion removes a boundary separating the *iab-6* and *iab-7 cis*-regulatory regions (the functions specific for parasegments 11 and 12) allowing the open configuration of *iab-6* to invade *iab-7* in parasegment 11. This is strongly supported by our finding that *Fab-7* can be caused to revert by lesions not only in *iab-7* but also in *iab-6*.

Key words: Abd-B/bithorax complex/*cis*-regulation/DNA boundary/gain-of-function mutation

Introduction

The bithorax complex (BX-C) specifies the identity of the region of the body of *Drosophila* extending from parasegment 5 (PS5) to 14 (PS14). These 10 parasegments together build up the 3rd thoracic (T3) and the 8 abdominal segments (A1–A8) of the adult fly (Lewis, 1978; for a definition of parasegments, see Martinez-Arias and Lawrence, 1985). Mutations have been identified that affect each of the parasegments under the control of the BX-C. They define nine parasegment specific functions named *abx/bx, bxd/pbx* and *iab-2–iab-8,9* that are aligned on the chromosome in the same order as the parasegments on the body of the fly (Lewis, 1978; Bender *et al.*, 1983; Karch *et al.*, 1985; reviewed in Duncan, 1987).

Genetic and molecular analyses of lethal complementation groups reveal that the BX-C contains only three genes, *Ubx, abd-A* and *Abd-B*, which account for all the morphogenic functions of the BX-C (Sanchez-Herrero *et al.*, 1985; Tiong, *et al.*, 1985; Beachy *et al.*, 1985; Casanova *et al.*, 1987; Celniker and Lewis, 1987; O'Connors *et al.*, 1988; De Lorenzi *et al.*, 1988; Kuziora and McGinnis, 1988; Kornfeld *et al.*, 1989; Karch *et al.*, 1990). Complementation analysis and staining of whole embryos with antibodies directed against the *Ubx* or *abd-A* proteins indicate that the parasegment-specific functions are positive *cis*-regulatory elements that specify the spatial distribution of *Ubx, abd-A* or *Abd-B* in the parasegment they control (see Peifer *et al.*, 1987 and Sanchez-Herrero *et al.*, 1988).

Despite extensive attempts, the *Abd-B* region remains poorly mapped in mutants. Recent reports showed that the *Abd-B* domain can encode multiple transcripts with two different spatial distributions, all sharing the *Abd-B* homeobox at their 3′ end. A short transcription unit is expressed from parasegment 10–13 (A transcript; see Figure 3) and three longer transcription units only in parasegment 14 (B, C and γ transcripts; see Figure 3; Celniker and Lewis, 1987; Sanchez-Herrero and Crosby, 1988; DeLorenzi *et al.*, 1988; Kuziora and McGinnis, 1988; Zavortink and Sakonju, 1989). The *Abd-B* transcription unit is not sufficient to achieve proper development of parasegments 10–14 as the *iab-5,6,7* and *8,9* regions are also required in *cis* (Karch *et al.*, 1985; Duncan, 1987; see Figure 3).

In this paper we describe a dominant gain-of-function mutation, *Frontabdominal-7 (Fab-7)* that misregulates *Abd-B* by ectopic activation of the parasegment 12 specific function (*iab-7*) in PS11. The molecular genetics of *Fab-7* suggest a possible model for the functional organization of the parasegment-specific regulatory elements.

Results

The Fab-7 dominant gain-of-function mutation

Among the progeny of X-rayed Oregon R flies, we found a dominant homeotic mutation, *Frontabdominal 7 (Fab-7)*, transforming A6 into A7. In adult homozygous *Fab-7* females, A6 is replaced by an exact copy of A7. Males have only 5 abdominal segments because A7 does not contribute to the adult cuticle apart from a pair of tracheal openings and a pair of sensory hairs on the sternite also present in *Fab-7* posterior to A5 as duplicates (Figure 1b). Both sexes are fully viable and fertile. The larval phenotype suggests that the effect of *Fab-7* is parasegmental. In wild type larvae, the shape of the band of dorsal hairs belonging to posterior A5 (pA5) is intermediate between the corresponding bands of hairs of pA4 and pA6 (Figure 1c). In *Fab-7*, however, the pA5 band of hairs closely resembles the pA6 band (Figure 1d). Similarly, the band of hairs of anterior A6 (aA6) resembles that of aA7. *Fab-7* fails to rescue the haplo-

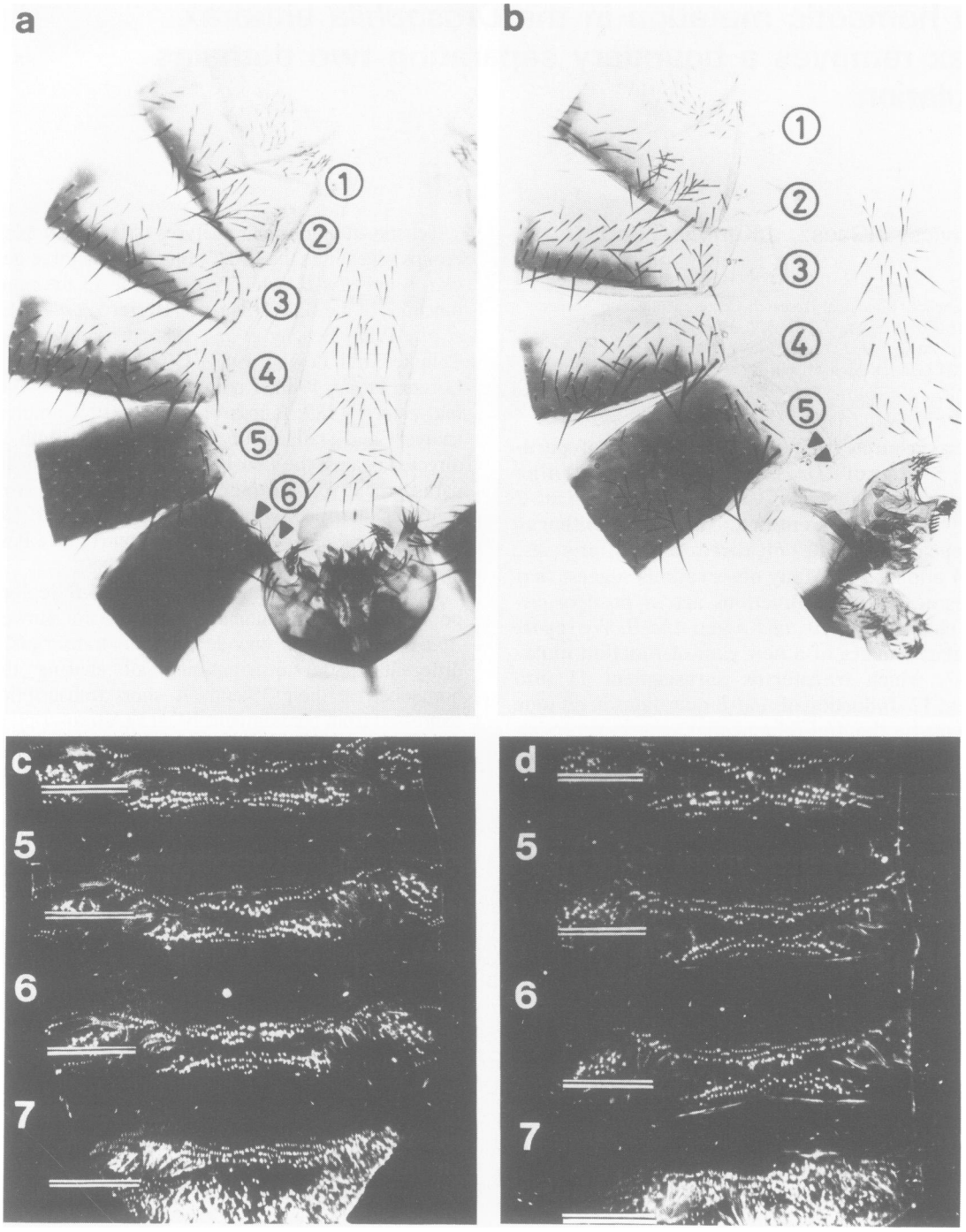

Fig. 1. Larval and adult segmental transformations in *Fab-7*. **Above**: Whole mounts of adult male abdominal cuticle (for a detailed description of the cuticle anatomy see Karch *et al.*, 1985). (**a**) wild-type Ore-R 369. (**b**) *Fab-7* homozygotes. The abdominal segments are numbered and the 6th and 7th tracheal openings are shown by arrowheads. The 6th tergite and sternite are completely absent in *Fab-7*. **Below**: The posterior part of dorsal larval cuticle (3rd instar). (**c**) wild-type Ore-R 369 and (**d**) *Fab-7* homozygotes. Abdominal segments are numbered. Each band of dorsal hairs is composed of a group of hairs pointing toward the front of the larvae which belong to the posterior compartment of one segment and a group of hairs pointing posteriorly belonging to the anterior compartment of the next segment. Segmental boundaries, indicated by horizontal lines, run somewhere in the narrow stripe of naked cuticle separating the two group of hairs (Jürgens, 1988). In wild-type larvae, the shape of the band of hairs belonging to posterior A5 and anterior A6 (pA5−aA6) is intermediate between the corresponding bands of hairs of pA4-aA5 and pA6−aA7. In *Fab-7*, however, the pA5−aA6 band of hairs closely resembles the pA6−aA7 band, suggesting that the transformation is parasegmental and effects parasegment 11.

insufficient phenotype in A7 of a deletion of the whole or distal part of the BX-C (like *Df(3R)P9* or *Df(3R)C4)*, indicating that the mutation is not a hypermorphic allele of the *Abd-B* gene.

We have mapped *Fab-7* by recombination to ~0.015 map units (data not shown) proximal to a relatively distal *iab-7* mutation (*iab-7*^{R73}; see below). We have identified and cloned a 4 kb deletion on the *Fab-7* chromosome at position

+124, between the *iab-6* and *iab-7* segment-specific functions (Figure 3). As the molecular position of this lesion agrees well with the genetic mapping data, this deficiency is probably solely responsible for the Fab-7 phenotype.

Revertants of Fab-7

To analyze the relationships between *Fab-7* and *iab-6/iab-7* on the one hand and between *Fab-7* and *Abd-B* on the other hand, a set of revertants was induced (Table I). *Fab-7* revertants can be divided into three classes on the basis of their loss-of-function phenotype. Although we did not separate the *Fab-7* lesion from the second-site mutations, the loss-of-function phenotypes of these revertants could be fairly accurately inferred.

The first class, $Abd\text{-}B^{R3}$, $Abd\text{-}B^{R9}$, $Abd\text{-}B^{R41}$ and $Abd\text{-}B^{R45}$, corresponds to *Abd-B* alleles as defined by Duncan (1987). $Abd\text{-}B^{R3}$ is indistinguishable from deficiencies deleting *Abd-B* (e.g. *DfC4*) and thus is equivalent to group 3 of Sanchez-Herrero and Crosby (1988; see Table 1). This is in agreement with our finding that $Abd\text{-}B^{R3}$ carries a 0.5 kb deletion, removing the homeobox at position +154 (see Figure 3). Since none of the available mutations from *iab-5* to *iab-8,9* can complement $Abd\text{-}B^{R3}$, we suggest that all functions of the *Abd-B* domain depend on the presence of the homeobox.

Two revertants, $Abd\text{-}B^{R41}$ and $Abd\text{-}B^{R9}$, are similar to $Abd\text{-}B^{S1}$ (Tiong *et al.*, 1985, 1988) and $Abd\text{-}B^{D14}$ (Karch *et al.*, 1985) and thus belong to group 1 of *Abd-B* alleles (Table I). $Abd\text{-}B^{R41}$ and $Abd\text{-}B^{R9}$ were localized and cloned. Both of them carry an insertion of foreign DNA of 0.5 kb at positions +155 and +157.5 respectively (Figure 3). Since many other mutations (apparently true point mutants) have very similar phenotypes (Sanchez-Herrero *et al.*, 1985; Karch *et al.*, 1985; Tiong *et al.*, 1985), it is possible that $Abd\text{-}B^{R9}$ and $Abd\text{-}B^{R41}$ interfere with a particular subfunction of *Abd-B* transcripts also called 'm element' by Casanova *et al.* (1986).

Based on its larval phenotype and complementation behavior, $Abd\text{-}B^{R45}$ resembles mutations of group 4 of Sanchez-Herrero and Crosby (1988; see Table 1). However, $Abd\text{-}B^{R45}$ differs from all the other members of this group in giving rise to occasional adult survivors with a unique phenotype. In adult males, A7 is incompletely transformed into A6, whereas A5 and A6 are almost wild-type (note that A6 and A7 have characteristics of both segments due to the presence of *Fab-7*). There is a small tergite in A8 and a sternite similar in shape to that of the transformed A7. Posterior to A8 a small 9th tergite-like structure is visible. There is no trace of a 9th sternite (see Figure 2). In most cases the genitalia and the analia are missing in both sexes. Very rarely, however, almost normal male genitalia and analia develop. In this case, the 9th tergite-like structure seems to replace the phragma of the genital arch (see Figure 2c). $Abd\text{-}B^{R45}$ carries a 5 kb insertion at position +155, within the same 1.25 kb restriction fragment, where the $Abd\text{-}B^{R41}$ insertion was found (Figure 3).

Revertants of the second class are similar to $iab\text{-}7^{SGA62}$ in which A6 and A7 are transformed into A5 as illustrated in Figure 4. In this case, males hemizygous for a deletion (*Df(3R)P9*) exhibit seven abdominal segments (Awad *et al.*, 1981; Karch *et al.*, 1985; Duncan, 1987). We have identified the lesion of $iab\text{-}7^{R73}$ as a small deletion of 1 kb at +144 (Figure 3). A second such revertant, $iab\text{-}7^{R7}$, is due to an inversion that breaks the bithorax DNA at +142 (Figure 3). Other revertants of this class ($iab\text{-}7^{HR1}$ and $iab\text{-}7^{HR3}$) result from rearrangement breaks which probably also disrupt the continuity of the *iab-7* region, indicating that *Fab-7* activates the *iab-7* function in A6 (PS 11).

The third class of revertants, $iab\text{-}6^{R44}$ and $iab\text{-}6^{HR2}$, are rearrangement breaks in the *iab-6* region (Karch *et al.*, 1985; Duncan, 1987). They cause the dominant Fab-7 phenotype to revert completely, yet they only partially inactivate *iab-7*. Figure 4 shows a hemizygous male of this class ($iab\text{-}6R^{44}/Df(3R)P9$). A marked difference is visible between the morphology of A6 and A7 (Karch *et al.*, 1985; Tiong *et al.*, 1988). We have cloned $iab\text{-}6^{R44}$ and found an inversion breakpoint at +121 (Figures 3 and 4). The leftmost chromosomal rearrangement break that causes the reversion of *Fab-7*, *T(Y;3)HR26*, is localized at position +106, near the proximal border of *iab-6* (Figures 3 and 4). In hemizygous males, A4, A5 and A6 are transformed into A3 (Figure 4). The transformation of A6, however, is incomplete: in some males, areas of the 6th tergite and sternite are missing, i.e. transformed into A7 (Figure 4b). Thus, with respect to segmental identity, cells in A6 can

Table 1.

Mutant	Cytology	Molecular map	Notes
Fab-7	Normal	123.5–127.5 deletes 4 kb	
$iab\text{-}6^{R44}$	Inversion 89C1-2, 89E3-4	121–122.5	
$iab\text{-}6^{HR2}$	Y; 3 translocation		
$iab\text{-}6^{HR26}$	Y; 3 translocation	105.5–106.5	
$iab\text{-}7^{R73}$	Normal	143–144 deletes 1kb	
$iab\text{-}7^{R7}$	Inversion 87D1-4; 89E3-4	141.5–143	
$iab\text{-}7^{HR1}$	Translocation 89E-1-4;Y	124–139.5	
$iab\text{-}7^{HR3}$	Multiple rearrangement 89E3-4		
$Abd\text{-}B^{R3}$	Normal	154–154.5 deletes 0.5 kb	*Abd-B* group 3
$Abd\text{-}B^{R9}$	Normal	157–158.76 0.5 kb insert	*Abd-B* group 1
$Abd\text{-}B^{R41}$	Normal	154.5–156 0.5 kb insert	*Abd-B* group 1
$Abd\text{-}B^{R45}$	Normal	154.5–156 5 kb insert	*Abd-B* group 4
Df(3R)R59		right endpoint 152.5	Deletes from *bxd* to *iab-7*

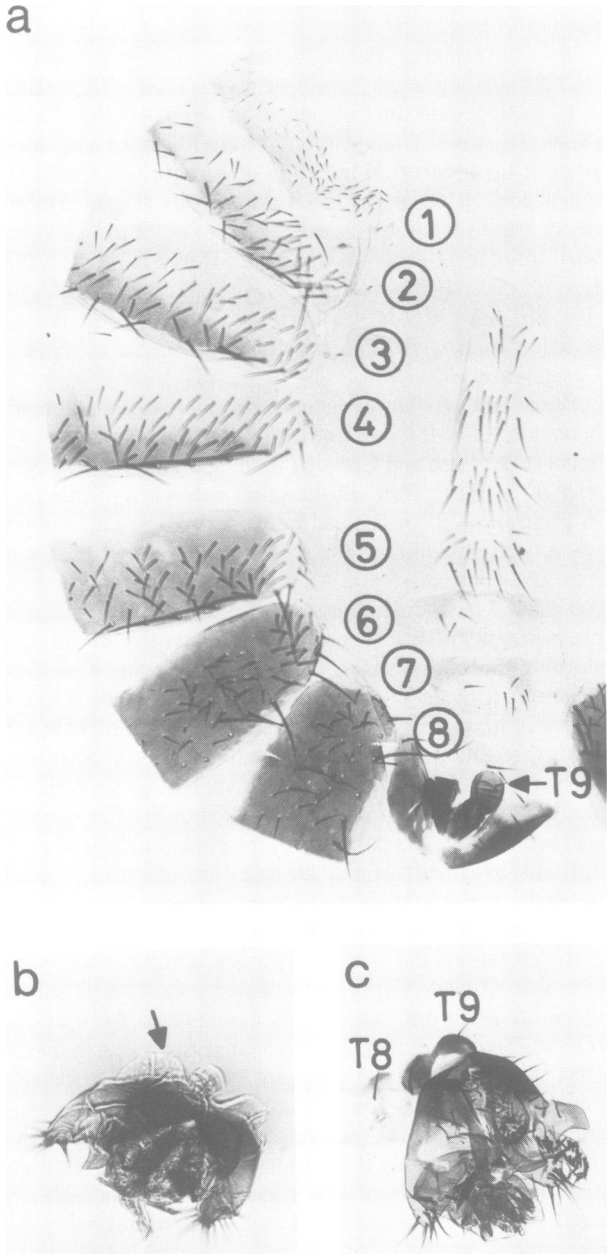

Fig. 2. Segmental transformations in adult *Abd-BR*^{45} (**a**) Photograph of whole mount of homozygous *Abd-BR*^{45} adult male abdominal cuticle (see also legend to Figure 1). Each abdominal segment is numbered and the 9th tergite is indicated by an arrow (**b**) wild-type male genitalia and analia (**c**) male genitalia and analia of *Abd-BR*^{45} homozygote. Note that the phragma indicated by an arrow in the wild-type is transformed into a 9th tergite in *Abd-BR*^{45} (T9).

assume one of two alternative states: either A3 or A7, depending on whether *iab-6* is active or not. This variegating phenotype is unlikely to be due to a heterochromatic effect of the Y chromosome because it cannot be modified by the addition of strong dominant enhancers or suppressors of variegation (Reuter,G. and Gyurkovics,H. unpublished observations). It is not clear why *T(Y;3)HR26* also inactivates *iab-5* and *iab-4*. It should here be added that we did not find revertants of *Fab-7* belonging to the canonical *iab-5* class as defined by Duncan (1987).

Finally, one revertant of *Fab-7, Df(3R)R59*, is a deficiency that lacks all the functions from *pbx* to *iab-7*. *Trans*-heterozygotes of this deficiency over *Abd-B*^{R3} or *DfC4* survive to adulthood with A5-A7 transformed into more anterior segments. Since the right endpoint of this deficiency is localized at +152.5, we conclude that all the essential coding sequences of *Abd-B* lie distal to +152.5 (Figure 3).

Discussion

Fab-7 misregulates Abd-B

Fab-7 is a dominant homeotic mutation that transforms A6 into A7. Based on the observation that the dominant phenotype can be made to revert fully by *Abd-B* mutations in *cis*, we conclude that *Fab-7* misregulates *Abd-B*. One of the revertants, *Abd-B*^{R3}, is a small deletion that removes the homeobox. This mutation fails to complement any other mutation in the *Abd-B* domain and is phenotypically indistinguishable from *Df(3R)C4*, a deletion that removes all the DNA distal to *iab-6*, demonstrating that all functions of the *Abd-B* domain depend on the presence of the homeobox. Three other revertants of *Fab-7*, *Abd-B*^{R9}, *Abd-B*^{R41} and *Abd-B*^{R45}, also appear to interfere with different subsets of the *Abd-B* functions. *Abd-B*^{R41} and *Abd-B*^{R9} transform A5, A6, A7 and A8 (PS10 to 13) into A4 (PS9). They both carry an insertion of 0.5 kb DNA at positions 155 and 157.5 respectively. These inserts are localized within the short A transcription unit (Figure 3), suggesting that *Fab-7* misregulates this class of transcripts that probably corresponds to the m element of Casanova *et al.* (1987). Although the number of mutants analyzed is small, it is interesting to note that no revertants of *Fab-7* were found distal to +157.5, raising the possibility that there is a promoter near that position. In this case any lesion to the right of this position would not prevent the production of the class of transcripts/proteins required for the proper identity of A7 (PS12). This is in agreement with the finding by Kuziora and McGinnis (1988) of the small α or A transcript that initiates at position +158 as determined by Zavortink and Sakonju (1989), and also with the observation that sequences to the right of +160 hybridize only to transcripts in PS14 (Sanchez-Herrero and Crosby, 1988).

Abd-B^{R45} only partially transforms A5, A6, A7 and A8. In addition, it also transforms A9 into more anterior structures. *Abd-B*^{R45} carries a 5 kb insertion at +155. Considering the phenotypic dissimilarities between *Abd-B*^{R41} and *Abd-B*^{R45}, further analysis of their precise localization relative to the position of the exons in this region is required to interpret the proximity of their lesions. It is tempting to speculate that *Abd-B*^{R45} primarily affects the B, C or γ transcripts, and only partially inactivates the A transcript (Kuziora and McGinnis, 1988; Zavortink and Sakonju, 1989). We note that *Abd-B*^{65}, another mutation with similar properties, is localized 12 kb distal to the *Abd-B*^{R45} insertion (see Figure 3; Karch *et al.*, 1985).

Fab-7 may remove a boundary separating two DNA domains

Examination of the larval morphology reveals that PS11 is transformed into PS12 in *Fab-7* homozygotes. Because of

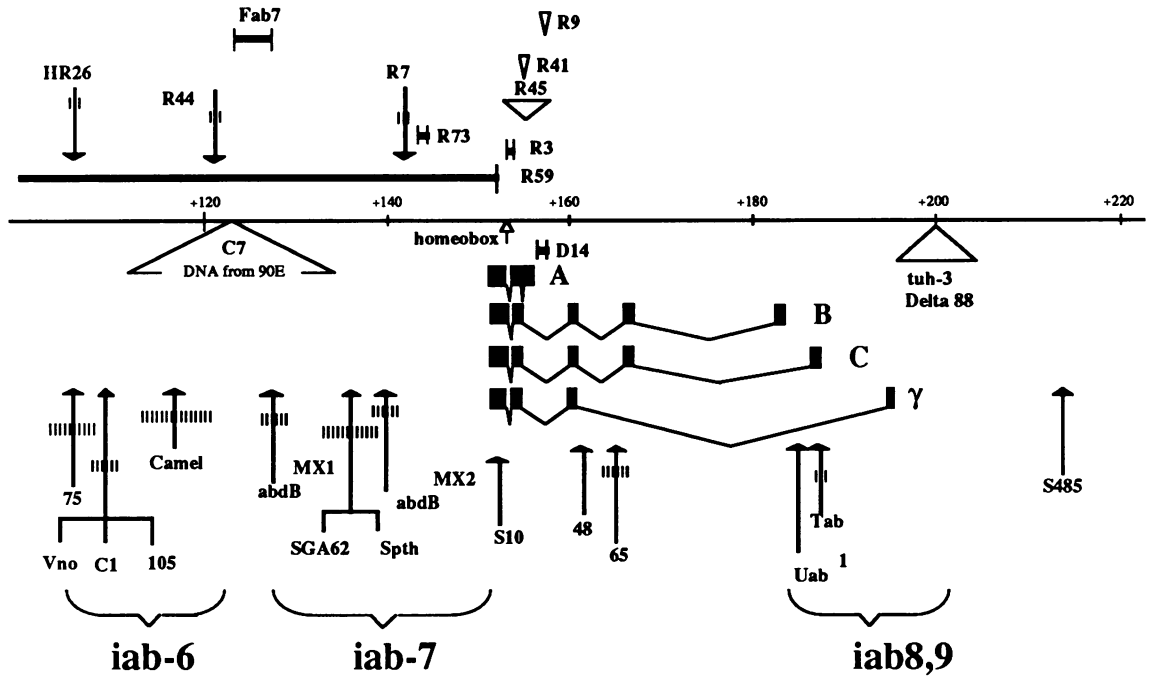

Fig. 3. Molecular map of *Fab-7* and *Fab-7* revertants. The thin line represents the DNA walk in kilobases; the sites of mutations previously localized are shown below the DNA line (Karch *et al.*, 1985) as well as the four classes of *Abd-B* transcripts (Celniker and Lewis, 1987; Kuziora and McGinnis, 1988; DeLorenzi *et al.*, 1988; Zavortink and Sakonju, 1989). The sites of the *Fab-7* mutation and its revertants are drawn above the DNA line. All DNA lesions were cloned. Vertical arrows indicate the location of chromosomal rearrangement breaks; the limits of uncertainty are represented by hatched horizontal lines. Triangles indicate DNA insertions. The thick lines represent the sequences deleted in various deficiencies. The homeobox sequence is shown by an arrow. The various classes of *iab* transformations are indicated by brackets (note that we use the terminology of Duncan, 1987).

the paucity of morphological markers, distinction between segmental and parasegmental transformation is not possible in the case of the adult abdomen posterior to A1. However, *iab-7* mutations cause the reversion of both the larval and the adult phenotype of *Fab-7*, indicating that the PS12 specific *iab-7* function is activated ectopically in PS11. The *Fab-7* mutation could be a hypermorphic allele of the *Abd-B* gene that overproduces the class of *Abd-B* protein expressed normally from PS10−13. We consider this possibility unlikely, since *Fab-7* fails to rescue the haploinsufficient phenotype of *Abd-B* mutations in A7. It is conceivable that the sequences deleted in *Fab-7* bind a factor which negatively regulates *iab-7* in PS11. However, it is not possible to reconcile this with the unexpected finding that removal (or inactivation) of *iab-6* can also cause reversion of the *Fab-7* phenotype without inactivating *iab-7* in PS12 (see Figure 4). This observation indicates that the effect of *Fab-7* depends upon the presence of *iab-6* in *cis*. The fact that no revertants were found in *iab-5* or further to the left implies that *iab-6* is autonomous with respect to ectopic activation of *iab-7*. In this context, it seems significant that the 4 kb deletion in *Fab-7* is located between *iab-6* and *iab-7*, suggesting that the two *cis*-regulatory regions are fused into a single functional unit with mixed characteristics: parasegment specificity is provided by *iab-6*, while identity is conferred by *iab-7*. Sanchez-Herrero and Akam (1989) recently defined broad regions that are transcribed in a spatially restricted fashion in embryos. They noted that *Fab-7* is localized between two such regions providing further evidence that the region deleted in *Fab-7* separates two functional domains.

In our current working hypothesis, the segment/parasegment-specific functions of the BX-C are *cis*-regulatory elements that regulate only three protein-coding genes, *Ubx*, *abd-A* and *Abd-B*. A two-step mechanism for this regulation was proposed (Peifer *et al.*, 1987). In the first step, the parasegmental address is conferred by the opening of a specific DNA domain. Then enhancer-like elements residing in that domain act on the transcription unit. The segment/parasegment-specific functions are lined up on the chromosome in the same order as the segments on the body of the fly. Consequently, as one proceeds from anterior to posterior, new domains become accessible sequentially and remain open in more posterior parasegments. This model implies the notion of boundaries that insulate inactive domains from active ones. We propose that the *Fab-7* lesion removes such a boundary between *iab-6* and *iab-7*. As a consequence, the open configuration of *iab-6* spreads over *iab-7*, thus activating it in PS11. Since *iab-7* remains active in PS12 of *iab-6* revertants, the opening up of each domain may occur independently in the wild-type as well.

In our view, Mcp^1 (*Miscadestral pigmentation*, a dominant mutation in the BX-C that transforms A4 into A5; Lewis, 1978) deletes a similar boundary between *iab-4* and *iab-5*. Both *Fab-7* and Mcp^1 are deletions of ~4 kb and can be made to revert by breaks which separate two adjacent DNA domains (Karch *et al.*, 1985). According to this interpretation, there are two types of dominant gain-of-function mutations in the BX-C. Firstly, chromosomal breaks like *Cbx2* or *Uab5* bring foreign DNA into the BX-C and cause misregulation of parts of it. Secondly, segment/parasegment-specific functions are misregulated by trans-

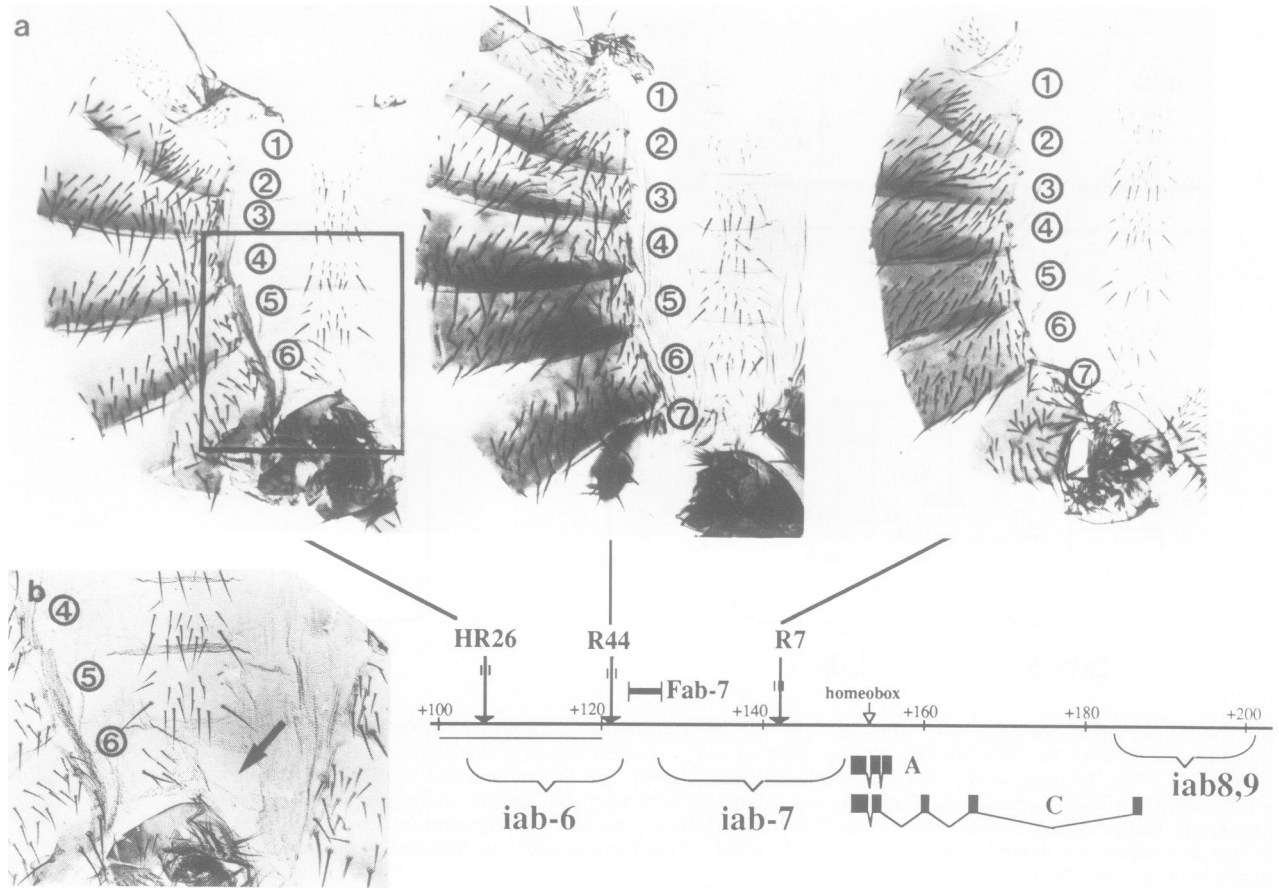

Fig. 4. *iab-6* and *iab-7* revertants of *Fab-7*. (**a**) Abdomens of hemizygous males (*iab-6^{HR26}/Df(3R)P9*, *iab-6^{R44}/Df(3R)P9* and *iab-7^{R7}/Df(3R)P9*) are shown on the top panels as well their respective localization on the molecular map (bottom panel, refer to Figure 3 for the legend of map). Note the presence of a complete A7 identical to A6 and A5 in the revertant *iab-7^{R7}*. In *iab-6^{R44}* however, only a rudimentary A7, significantly different from A6, is visible. (**b**) Enlargment of the last three segments of the mutant *iab-6^{HR26}/Df(3R)P9* shown in (a). The arrow points to an area in A6 exhibiting A7 characteristics (the 7th sternite is devoid of both chaetae and trichomes).

position from one domain to another (*Cbx1*; Peifer *et al.*, 1987) or by fusion of one domain to another (*Mcp^1*, *Fab-7* and perhaps *Ubx^{C1}*: Rowe and Akam, 1988; Casanova *et al.*, 1988; Karch *et al.*, 1990).

Our model predicts that significant weakening (functional removal) of all boundary regions simultaneously would result in an 'all out' situation: genes of the BX-C would be expressed indiscriminately along the body axis. However, since parasegmental clues (provided by the segmentation genes, see Akam, 1987; Ingham, 1988) alone are responsible for the initial opening of the individual domains, derepression of the BX-C genes could be delayed. This is precisely what seems to happen (Struhl and Akam, 1985) in mutations belonging to the *Polycomb*-like group (Jürgens, 1985). Therefore, we propose that the boundary regions are the primary sites of action of at least some *Polycomb*-like genes.

Recently, homologs to the *Antp* and bithorax complex proteins were isolated in mammals. As in flies, the order of expression along the body plan reflects the order of the genes on the chromosome (Boncinelli *et al.*, 1989; Graham *et al.*, 1989; Duboule and Dolle, 1989). It will be interesting to see if boundary regions play a similar role in these mammalian gene clusters. Morover, *Fab-7* may be regarded as a specific example of the so-called position effect (the term position effect refers to the observation that expression of a given gene can often be modified by changing the chromosomal surroundings). Since position effect is a widespread phenomenon in eukaryotes (see for example Pirrotta *et al.*, 1985; O'Kane and Gehring, 1987; Kollias *et al.*, 1987), it seems reasonable to assume that many genes are bracketed by insulators, with a similar function to those found in the BX-C, which protect them from the influences of their chromosomal environment.

Materials and methods

Drosophila cultures and stocks

Fly stocks were maintained on standard yeast–cornmeal medium. For complementation and phenotypic analysis the following mutations and deficiencies described in Lewis (1978), Tiong *et al.* (1986), Karch *et al.* (1985) and Duncan (1987) were used: *abd-A^{S1}*, *iab-4^{301}*, *iab-6^{Camel}*, *iab-7^{SGA-62}*, *Abd-B^{S1}*, *Abd-B^{D14}*, *Abd-B^{S4}*, *Abd-B^{65}*, *iab-8,9^{Tab}*, *iab-8,9^{Uab1}*, *Df(3R)P9* and *Df(3R)C4*.

Isolation of *Fab-7* and its revertants

Fab-7 was found among the progeny (~40 000 males) of X-rayed Oregon-R-369 (4000 rads; 1000 rads/min, 0.5 mm Al filter). *Fab-7* males were X-rayed and mated *en masse* to *CxD/TM3, Sb Ser* virgins. Individuals with A6 approaching wild-type were collected and maintained in stocks with *CxD*, *TM3*, or *Dp(3;3)P5*.

Mapping of *Fab-7* relative to *iab-7^{R73}*

Virgins of the genotype *Fab-7 iab-7^{R73}/sbd ss pbx^1 Mc e^s* were mated to *CxD/TM3, Sb Ser* males *en masse*. Among ~40 000 F1 flies, 3 individuals with *Fab-7* phenotype were found. Subsequent test crosses showed that the

Fab-7 bearing chromosomes also carried *Mc* and e^s indicating the position of *Fab-7* relative to $iab\text{-}7^{R73}$. Whole genome Southern analysis confirmed the separation of the two lesions.

Mounting larvae and abdominal cuticle

Larvae were fixed and cleared according to the procedure of Van der Meer (1977). They were then cut along the lateral midlines and only the dorsal cuticles were mounted. The adult abdomens were cut along the dorsal midline, opened and flattened on a slide as described in Duncan (1982).

Whole genome Southern blots and construction of genomic libraries

The preparation of fly DNA, the growth of phage stocks, the analysis of the recombinant phages by heteroduplex and restriction digests were described previously (Bender *et al.*, 1983). Genomic libraries of the mutants were constructed by inserting *Sau*3A partially digested genomic DNA into the *Bam*H1 sites of the λ phage vector EMBL3 (Frieschauf *et al.*, 1983). Probes were labelled by random priming with hexanucleotides (Feinberg and Vogelstein, 1984) and Southern blots were transferred onto Zeta probe or Nytran nylon membranes in 0.4 M NaOH (Khandjian, 1987).

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