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Developmental analysis of a hybrid gene composed of parts of the *Ubx* and *abd-A* genes of *Drosophila*

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***C1* is a mutation in the bithorax complex (BX-C) of *Drosophila* resulting from the deletion of parts of the *Ubx* and *abd-A* genes. We show that the 'hybrid' gene formed by the fusion of the remaining parts of *Ubx* and *abd-A* (5'*abd-A/Ubx*3') is functional and developmentally active. It specifies parasegment patterns with a mixture of thoracic and abdominal identities. The hybrid gene also has other properties typical of conventional bithorax genes: it can be spatially derepressed in the absence of *trans*-acting genes like *extra Sex combs* or *Polycomb* and in turn represses other homeotics like *Sex combs reduced*. The comparison of embryos containing exclusively hybrid gene activity with others having no BX-C function indicates that the hybrid gene is active in the body region defined by PS5 to PS14. The expression in PS5 and PS6 suggests that one control region (*abx*) of *Ubx* can regulate the transcription of the *abd-A* promoter.**

Key words: bithorax complex/Ultrabithorax/abdominal-A/hybrid gene

Introduction

The genes of the bithorax complex (BX-C) establish the characteristic development of the region of the body extending from parasegment 4 (PS4) to PS14 (Lewis, 1978; Sánchez-Herrero *et al.*, 1985a) (for the definition of parasegment, see Martínez-Arias and Lawrence, 1985). The BX-C contains three independent genes: Ultrabithorax (*Ubx*), abdominal-A (*abd-A*), and Abdominal-B (*Abd-B*) (Sánchez-Herrero *et al.*, 1985a,b; Tiong *et al.*, 1985; Casanova *et al.*, 1987) which account for all the developmental functions of the complex.

The gene *Ubx* can only determine the morphological pattern of PS5 and PS6, which are thoracic except for the anterior compartment of the first abdominal segment. The gene *abd-A* establishes a different set of patterns, all abdominal, in PS7, PS8 and PS9. In the absence of the two genes, parasegments 5 to 9 develop equally as PS4 (Lewis 1978; Morata *et al.*, 1983; Casanova *et al.*, 1987).

There is a mutation in the BX-C, discovered by G.Struhl, originally called Ubx^{C1} hereafter *C1*, which is very atypical, for it shows a recessive phenotype consisting of thoracic and abdominal transformations unlike any other BX-C mutation (G.Struhl, personal communication). In addition, the *C1* mutation shows a dominant phenotype which can be interpreted as ectopic BX-C function outside its normal domain.

The molecular characterization reported in the accompanying paper (Rowe and Akam, 1987) indicates that the *C1* mutation is a deletion of parts of the *Ubx* and *abd-A* genes, which results in a fusion gene formed by the remaining parts of *Ubx* and *abd-A*.

We have analyzed the developmental properties of this gene. Surprisingly we find that the gene product(s) encoded by a hybrid gene formed by pieces of two different genes is functional. It exhibits a set of pattern-determining properties and *trans* interactions with other homeotic genes which are characteristic of the BX-C genes. Our results also demonstrate that the *Ubx* 3' end, and possibly the *abx* regulatory region, can act through the 5' end of *abd-A*, indicating a functional homology between the two genes.

Results

Genetic and phenotypic characterization of the C1

The mutation *C1* is lethal when homozygous. It is also lethal over the *DfP9*, but complements the two flanking lethals of the BX-C, *llb* and *lrb* (Sánchez-Herrero *et al.*, 1985a). It complements *Abd-B* mutations ($Abd\text{-}B^{M1}$, $Abd\text{-}B^{M7}$) completely but does not complement the lethality or mutant syndrome of *Ubx* (Ubx^{1}, Ubx^{130}, Ubx^{M1}, $Dfbxd^{100}$) or *abd-A* ($abd\text{-}A^{M1}$, $abd\text{-}A^{M2}$, $abd\text{-}A^{M3}$, *TpP10*, *C26*) mutations. Altogether this indicates that the *C1* lesion is restricted to the *Ubx* and *abd-A* genes.

The extent to which the functions of *Ubx* and *abd-A* are affected was investigated by studying in detail the phenotypes of *trans* combinations of *C1* with viable mutations at the *abx* and *bxd* subunits (Casanova *et al.*, 1985) of *Ubx* (abx^{2}, bx^{2}, bx^{34e}, bxd^{1}, $Tpbxd^{100}$, pbx^{1}) and with the mutation $iab\text{-}2^{k}$ (Kuhn *et al.*, 1981) which reduces *abd-A* activity in the A2 segment.

The results with respect to *Ubx* can be summarized as follows: *C1* almost complements (Figure 1) the mutations at *abx* (abx^{2}, bx^{3}, bx^{34e}) for both the *ppx* and *bx* transformations (Casanova *et al.*, 1985) but gives very strong mutant phenotype with those at *bxd*. Thus, it allows for virtually normal *Ubx* activity in PS5 but little or none in PS6.

The phenotype of $C1/iab\text{-}2^{k}$ indicates a partial loss of function of *abd-A* in A2, which is incompletely transformed towards A1. This is similar but weaker than that produced by combinations of $iab\text{-}2^{k}$ with *DpP10Df109* (a deletion of the *abd-A* gene, Morata *et al.*, 1983) or with strong *Abd-A* mutations. Also, adult flies of genotype $C1/abd\text{-}A^{M3}$ ($abd\text{-}A^{M3}$ is a weak allele which allows the survival of some adult hemizygotes) show a weaker phenotype than $DfP9/abd\text{-}A^{M3}$ or $abd\text{-}A^{M1}/abd\text{-}A^{M3}$ ones.

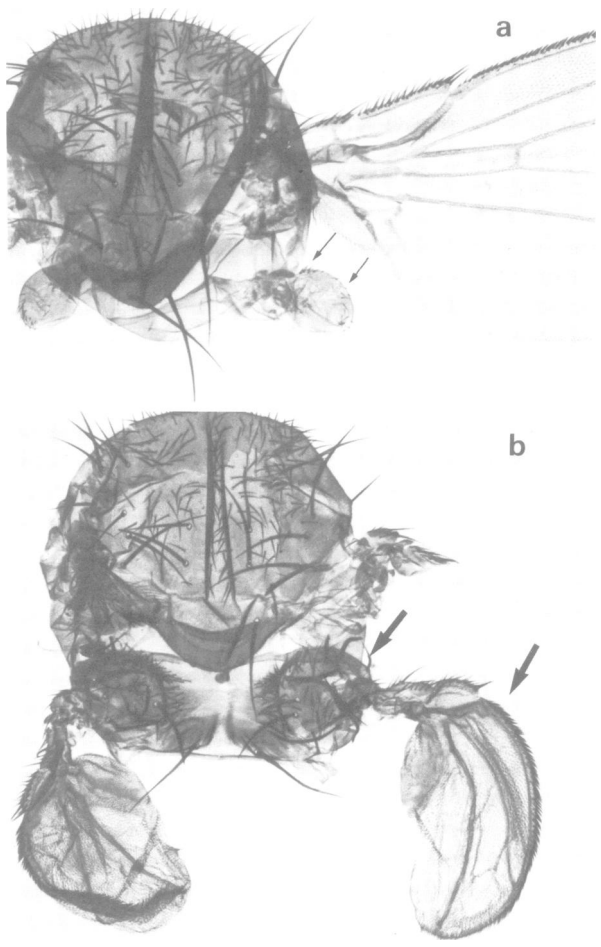

Fig. 1. Mesothoracic transformation of the dorsal methathorax of (**a**) $C1/bx^3$ and (**b**) Ubx^{130}/bx^3. Notice that in (**a**) there is a weak transformation of the haltere as indicated by the presence of some wing bristles in the anterior margin (thin arrows). The proximal methathorax is not transformed. By contrast bx^3/Ubx^{130} (**b**) shows a strong transformation of the anterior haltere into anterior wing and also the proximal methathorax is transformed into proximal mesothorax (notum). These transformations are marked by thick arrows.

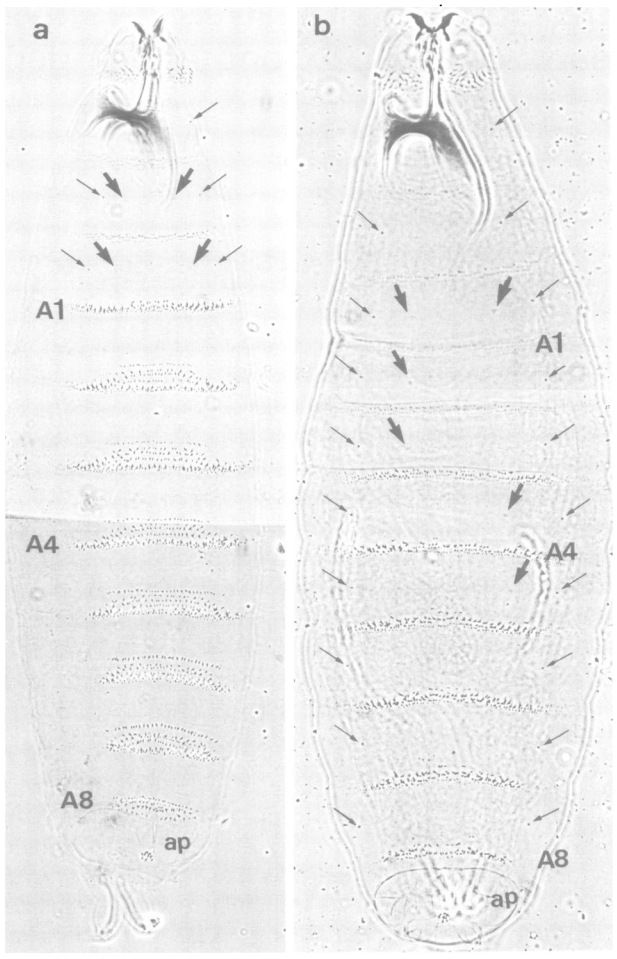

Fig. 2 Ventral aspect of embryos of genotype: (**a**) wild type and (**b**) *C1/DfP9*. In the mutant all the denticle belts posterior to A1 are modified; A1 and A2 are completely transformed into a thoracic belt. From A3 downwards there is a mixture of thoracic and abdominal pattern elements; there are denticles of abdominal and others of thoracic size. There are thoracic markers like Keilin's organs (marked by thick arrows) and ventral pits (thin arrows) which may appear in all abdominal segments (except A8).

Thus, complementation and phenotypic analyses indicate that the *C1* chromsome retains partial activity of *Ubx* and *abd-A* genes and a full function of *Abd-B*. The characteristics of the *C1* syndrome were further defined by the study of larval and adult phenotypes of the homo- or hemizygous combinations.

Transformation of the larval segments. Larvae homo- or hemizygous for *C1* are normal in the head and in the thoracic segments, but the abdominal ones appear transformed (Figure 2). Segment A1 shows a thoracic pattern as indicated by the denticle size, the presence of ventral pits and fully developed Keilin's organs. This pattern is probably methathoracic (T3) but it is difficult to discriminate from mesothoracic. In homozygous larvae, segments A2−A8 show a mixture of thoracic and abdominal pattern elements. Overall, there is a gradient from A2, which is mostly thoracic, to A8, mostly abdominal, but even A8 remains clearly different from wild type. Hemizygous larvae exhibit a more extreme phenotype than the homozygotes. Segments A1 and A2 develop a complete thoracic pattern and the remaining abdominal segments present more thoracic features than their counterparts in *C1*/C1, Ventral pits and defective Keilin's organs can be observed down to A7 in both homo- and hemizygous larvae. More posterior structures like spiracles and filzkorper are entirely normal (Figure 2).

The transformations of *C1* larvae can be described as a mixture of partial *Ubx* and *abd-A* phenotypes. To discriminate the contribution of each of these components to the *C1* phenotype we studied larvae of genotypes $C1/Ubx^{130}$ and $C1/abd\text{-}A^{M1}$ in which a different component was lacking in each case. In $C1/Ubx^{130}$ larvae the A1 segment develops very much as in *C1/DfP9* but in the A2−A8 region the denticle belts are much more normal. We found no evidence of prothoracic transformation normally observed in the dorsal side of Ubx^- larvae (Hayes *et al.*, 1984). The only sign of thoracic transformation is the presence of ventral pits in the abdomen (except in A8). The phenotype of $C1/Ubx^{130}$ in the abdominal segments is very much like that described for Ubx^- (Lewis, 1978; Struhl, 1981) except that the A1 denticle belt resembles T3 more than T2.

$C1/abd\text{-}A^{M1}$ larvae show a normal pattern in the thorax

Table I. Patterns generated by hemizygous *C1* clones in different leg compartments

Time of clone initiation	T1a	T1p	T2a	T2p	T3a	T3p
Early embryonic age (4 ± 2 h of age)	T1a(3)	T1p(2)	T2a(6)	T2p(4)	T3a(2)	*T2p*(2)
Larval period (48–96 h of age)	T1a(16)	T1p(7)	T2a(15)	T2p(5)	T3a(15)	*T2p*(5)

The table shows the number of clones (in parenthesis) found in each leg compartment and the characteristic bristle pattern that they produce. Only the clones found in T3p (italicized for emphasis) show a homeotic transformation towards T2p.

and in A1. The A2–A8 segments appear partially transformed towards A1. The effect is more extreme in A2 and decreases gradually in the posterior direction. Monohairs are sometimes seen in A2–A6 indicating a thoracic transformation of posterior compartments (Struhl, 1984; Sanchez-Herrero *et al.*, 1985a). This phenotype is much weaker than that seen in *abd-A*^{M1} homozygotes.

There are two conclusions that can be derived from the larval phenotypes: (i) the phenotype of *C1* is produced in part by a defect in *Ubx* and in part by a defect in *abd-A*; (ii) the transfomations observed are less extreme than those produced by the elimination or complete inactivation of the *Ubx* and *abd-A* genes (Lewis, 1978; Morata *et al.*, 1983; Casanova *et al.*, 1987) indicating that at least some functions of these genes remain. Accordingly, the hemizygous phenotype is stronger than the homozygous one.

Transformations of the adult segments. These have been studied by generating *C1/DfP115* cell clones in the adult cuticle (see Materials and methods for details). Clones were initiated at around blastoderm (4 ± 2 h) and also during the larval period, 48–98 h and 96–120 h after egg laying. No difference was found for clones induced at blastoderm or later. They were found in all body segments. Special attention was paid to the clones found within the area of the body where the BX-C genes act, that is parasegments 5–14. Clones anterior to this region always developed normally according to the position in which they appear (data not shown except for PS4, Table I). Clones in PS5 showed very little effect. In the anterior haltere they presented a slight increase of the haplo-insufficient phenotype. No sign of *post-prothorax* transformation (Morata and Kerridge, 1981) was found in blastoderm clones (Table I) in T2p or in T3p, in contrast with regular *Ubx* alleles (Kerridge and Morata, 1982). In T3p the clones showed a transformation towards T2p; for example in the posterior haltere they develop as posterior wing and in the posterior third leg as posterior second.

The number and size of *C1/DfP115* clones found in different abdominal segments are shown in Table II. In segments A3–A6 we observe the expected number of clones for the dose of irradiation and markers used. Also the size is that expected for clones induced during the larval period. However, some of these clones develop abnormal patterns, differentiating small bristles (intermediate between those of A1 and more posterior segments) and having less pigment than normal (Figure 3). Thus we probably recover all the *C1/DfP115* clones in A3–A6 but they exhibit a pattern which does not correspond to any normal segment. In A1 and A2 the clone's size is clearly smaller than in other segments (Table II), suggesting that part of the clone is lost. These clones are frequently associated with pieces of invaginated cuticle that we interpret as a part of the clone transformed into thorax and that cannot develop in abdominal environment (Morata and García-Bellido, 1976). In A1 we find few clones, indicating a strong thoracic transformation, in parallel with the larval phenotype. Some of the A1 clones show bristles longer than those of A1, indicative of a transformation towards a more posterior abdominal segment. This partial rescue of A1 clones and their phenotype suggests an adventitious abdominal function operating in some of the clones.

Table II. Number and size of *C1* cell clones in the abdomen

	Abdominal segments					
	A1	A2	A3	A4	A5	A6
No. of clones	12	30	34	26	23	27
Clone size	1.7	2.2	4.0	4.0	3.6	3.9

Number and average size (measured as number of bristles/clone) of *C1* hemizygous clones found in the abdominal segments after irradiation during larval period (72–120 h after egg laying). 320 flies were screened for clones.

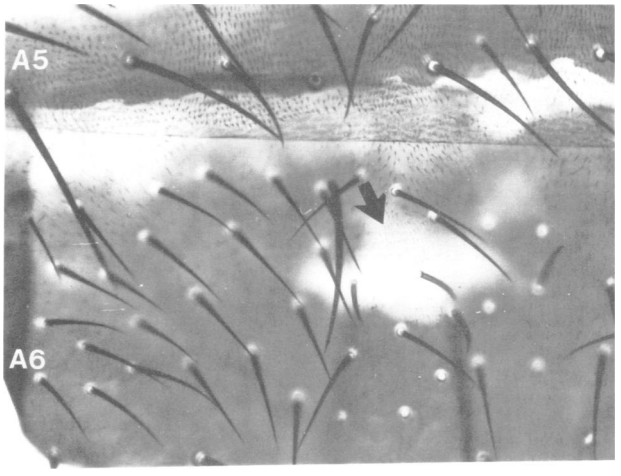

Fig. 3. Example of a clone of genotype *yf*^{36}; *C1/Df115* (arrow) in segment A6. Note that the pattern exhibited by the clone does not fit with that of segment A6; it has less pigment and differentiates trichomes, which are mostly absent in A6. The size of the bristles is intermediate between those of A1 and of a more posterior segment.

Elimination of the hybrid functions by Ubx$^{9\text{-}22}$

The genetic and phenotypic characterizations of *C1* indicate that in addition to an intact *Abd-B* activity, there is another BX-C function able to promote almost normal development of PS5 and also partial abdominal development of A1–A4 segments. This, together with the molecular data provided in the accompanying paper (Rowe and Akam, 1987), suggests that the *C1* chromosome contains a composite (hybrid) BX-C gene including parts of the *Ubx* and *abd-A* genes which encodes some of the functions of these two genes.

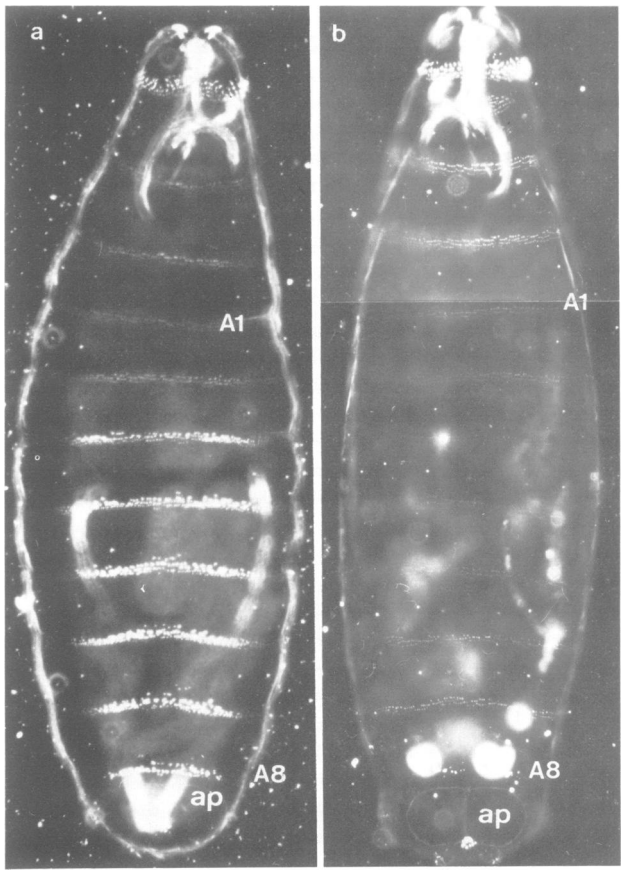

Fig. 4. Ventral view under dark field of embryos of genotype (**a**) *C1/DfP9* and (**b**) $Ubx^{9\text{-}22}$*C1/DfP9*. In (**b**) all the denticle belts from A3 to A8 are very different from those of (**a**) and much more thoracic in character. The phenotype of (**b**) is identical to that of *Df109* (Morata *et al.*, 1983), in which the genes *Ubx* and *abd-A* are deleted.

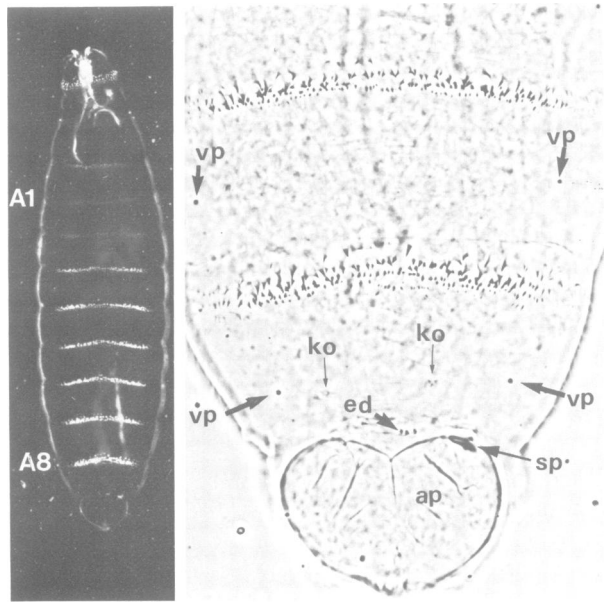

Fig. 5. Ventral view of an embryo of genotype *C1* $Abd\text{-}B^{M1}$/*DfP9*. Abdominal segments A1 and A2 have lost all the abdominal features. From A3 to A8 there is a mixed thoracic and abdominal pattern, becoming gradually more abdominal in the posterior region. The last two abdominal segments are amplified in the phase contrast picture. The denticle belts are intermediate between abdominal and thoracic as indicated by the size of the denticles. There also are thoracic structures like ventral pits (vp) and Keilin's organs (KO). There is a group of denticles (ed) just anterior to the anal pads (ap). On one side there is a piece of sclerotized cuticle (sp).

One prediction of the hybrid gene hypothesis is that it should be possible by a single mutation to eliminate at once its ability to promote thoracic (PS5) and abdominal patterns. To test this we constructed a chromosome carrying *in cis* (see Materials and methods) the mutations $Ubx^{9\text{-}22}$ and *C1*. $Ubx^{9\text{-}22}$ is a 1.6 kb deletion eliminating most of the homeobox and part of the exonic and intronic material in the 3′ end of the *Ubx* transcription unit (Hogness *et al.*, 1985; Beachy *et al.*, 1985; Weinzierl *et al.*, 1987). It complements all the *abd-A* mutations. Like other *Ubx* mutant alleles it has a very slight effect on abdominal embryonic segments (Lewis, 1978; Struhl, 1984).

We find that the $Ubx^{9\text{-}22}$*C1* combination shows a hemizygous phenotype dramatically different from that of *C1* (Figure 4). A2−A4 denticle belts lose the abdominal character and become transformed into thoracic belts. Those of A5−A8 are modified, showing much more marked thoracic features. The phenotype is identical to that described for *Df109* (Morata *et al.*, 1983) in which the *Ubx* and *abd-A* genes are completely eliminated. Moreover, the combination $Ubx^{9\text{-}22}$*C1*/abx^{2} exhibits a strong effect in T2p (*ppx* transformation) and T3a (*bx* transformation), indicating that this genotype produces a transformation of PS5 into PS4; a complete elimination of *Ubx* activity. Also, flies of genotype $Ubx^{9\text{-}22}$*C1*/*iab-2*^{K} show a transformation of A2 towards A1 like that of *Df109*/*iab-2*^{K} indicating a loss of *abd-A* function. Finally, cell clones of genotype yf^{36};$Ubx^{9\text{-}22}$*C1*/*DfP115* in adult flies show a strong wing transformation in the anterior haltere and they do not develop in the abdominal segments A2−A7, indicating a loss of the abdominal properties observed in *C1*/*DfP115* clones.

Thus, the $Ubx^{9\text{-}22}$ lesion removes all the thoracic (PS5) and abdominal properties of the *C1* chromosome attributable to the *Ubx* and *abd-A* genes. We consider this a conclusive proof of the existence of a functional *Ubx*−*abd-A* hybrid gene in the *C1* chromosome. Complementation analysis with several *Abd-B* mutations indicates that the $Ubx^{9\text{-}22}$*C1* chromosome contains a fully functional *Abd-B* gene, as expected from the phenotype.

It is worth noting that the expressivity of the *bx* transformation of the $Ubx^{9\text{-}22}$*C1* chromosome *in trans* with *abx* mutants is extreme and like that of *DfP9* or strong *Ubx* mutations. In contrast, $Ubx^{9\text{-}22}$ alone shows moderate transformations i.e. there is some *Ubx* activity. Since $Ubx^{9\text{-}22}$ effectively removes all the *Ubx* products (Hogness *et al.*, 1985), this partial complementation can only be due to the *abx* chromosome (Weinzierl *et al.*, 1987). Lewis (personal communication) has shown that this activity in the combinations of $Ubx^{9\text{-}22}$ with *abx* chromosomes depends on normal pairing. Our observation indicates that this activity is eliminated by *cis*-linking *C1* with $Ubx^{9\text{-}22}$.

Characterization of the hybrid C1 gene

We have removed the *Abd-B* activity present in the *C1* chromosome by recombining the strong mutation $Abd\text{-}B^{M1}$ *in cis* to *C1* (see Materials and methods). The resulting chromosome, *C1* $Abd\text{-}B^{M1}$, exclusively contains BX-C activity of the hybrid gene. We have studied its effect on the

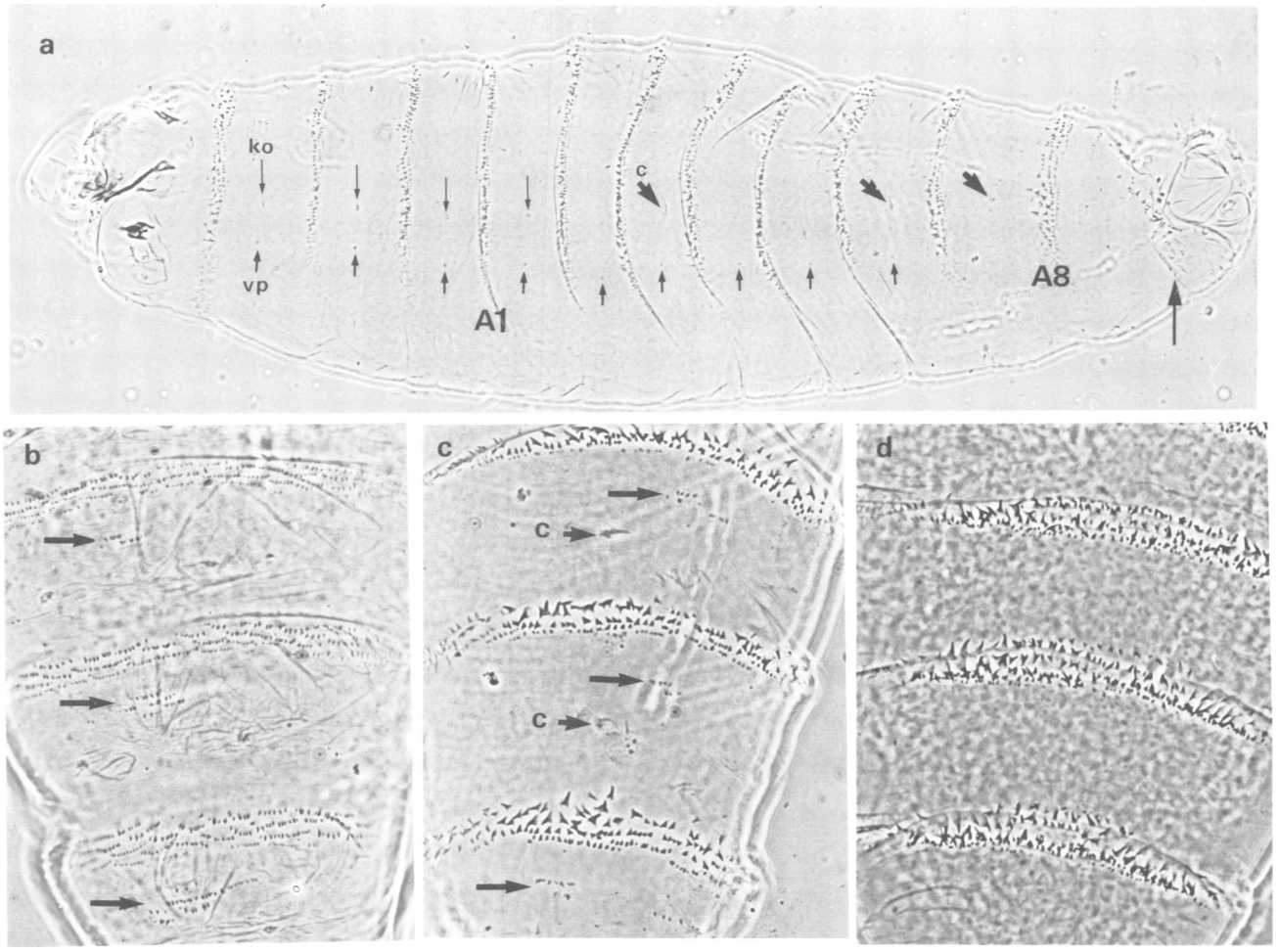

Fig. 6. (**a**) Embryos of genotype *esc*$^{-}$; *Cl Abd-B*^{M1}. All the thoracic and abdominal metameres develop similarly a mixed pattern with thoracic and abdominal features. Some Keilin's organs (KO) and ventral pits (vp) are marked by arrows. There is an extra denticle belt (arrow) posterior to A8 generated by the ectopic activity of the hybrid gene in PS14 in the absence of the *r* element of *Abd-B* (Casanova *et al.*, 1986). In some metameres there are some structures labelled c which resemble the *cirri* normally present in the head structures. These can more clearly be seen in picture (**c**). The lower part of the panel presents a comparison of the abdominal segments 6, 7 and 8 of the genotypes (**b**) *esc*$^{-}$; *Ubx*^{MX12}*abd-A*^{M1}*Abd-B*^{M1}, completely deficient in BX-C activity; (**c**) *esc*$^{-}$; *Cl Abd-B*^{M1} containing only hybrid gene activity and (**d**) *esc*$^{-}$; *Ubx*$^{9-22}$*Abd-B*^{M1} containing an intact *abd-A* gene. Note that the embryo (**b**) presents a centrally located second group of denticles (arrows), characteristic of the prothorax. They probably indicate an ectopic activity of *Scr* (Struhl, 1983; Sato and Denell, 1985) in the absence of BX-C genes. In embryo (**b**) this second group of denticles is less developed but there are some rudiments of cephalic structures (**c**). In (**d**) there is neither a second group of denticles nor cephalic structures.

larval and adult epidermis and also its response to the lack of function of *trans*-acting genes like *Polycomb* or *extra sex comb*.

The larval phenotype of *Cl Abd-B*^{M1} is illustrated in Figure 5. Thoracic structures appear normal both in dorsal and ventral sides. In the abdominal region, the denticle belts corresponding to A1 and A2 are of thoracic type and from A3 they show a mixture of thoracic and abdominal pattern elements, becoming gradually more abdominal. In the region posterior to the A8 belt, the phenotype of *Cl Abd-B*^{M1} is similar to that of *Ubx*$^{9-22}$*Abd-B*^{M1} embryos (Casanova *et al.*, 1987). There is a region of naked cuticle between the belt and the anal pads containing ventral pits, indicating a partial thoracic character. We see, however, some significant differences with the *Ubx*$^{-}$*Abd-B*$^{-}$ combination. One is the presence of Keilin's organs, which do not appear in the latter genotype. A second is that there often is a rudimentary belt of denticles (average 5 ± 3) of abdominal type in the position corresponding to the A9 belt (Duncan and Lewis, 1982; Casanova *et al.*, 1986). The presence of this extra belt is accompanied by the reduction or sometimes absence of the sclerotic plates at A8p which appear in strong *Abd-B*$^{-}$ mutations (Lewis, 1978; Struhl, 1983; Casanova *et al.*, 1986). This suggests an adventitious expression of the hybrid gene in *PS14*. We have checked this possibility by synthesizing the combination *Cl Abd-B*^{M5}. The mutation *Abd-B*^{M5} (unlike *Abd-B*^{M1}) contains an intact *r* element (Casanova *et al.*, 1986) able to suppress any BX-C activity in PS14. The phenotype of *Cl Abd-B*^{M5} is like that of *Cl Abd-B*^{M1} with the difference that the A9 belt and the sclerotic plates are always lacking and the region of naked cuticle posterior to the A8 belt is smaller.

We have further studied the expression of the hybrid gene by having it indiscriminately expressed using the mutation *extra sex combs* (Struhl, 1981). The phenotype of *esc*$^{-}$; *Cl Abd-B*^{M1} embryos is shown in Figure 6, which also includes the phenotypes of *esc*$^{-}$; *Ubx*^{MX12}*abd-A*^{M1}*Abd-B*^{M8} (containing no BX-C activity) and *esc*$^{-}$; *Ubx*$^{9-22}$*Abd-B*^{M1}

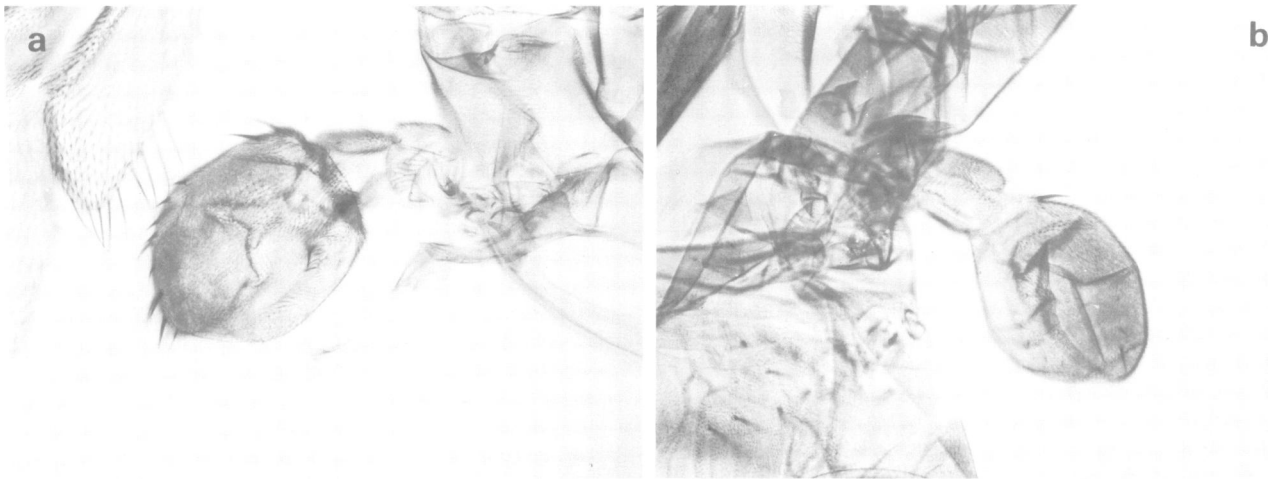

Fig. 7. Halteres of genotypes: (**a**) *C1*/+ and (**b**) $Ubx^{9\text{-}22}C1$+/. The haltere in (**b**) shows the typical haplo-insufficient phenotype of *Ubx* mutations indistinguishable from that of *DfP9*/+. Notice that this phenotype is enhanced in (**a**); the haltere is bigger and contains more wing bristles.

(containing only *abd-A* activity) embryos for comparison. In *esc*$^-$; *C1 Abd-B*^{M1} all thoracic and abdominal denticle belts develop similarly, showing a mixture of denticles of thoracic and abdominal character. There are Keilin's organs, sometimes defective, and ventral pits in all metameres. Frequently, there is a second group of thoracic denticles located centrally and posterior to the main belt. These denticles represent a prothoracic transformation. They appear in greater frequency and size in the *esc*$^-$; Ubx^{M12} *abd-A*^{M1}*Abd-B*^{M8} (Figure 6a). This prothoracic transformation does not appear in *esc*$^-$; $Ubx^{9\text{-}22}$*Abd-B*^{M1} (Figure 6d) indicating that *abd-A* alone is able to suppress this transformation. In this latter combination all the metameres develop with a marked abdominal character. One further difference among *esc*$^-$; *C1 Abd-B*^{M1} embryos and those of the other two genotypes (Figure 6a,c) is the presence in the former of some gnathal structures like *cirri*. In Pc^3 *C1 Abd-B*^{M1} homozygous embryos, we observe a similar but weaker phenotype. Thus, the hybrid gene can be derepressed by eliminating *esc* or *Pc* activities although the pattern produced does not correspond to the derepression of any intact BX-C gene.

We have also studied the effect of the hybrid gene in the adult cuticle by genetic mosaics, generating *C1 Abd-B*^{M1}/*Df115* cell clones in flies with normal segment pattern. All the clones in the thoracic segments were normal except in the posterior haltere and posterior third leg where they differentiated the corresponding posterior second leg structures.

In the abdominal segments a total of 97 marked clones were examined of which about 80% are of genotype *C1 Abd-B*^{M1}/*Df115* (see Materials and methods). They differentiate abdominal structures which can be defined as intermediate between A1 and a more posterior segment of the A2−A5 type. The pattern shown by these clones is similar to that of *C1*/*DfP115* clones although there are differences in the last abdominal segments, probably due to the presence in the latter of *Abd-B*$^+$. The clones are often associated with malformations and invaginated structures, suggesting that part of the clone is transformed towards thorax and subsequently lost.

Dominant syndromes caused by the hybrid C1 gene

The chromosomes carrying *C1* exhibit in heterozygous condition a set of dominant transformations.

In the first place there is the characteristic haplo-insufficiency of *Ubx*; the haltere is slightly enlarged and differentiates some bristles in the anterior margin. Curiously, this phenotype is stronger in *C1*/+ or *C1 Abd-B*^{M1}/+ than in *DfP9*/+ or Ubx^{130}/+ (Figure 7) indicating that the *C1* produces a debilitation of the *Ubx* activity of the homologous chromosome. This effect does not depend on the genetic background since it appears after outcrossing *C1* with many different stocks. However, it is eliminated in the combination $Ubx^{9\text{-}22}C1$/+, in which the haltere is like that of *DfP9*/+. This is a remarkable situation in which a *Ubx* mutation enhances rather than reduces *Ubx* activity (see Discussion).

In the second place, there are transformations of the gain-of-function type (Lewis, 1978, 1982). Flies of genotype *C1*/+ or *C1 Abd-B*^{M1}/+ frequently exhibit a partial transformation of wing territory into haltere (Figure 8a,b), a *Cbx*-like phenotype (see Lewis, 1963; Morata, 1975). Moreover, the A1 segment shows a partial transformation towards a more posterior one, a *Uab*-like (Lewis, 1978) phenotype (Figure 8c). As described above, clones *C1*/*Df115* sometimes show a phenotype which can also be described as *Uab*-like.

The penetrance and expressivity of the *Cbx*-like and *Uab*-like phenotypes are variable, depending on stocks and crosses. However both parameters can be greatly increased if there are two doses of the hybrid gene, as in the combination *DpP115*; *C1*/*C1*. In this genotype the recessive lethality and phenotype of *C1* are covered by the *DpP115*, which contains a full dose of the BX-C. *DpP115* does not affect the gain-of-function phenotypes which can now be studied in two doses. About 50% of the flies show *Cbx*-like and 100% *Uab*-like phenotype. In about half of these flies we observe patches of abdominal tissue anterior to and transformed in the same direction as A1. Also halteres and hindlegs are sometimes missing. All these traits are consistent with an abdominal transformation of metathoracic tissue; a Hyperabdominal (*Hab*) phenotype (Lewis, 1978).

There was the possibility that the *Cbx*-like and *Uab*-like

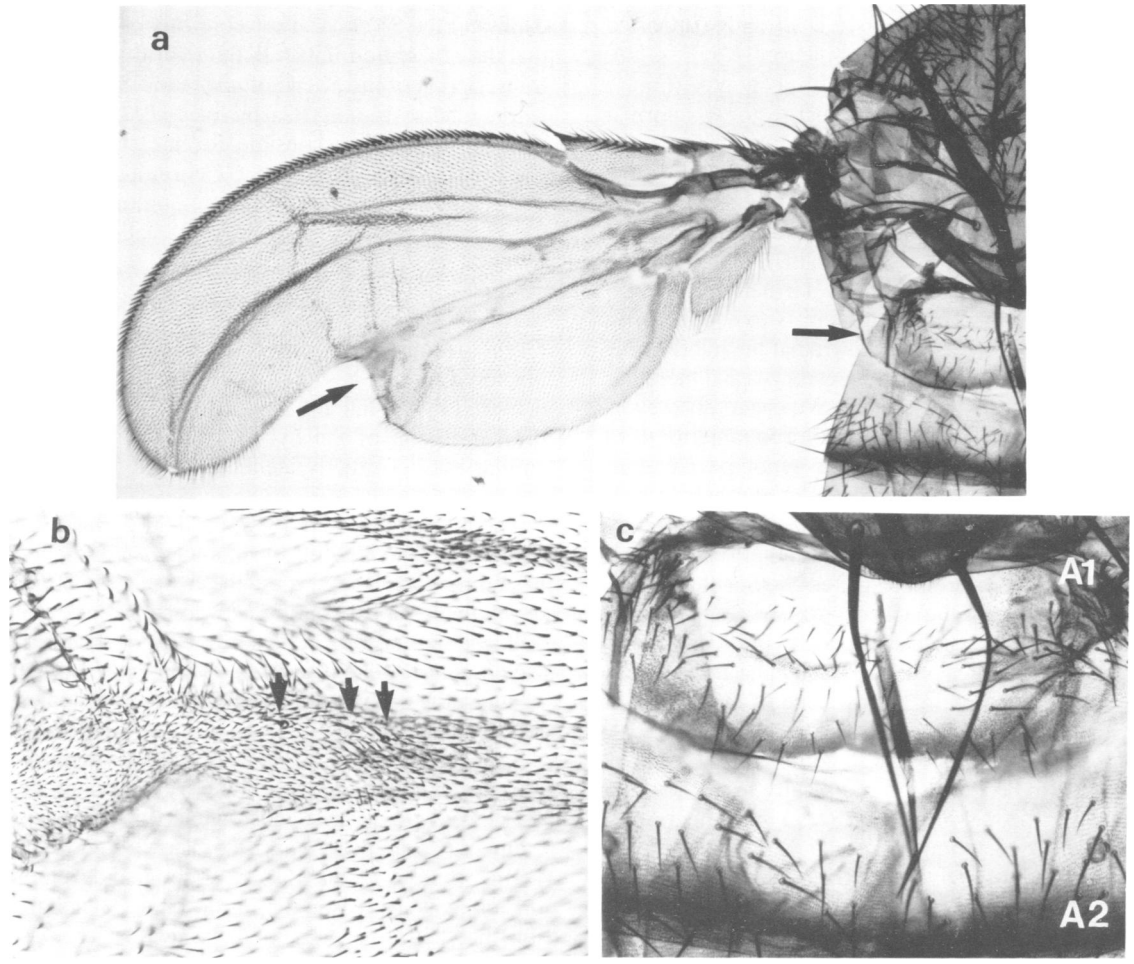

Fig. 8. (**a**) Dorsal aspect of the thoracic and first and second abdominal segments of a fly of genotype *DpP115*; *C1/C1*. The wing and first abdominal segments show gain of function *Cbx*-like and *Uab*-like phenotypes (arrows). (**b**) Detail (from another fly) of part of the wing showing *Cbx*-like phenotypes; the trichomes are smaller and more tightly packed than those of the wing, resembling haltere trichosomes. Typical haltere structures like sensilla trichoidea (arrows) are present. (**c**) Details of the segments A1 and A2. The bristles of A1 are bigger than normal and approach those of A2. Also the width and the amount of pigment of A1 are modified, resembling those of more posterior segments.

phenotypes might be due to some *trans*-acting factor outside the BX-C. Mutations like *Polycomb* (Lewis, 1978; Dunan and Lewis, 1982) and *Polycomb-like* (Duncan, 1982) are known to produce similar transformations. We have constructed stocks of *C1* with different combinations of first and second chromosomes and have eliminated the possibility that factor(s) located in these chromosomes may be responsible for the dominant phenotype. In addition, in the recombinant chromosomes sbd^2C1 and $C1\ abd\text{-}A^{M1}$, there has been a replacement of virtually all the third chromosome material outside the BX-C and they still show the same phenotype. These gain-of-function phenotypes are probably produced by the hybrid gene activity; it is strongly suggested by phenotype of flies $DpP115;Ubx^{9\text{-}22}C1/Ubx^{9\text{-}22}C1$, which having an inactivated hybrid gene, show no sign of *Cbx*-like or *Uab*-like phenotypes. They exhibit the normal haplo-insufficient *Ubx* phenotype.

Discussion

A functional hybrid Ubx – abd-A gene with mixed properties of both Ubx and abd-A

The results reported in the accompanying paper (Rowe and Akam, 1987) indicate that in the *C1* chromosome there is a deletion of parts of *Ubx* and *abd-A* and that the remaining parts of these genes form a composite gene able to produce hybrid RNA. Our results indicate that this hybrid gene is functional and produces a developmental response in larval and adult metameres. The $C1\ Abd\text{-}B^{M1}$ chromosome, whose only BX-C activity comes from the hybrid gene, is able to determine almost normal development of PS5 and partial abdominal development of A3–A8 segment in the larvae and in the adult abdominal segments. Its developmental properties appear to be a mixture of those of *Ubx* and *abd-A*. We have shown that $Ubx^{9\text{-}22}$, which eliminates all the *Ubx* functional products (Hogness *et al.*, 1985; Weinzierl *et al.*, 1987), eliminates completely the thoracic and abdominal properties of *C1*. This result provides strong support for the view that there is a functional hybrid *5′-abd-A–3′Ubx* gene. It also shows that at least partial *abd-A* function can be obtained through the *Ubx* 3′ exon.

The fact that the hybrid gene contains properties of both *Ubx* and *abd-A* can explain the extreme haplo-insufficient *Ubx* phenotype of *C1/+*. Our intepretation is that the *abd-A* properties of the hybrid product repress the in part normal *Ubx* activity of the homologous chromosome. There is

evidence that *abd-A* reduces the level of *Ubx* products (Struhl and White, 1985). This interpretation is strongly supported by the observation that the combination $Ubx^{9\text{-}22}$ *C1*/+ presents a normal haplo-insufficient *Ubx* phenotype (Figure 7); the elimination of the hybrid product restores a normal *Ubx* activity in the homologous chromosome.

One perplexing aspect of the properties of the hybrid gene is that it promotes a virtually normal development of PS5 (T2p−T3a) even though its *Ubx* component is only a small part of the *Ubx* product (Rowe and Akam, 1987). That the T2p compartment of the leg develops normally can be explained if the only BX-C function there is to suppress *Scr* activity (Struhl, 1982). We have shown that the hybrid gene represses prothoracic development in embryos and therefore is likely to do so in adult cells as well. We cannot, however, explain the quasi normal development of T3a (anterior third leg and haltere) in clones of cells of genotype *C1* $Abd\text{-}B^{M1}$/*Df115*. Either the *Ubx* component of the hybrid product is the critical part of the *Ubx* protein or the homology between *Ubx* and *abd-A* is greater than hitherto suspected.

The *Ubx* gene cannot promote patterns other than those of PS5 and PS6, even in the absence of *abd-A* and *Abd-B* (Casanova *et al.*, 1987). The fact that the hybrid gene determines patterns with features of PS7−PS10 indicates that they are due to the presence in the hybrid gene of the 5′ end of *abd-A*.

The hybrid gene has properties in common with normal BX-C genes. It can be expressed ectopically in the absence of the products of *Polycomb* or *extra sex comb*. This results in most segments developing alike and with a mixture of thoracic and abdominal pattern elements (Figure 6). The hybrid gene can also repress, though not very efficiently, other homeotics which are down-regulated by conventional BX-C genes This can be deduced by comparing the esc^- phenotypes of $Ubx^{9\text{-}22}$ $Abd\text{-}B^{M1}$ (with a fully active $abd\text{-}A^+$), *C1* $Abd\text{-}B^{M1}$ (with hybrid gene activity) and Ubx^{MX12} $abd\text{-}A^{M1}$ $Abd\text{-}B^{M8}$ (with no BX-C activity). In the latter all the metameres show some prothoracic development (Figure 6b) probably due to the expression of the *Sex combs reduced* (*Scr*) gene (Struhl, 1983; Sato and Denell, 1985) in the absence of BX-C genes. The prothoracic development is completely eliminated in the esc^-; $Ubx^{9\text{-}22}$ $Abd\text{-}B^{M1}$ embryos indicating that normal *abd-A* activity efficiently suppresses *Scr* activity. In esc^-; *C1* $Abd\text{-}B^{M1}$ embryos there is some prothoracic development though much less than in the genotype with no BX-C activity. Therefore the hybrid gene is able to suppress *Scr* in part. In these embryos there sometimes appear gnathal structures like cirre (Figure 6) indicating some expression of cephalic genes.

Spatial expression of the hybrid gene

The spatial expression of the gene can be deduced by morphological criteria from the comparison of embryos totally deficient for the BX-C with those containing only hybrid gene activity. Also from the effects of *C1* $Abd\text{-}B^{M1}$/*DfP115* cell clones in the adult cuticle and the phenotype of some viable combinations. Taking all these data together, the hybrid gene is expressed in an area of the body spanning from PS5 to PS14. The anterior limit of expression, PS5, coincides with that of *Ubx* and is a domain of the body where there is no *abd-A* activity. The expression of *Ubx* in PS5 is dependent on the regulatory role of the *abx* region (White and Wilcox, 1985; Cabrera *et al.*, 1985), defined by the position of the *abx* and *bx* mutations in the intron between the second microexon and the 3′ exon. Therefore this observation suggests that the *abx* region, intact in the hybrid gene, promotes the transcription of the 5′ end of *abd-A* in PS5. Conversely, the *bxd* regulatory region (Beachy *et al.*, 1985) has been deleted and this may be responsible for the strong *pbx* and *bxd* phenotypes associated with *C1* chromosomes.

The observation that the *abx* region of *Ubx* can regulate the 5′ end of *abd-A* suggests a functional homology between *Ubx* and *abd-A*. However, a normally regulated *Ubx* would not be expressed in the posterior wing whereas our results suggest some expression of the hybrid gene in this territory; *C1*/+ or, more frequently *C1*/*C1* wings, present a *Cbx*-like phenotype which is suppressed when the hybrid gene is inactivated by *cis*-linking $Ubx^{9\text{-}22}$ to *C1*. Thus, it appears that the expression of the hybrid gene in the *Ubx* domain is similar but not exactly identical to that of the normal *Ubx* gene.

The hybrid gene activity in the *Ubx* domain can account for the *Uab* and *Hab*-like phenotypes. Since the hybrid gene has some *abd-A* properties, these will promote abdominal development in parts of the *Ubx* domain. For example, they will tend to transform A1 towards a more posterior segment, yielding a *Uab*-like phenotype. Their expression in T3 may result in the disappearance of thoracic appendages, a *Hab*-like phenotype.

In the posterior limit there is also a difference between normal *Ubx* and *abd-A*, which terminate their function in PS13, and the hybrid gene, which in the absence of a *r* element (Casanova *et al.*, 1986) shows some expression in PS14, where it produces some abdominal development. We do not know the reason for this difference although it is possible that the lack of some of the regulatory regions of *abd-A* may result in abnormal expression.

Within the area PS5−PS14, the hybrid gene is active but its developmental effects appear to be different in the distinct metameres. In the larvae, PS6 and PS7 are completely transformed into PS5 whilst the rest of the parasegments develop with a mixture of thoracic and abdominal pattern elements (Figure 5), becoming gradually more abdominal in the more posterior parasegments. This indicates that the expression of the hybrid gene, in the absence of any other BX-C activity, is developmentally regulated. Part or all this regulation may be attributed to the *iab-3* and *iab-4* regions which are present in the hybrid gene. These are part of the *abd-A* gene and possibly have a regulatory role. They may modulate the amount of hybrid gene product and/or perhaps the nature of the product in the different segments. We cannot exclude that this regulation may in part be due to factors extrinsic to the BX-C.

Materials and methods

Mutant stocks

A large number of mutations at the BX-C and others have been used for complementation and phenotypic analyses. The mutations abx^2, bx^3, bx^{34e}, bxd^1, pbx^1, $iab\text{-}2^k$, Ubx^1, Ubx^{130}, Ubx^{M1}, $abd\text{-}A^{M1}$, $abd\text{-}A^{M2}$, $abd\text{-}A^{M3}$, *C26*, $Abd\text{-}B^{M1}$, $Abd\text{-}B^{M7}$, and the deletions and rearrangements $Df(3R)bxd^{100}$, *Df(3R)P9*, *Df(3R)C4*, $Tp(3;3)bxd^{100}$, Tp(3;3)146, T(2;3)P10 and *T(1;3)P115* have already been described in Lewis (1978), Sánchez-Herrero *et al.* (1985), Morata *et al.* (1983), Kuhn *et al.* (1981), Karch *et al.* (1985) and Casanova *et al.* (1986). Other mutations like *extra sex comb* (*esc*) and *Polycomb* (*Pc*) have been described by Struhl (1981, 1983) and Duncan and Lewis (1982).

Chromosomes carrying C1

The EMS induced mutation *C1* was obtained from its discoverer, Dr Gary Struhl. We have *C1* in various stocks with different combinations of markers and balancers. We have also constructed several *cis* combinations of *C1* with other mutations at the BX-C, namely $Ubx^{9\text{-}22}$*DfC1*, *DfC1 Abd-*B^{M1} and *DfC1 Abd-*B^{M5}. The detection of the recombinants was based on the dominant phenotype of *Ubx* and *Abd-B* mutations. To synthesize the recombinant $Ubx^{9\text{-}22}$ *C1* we have made use of a $Ubx^{9\text{-}22}$*Abd-*B^{M1} chromosome (a gracious gift of Dr Gary Struhl). By appropriate crosses females of genotype *DpP10*/+; $sbd^2Ubx^{9\text{-}22}$*Abd-*B^{M1}/*C1* are generated. In these, the *DpP10*, carrying Ubx^+ in the second chromosome, covers the lethality of the third chromosome. They were mated to sbd^2 males. The progeny that was sbd^2 but did not show a dominant *Abd-B* phenotype result from a recombination event between *sbd* and *Abd-B* (~0.5 cM). Every one of these recombinants was progeny-tested for lethality with *abd-*A^{M1} and also for phenotype with bx^{34e}, with which $Ubx^{9\text{-}22}$ gives a moderate phenotype but *C1* gives a very slight one. Out of a total of 11 recombinants seven were found to be sbd^2*C1* (lethal over *abd-*A^{M1} but slight *bx* phenotype), three were $sbd^2Ubx^{9\text{-}22}$ (moderate phenotype ovex bx^{34e} but it complements *abd-*A^{M1}) and one was $Ubx^{9\text{-}22}$*C1* as it gives a strong *bx* phenotype and is lethal over *abd-*A^{M1}.

The recombinant *C1 Abd-*B^{M1} was obtained by generating females *C1*/*Abd-*B^{M1} that were mated to bx^{34e}. Flies with slight *bx* and *Abd-B* phenotypes were selected as possible recombinants and subsequently tested for lethality with *abd-*A^{M1}. The same method was used for *C1 Abd-*B^{M5}.

Genetic mosaics

We have studied the effect of *C1* in the adult cuticle by generating by mitotic recombination cell clones hemizygous for *C1*, $Ubx^{9\text{-}22}$*C1* and *C1 Abd-*B^{M1}.

Two different methods were used (see Sánchez-Herrero *et al.*, 1985b for details and mitotic recombination schemes). In the first case embryos and larvae of genotype *M(1)*o^{Sp} *DpP115*/yf^{36}; *C1*/*DfP115* were irradiated (500 or 1000 R). After mitotic recombination in the first chromosome, cell clones marked with yf^{36} were produced, of which ~80% have lost the *DpP115* (carrying a full dose of the BX-C) and are therefore hemizygous for *C1*. The same scheme was used for clones of genotype $Ubx^{9\text{-}22}$*C1*/*DfP115* and *C1 Abd-*B^{M1}/*DfP115*.

The second method utilizes mitotic recombination in the left arm of third chromosome. The clones are generated in larvae of genotype *y* ; *Dp*Sc^{J4}*Dp146 M(3)*i^{55}*DfP115*/*mwh jv C1* and clones hemizygous for *C1* are marked with *y*, *mwh* and *jv*. This method has the advantage that virtually 100% of the marked clones have lost the *Dp146* and are mutant for the BX-C combinations.

Preparation of the larval and adult epidermis

For the study of larval cuticle, embryos were dechorionated, fixed and cleared as described by van der Meer (1977).

For the adult cuticle, flies were cut in pertinent pieces, internal organs digested with hot 10% KOH, washed with alcohol and mounted in Euparal.

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