The EMBO Journal vol.6 no.10 pp.3103-3109, 1987

Double and triple mutant combinations of the bithorax complex of *Drosophila*

Jordi Casanova, Ernesto Sánchez-Herrero[1], Ana Busturia and Ginés Morata

Centro de Biología Molecular C.S.I.C., Universidad Autónoma de Madrid, Canto Blanco, Madrid 28049, Spain and [1]Genetics Department, University of Cambridge, Downing Street, Cambridge CB2 3EH, UK

Communicated by G.Morata

We have constructed double and triple mutant combinations for the *Ubx, abd-A* and *Abd-B* genes of the bithorax complex and have examined the homeotic transformations they produce in the larval and adult patterns. Embryos hemizygous for the triple combination exhibit a metameric pattern consisting of parasegments 5-12 being transformed into parasegment 4. In addition, parasegment 13 develops like a mixture of parasegment 3 and 4, and parasegment 14 is abnormal. The same phenotype is displayed by embryos homozygous for *DfP9*, lacking all the BX-C DNA, >300 kb. This result strongly supports the notion that the BX-C contains only three genes which account for all the developmental functions of the complex. The phenotypes of the different double combinations also support the same view; the *Ubx abd-A* combination shows the same phenotype as *Df109*, which lacks all thoracic and several abdominal functions. The *abd-A Abd-B* combination exhibits the same phenotype of *DpP10 DfP9*, lacking all the abdominal functions except those specific for A1. Our results also indicate that each BX-C gene becomes active autonomously regardless of the presence or functional state of the other BX-C genes.

Key words: Drosophila/bithorax complex/mutants/homeotic transformations

Introduction

The genes clustered in the bithorax complex (BX-C) specify the development of part of the thorax and the abdomen of *Drosophila* (Lewis, 1978; Sánchez-Herrero *et al.*, 1985a). There is some dispute concerning the number and structure of BX-C genes. The model of Lewis (1978, 1982) proposes that the characteristic development of each segment within the bithorax domain is specified by a particular BX-C gene; a one metamere/one gene hypothesis. Modifications of this model (Struhl, 1984; Hayes *et al.*, 1984) have been suggested to accommodate a more precise definition of some mutant phenotypes (Morata and Kerridge, 1981; Struhl, 1984) but the essence of one metamere/one gene remains the same. However recent results based on complementation analyses of mutations within the complex (Sánchez-Herrero *et al.*, 1985a; Tiong *et al.*, 1985) indicate that the BX-C consists of only three genes, Ultrabithorax (*Ubx*), abdominal-A (*abd-A*) and Abdominal-B (*Abd-B*) which account for all the developmental functions of the BX-C. These results challenge the one metamere/one gene view and suggest that each BX-C gene specifies the development of several metameres. It is important to distinguish these two models for they suggest different approaches to understanding the functional organization of the BX-C. One test to resolve this question is to synthesize a triple combination Ubx^- $abd\text{-}A^-$ $Abd\text{-}B^-$ and ask whether it is equivalent to the deletion of all the BX-C DNA. Also the double mutant combinations should present the phenotype of the deletion of the corresponding part of the BX-C DNA. We have generated two triple and all the possible double mutant combinations. Our results strongly support the view that the BX-C comprises only three independent genes. We also suggest that each BX-C gene contains several controlling elements that modulate its expression on a parasegmental basis.

Results

Construction of double and triple mutant combinations of the BX-C genes

We started with $abd\text{-}A^{M1}$, a strong ethylmethane sulphonate (EMS)-induced allele equal in phenotype to the physical deletion of the gene (Sánchez-Herrero *et al.*, 1985a; Morata *et al.*, 1983) and caused by a breakpoint in the interval, +41.5-43 kb of BX-C DNA (B.Weiffenbach and W.Bender, personal communication). It fully complements *Ubx* and *Abd-B* mutant alleles. After further EMS treatment of the $abd\text{-}A^{M1}$ chromosome, two *Abd-B* alleles were recovered, $Abd\text{-}B^{M8}$ and $Abd\text{-}B^{M9}$. By appropriate tests with other *Abd-B* mutations (Casanova *et al.*, 1986), we found that $Abd\text{-}B^{M8}$ is a strong allele like $Abd\text{-}B^{M1}$, equivalent to the deletion of the gene. We therefore used the double $abd\text{-}A^{M1}Abd\text{-}B^{M8}$ for further mutagenesis and for phenotypic description. The $abd\text{-}A^{M1}Abd\text{-}B^{M8}$ chromosome was X-irradiated to produce *Ubx* mutations (EMS was not used because it is very inefficient in producing strong *Ubx* mutations, see Sánchez-Herrero *et al.*, 1985a). Two triple combinations $Ubx^{MX11}abd\text{-}A^{M1}Abd\text{-}B^{M8}$ and $Ubx^{MX12}abd\text{-}A^{M1}Abd\text{-}B^{M8}$ were obtained. The two new *Ubx* mutations were characterized by their phenotype over *abx* or *bxd* mutations. Ubx^{MX11} is a moderate allele like Ubx^1 or Ubx^{9-22} (Kerridge and Morata, 1982) while Ubx^{MX12} is a strong mutation equivalent to the deletion of the gene. Both triple combinations fully complement lethal alleles of *11b* and *1rb* (Sánchez-Herrero *et al.*, 1985a), the genes that flank the BX-C at either side. This result indicates that the defects associated with both triple chromosomes are restricted to the BX-C.

In a separate mutagenesis experiment the $abd\text{-}A^{M1}$ chromosome was irradiated to obtain a *Ubx* mutation. The allele obtained in this way, Ubx^{MX6}, shows moderate adult phenotypes over *abx* and *bxd* alleles.

Finally two double combinations were obtained by recombining the pre-existing alleles Ubx^{9-22}, $Abd\text{-}B^{M1}$ and $Abd\text{-}B^{M5}$; $Ubx^{9-22}Abd\text{-}B^{M1}$ (kindly provided by Professor G.Struhl) and $Ubx^{9-22}Abd\text{-}B^{M5}$.

Particularly in the case of the strong triple combination $Ubx^{MX12}abd\text{-}A^{M1}Abd\text{-}B^{M8}$ it was important to demonstrate that it is not a deletion of the BX-C DNA. By Southern analysis we assayed the presence in the triply mutant combination of two genetic fragments, one located near the intersection of the *Ubx*

© IRL Press Limited, Oxford, England

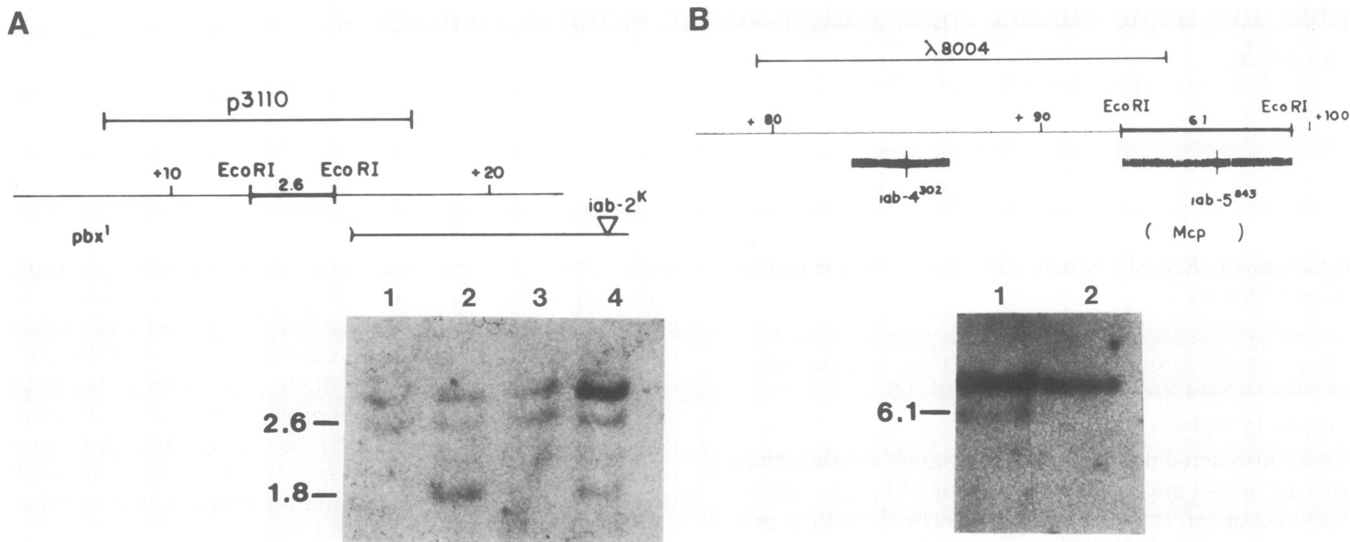

Fig. 1. Demonstration that there are no deficiencies between *Ubx* and *abd-A* (**A**) and between *abd-A* and *Abd-B* (**B**) in the triple mutant chromosome *Ubx*^{MX12}*abd-A*^{M1}*Abd-B*^{M8}. The data on restriction maps and localization of the mutants is taken from Karch *et al.* (1985). (**A**) Southern blot of *Eco*RI-digested DNA and hybridized with DNA from p3110 plasmid of the following stocks. (**1**) Multiple marked chromosome, *mm*, the original chromosome on which all our BX-C mutants have been induced (Sánchez-Herrero *et al.*, 1985a). (**2**) *Ubx*^{MX12}*abd-A*^{M1}*Abd-B*^{M8}/*TM1*. (**3**) *Ubx*^{MX12}*abd-A*^{M1}*Abd-B*^{M8}/*DpP5*. (**4**) *Ubx*$^{9-22}$/*TM1.B*. *Eco*RI-digested DNA hybridized with DNA from phage λ8004 from the stocks: (**1**) *Ubx*^{MX12}*abd-A*^{M1}*Abd-B*^{M8}/*Mcp* and (**2**) *Mcp*/*Mcp*.

and *abd-A* genes and the other around the junction of *abd-A* and *Abd-B*.

In the first case we used a 2.6-kb *Eco*RI fragment (Figure 1), part of the DNA deleted in *pbx*^{1}, the most distal mutation at the *Ubx* gene (Bender *et al.*, 1983). This DNA exhibits polymorphism in the TM1 chromosome (M.Akam, personal communication), yielding a 1.8-kb *Eco*RI fragment. Digested genomic DNA from the triple/TM1 stock and from other appropriate controls (Figure 1A) were hybridized with nick-translated DNA from the p3110 plasmid. The presence of the 2.6-kb fragment in the triple/TM1 stock can only originate from the DNA of the triple mutant combination, thus eliminating the possibility of a deletion of this region of the BX-C.

In the second case we took advantage of the fact that the *Mcp* mutant chromosome has a 3.6-kb deletion (Karch *et al.*, 1985) included in the 6.1-kb *Eco*RI fragment as indicated in Figure 1B. This DNA includes part of the most proximal region of the *Abd-B* gene. DNA from the triple/*Mcp* and *Mcp*/*Mcp* flies was digested with *Eco*RI and hybridized with nick-translated DNA from phage λ8004 (Karch *et al.*, 1985). The 6.1-kb fragment present in the triple/*Mcp* (Figure 1B) can only have originated from the triple mutant chromosome.

Homeotic transformations caused by the triple and double mutant combinations

Embryonic phenotypes. Embryos hemizygous for the triple combinations *Ubx*^{MX12}*abd-A*^{M1}*Abd-B*^{M8} and *Ubx*^{MX11}*abd-A*^{M1}*Abd-B*^{M8} were examined in detail. We describe the metameric pattern of these embryos in terms of parasegments (Martínez-Arias and Lawrence, 1985) because the embryonic activity of the BX-C genes is defined spatially by parasegmental boundaries. However the morphological features that can easily be distinguished (denticle belts, ventral pits) belong to anterior compartments (the posterior parts of parasegments). Thus, parasegment 3 (PS3) is identified by the denticle belt of the prothorax, T1a, PS4 by T2a, PS5 by T3a, PS6 by the belt of the first abdominal segment (A1a), PS9 by A4a and so forth.

The homeotic transformations in *Ubx*^{MX12}*abd-A*^{M1}*Abd-B*^{M8}

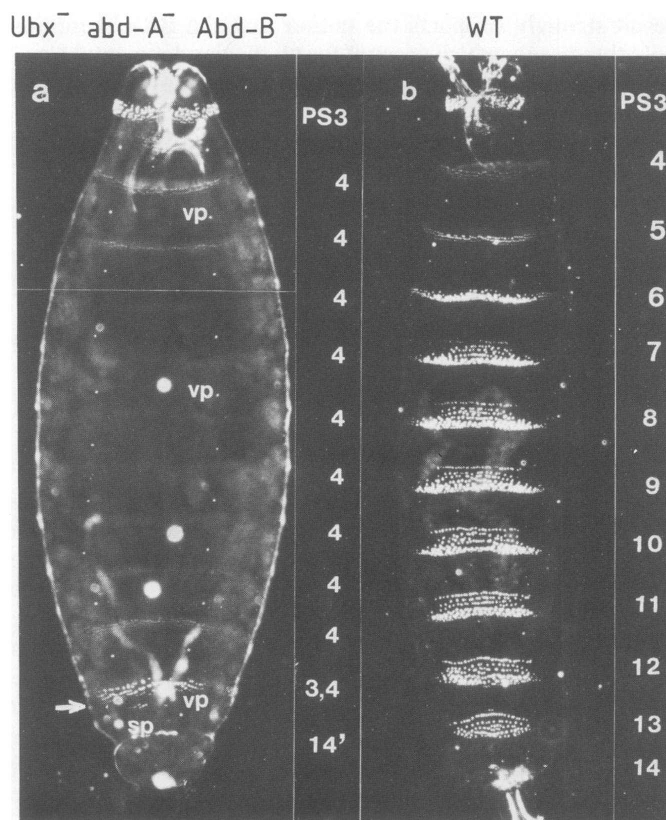

Fig. 2. Ventral view of embryos of genotype (**a**) *Ubx*^{MX12}*abd-A*^{M1}*Abd-B*^{M8}/*DfP9* and (**b**) wild-type. In (**a**) the homeotic transformations resemble in every aspect those described for *DfP9*. All parasegments posterior to PS4 are transformed into PS4, as indicated by a number of diagnostic criteria like denticle size, ventral pits (vp), Keilin's organs, dorsal spinules, etc. The last denticle belt (arrow) shows features intermediate between T1a and T2a as indicated by the presence of a second group of denticles characteristic of T1a. Also PS14 is abnormal (PS14'), but not transformed towards PS4, in that it presents sclerotic plates (sp). (**b**) Normal metameric pattern.

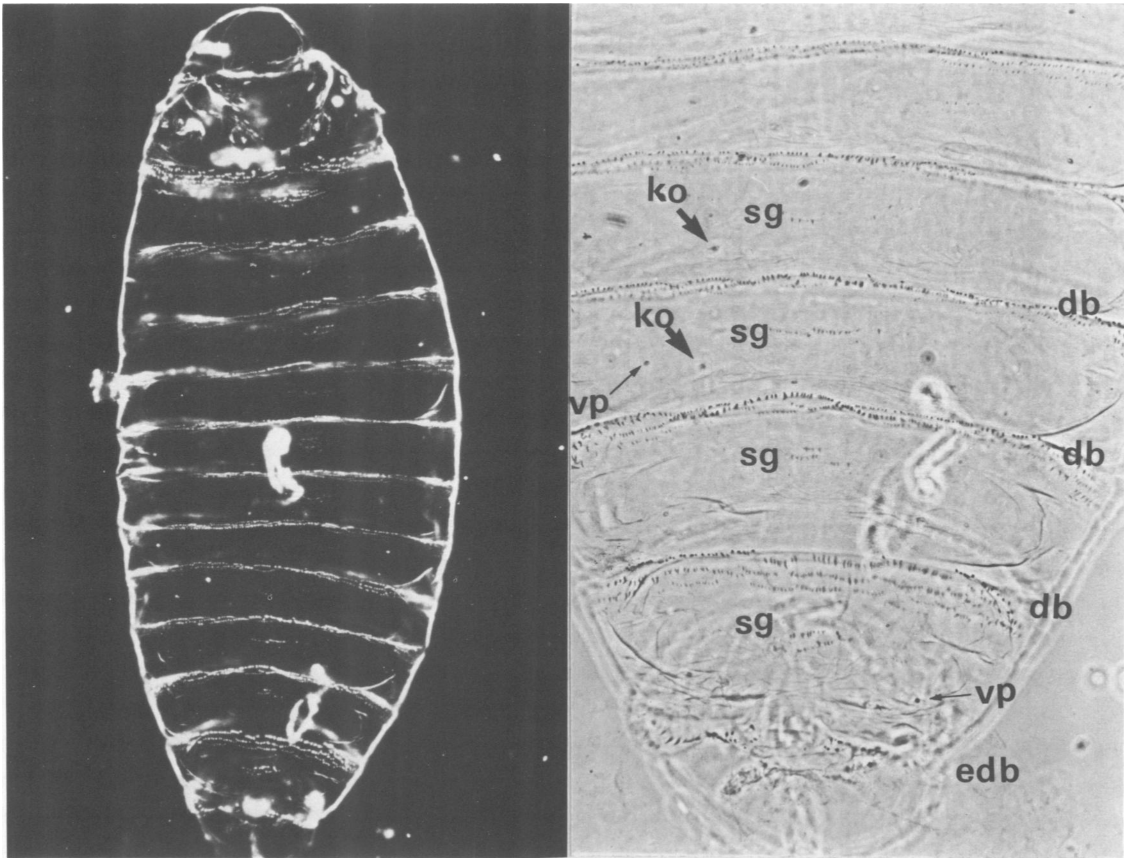

Fig. 3. Embryo of genotype *esc*$^-$; *Ubx*^{MX12}*abd-A*^{M1}*Abd-B*^{M8}. All cephalic, thoracic and abdominal metameres are profoundly modified. A region including parasegments 9–13 is magnified in the right hand side of the figure. They all develop with a mixture of prothoracic and mesothoracic patterns as indicated by the rudimentary second group of denticles (sg), the ventral pits (vp) and Keilin's organs (ko) some of which are marked. There is also an extra denticle belt (edb) developing in PS14. The homeotic transformations observed in these embryos are identical to those described for *esc*$^-$; *DfP9* (Struhl, 1983).

(heretofore referred to as *Ubx*$^-$*abd-A*$^-$*Abd-B*$^-$) resemble in every respect those described for *DfP9* (Lewis, 1978; Morata *et al.*, 1983; Struhl, 1983). PS5–PS12 develop like PS4 (Figure 2a) as indicated by denticle size, ventral pits, Keilin's organs, dorsal spinules, etc. The denticle belt of PS13 shows features intermediate between PS3 and PS4; the denticles are bigger and there is a centrally located second group of denticles, characteristic of T1a (PS3). PS14 is also abnormal, showing sclerotic plates. The other triple combination, *Ubx*^{MX11}*abd-A*^{M1}*Abd-B*^{M8}, is almost identical, with the difference that the second group of denticles in PS13 appears less frequently than in the former combination.

In the case of the triple combination *Ubx*$^-$*abd-A*$^-$*Abd-B*$^-$ we also performed an experiment to detect any BX-C function which might remain in the chromosome: Struhl (1981, 1983) showed that the elimination of *extra sex combs* (*esc*) product results in full and indiscriminate expression of all BX-C functions present. We therefore studied *esc*$^-$ embryos (from *esc*$^-$ mothers) hemizygous for the triple combination *Ubx*$^-$*abd-A*$^-$*Abd-B*$^-$. All metameres present thoracic denticle belts, Keilin's organs and ventral pits indicating a thoracic identity (Figure 3). They also show a rudimentary second group of denticles, typical of the anterior prothorax (PS3). Thus, the metameric pattern can be best described as intermediate between PS3 and PS4. This is the pattern found by Struhl (1983) in *esc*$^-$; *DfP9* embryos.

Embryos of the three double combinations *abd-A*^{M1}*Abd-B*^{M8}, *Ubx*^{MX6}*abd-A*^{M1} and *Ubx*$^{9-22}$*Abd-B*^{M1} are shown in Figure 4a,b,c. Embryos of genotype *abd-A*^{M1}*Abd-B*^{M8} (which can be described as *Ubx*$^+$*abd-A*$^-$*Abd-B*$^-$) show a phenotype identical to that of the synthetic deletion *DpP10 DfP9* (Lewis, 1978; Morata *et al.*, 1983): PS5–PS6 are normal, then PS7–PS13 develop as a chain of reiterated PS6. We often see monohairs in between transformed denticle belts indicating thoracic transformation (T3p) of posterior compartments (Struhl, 1984). The comparison of this phenotype with that produced by the triple *Ubx*$^-$*abd-A*$^-$*Abd-B*$^-$ (Figure 2a) can be used to demarcate the region of the body in which the *Ubx* gene, in the absence of *abd-A* and *Abd-B*, is morphogenetically active. By this criterion *Ubx* activity spans from PS5 to PS13. This is the same region defined by *in situ* hybridization (Akam and Martínez-Arias, 1985) or anti-*Ubx* antibodies (White and Wilcox, 1984; Beachy *et al.*, 1985).

In the combination *Ubx*$^{9-22}$*Abd-B*^{M1} (*Ubx*$^-$*abd-A*$^+$*Abd-B*$^-$) PS5 and PS6 (Figure 4b) develop as PS4, as expected for *Ubx*$^-$. From PS7 down to PS13 all the metameric patterns are abnormal. They present a mixture of thoracic and abdominal features. The denticles are of abdominal type, but there are ventral pits, a typical thoracic character. We nearly always observe ventral pits in PS13 which are never present in either *Ubx*$^-$ or in *Abd-B*$^-$ embryos. This illustrates the combinatorial manner in which these patterns are produced (Struhl, 1982). The comparison of these embryos with the *Ubx*$^-$*abd-A*$^-$*Abd-B*$^-$ ones permits us to

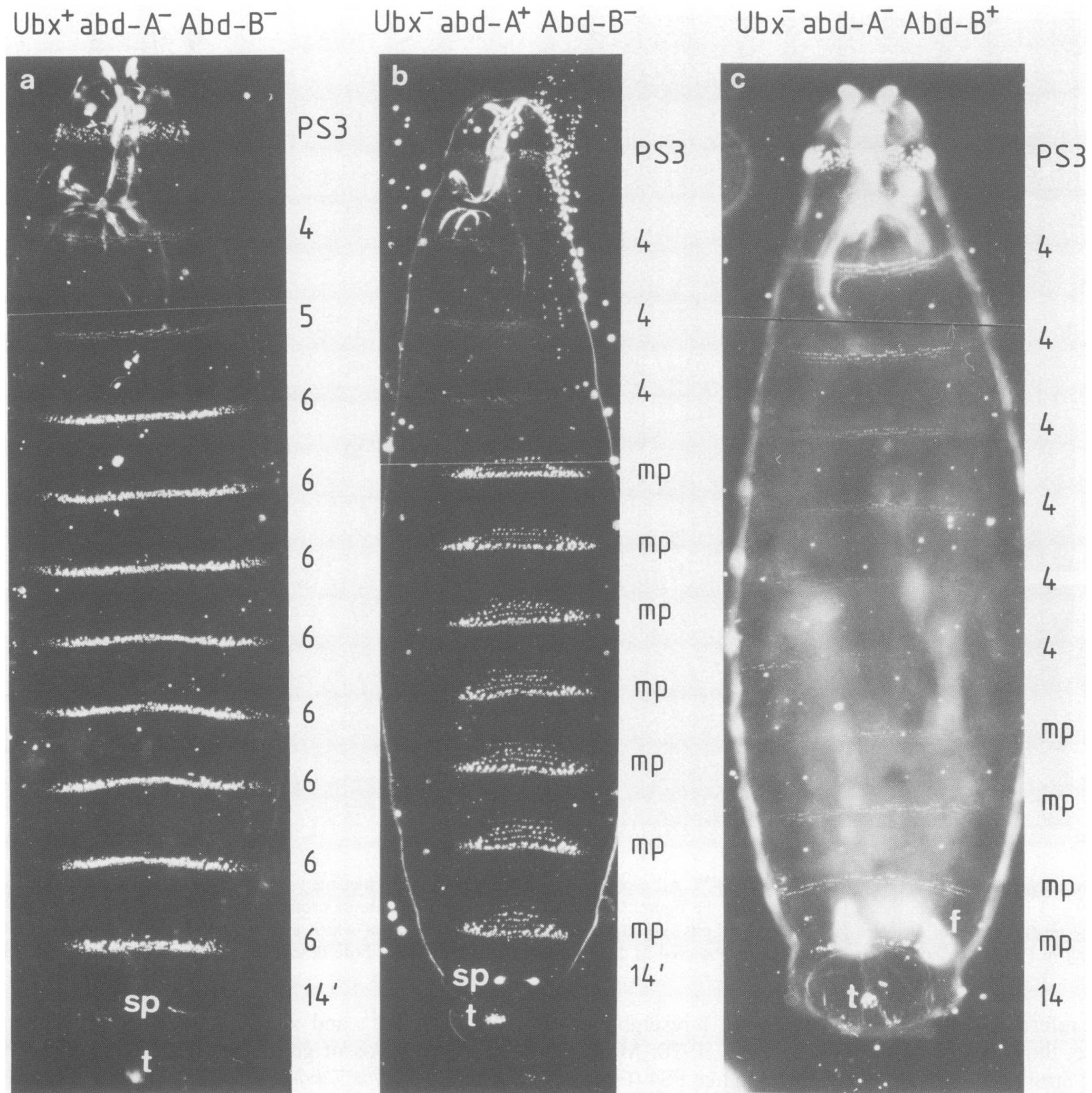

Fig. 4. Ventral view of embryos hemizygous for the three double combinations (**a**) *abd-A*^{M1}*Abd-B*^{M8} (*Ubx*$^+$*abd-A*$^-$*Abd-B*$^-$). (**b**) *Ubx*$^{9-22}$*Abd-B*^{M1} (*Ubx*$^-$*abd-A*$^+$*Abd-B*$^-$) and (**c**) *Ubx*^{MX6} *abd-A*^{M1} (*Ubx*$^-$*abd-A*$^-$*Abd-B*$^+$). (**a**) The phenotype is identical to that of the synthetic deletion *DpP10DfP9* (Morata *et al.*, 1983) in which 160–180 kb of BX-C DNA are lacking. PS5–PS6 are normal, then all more caudal parasegments develop as PS6. PS14 is abnormal, presenting sclerotic plates (sp). (**b**) In these embryos, PS5 and PS6 develop as PS4 as expected for *Ubx*$^-$. From PS7 downwards to PS13, all the metameres are alike but exhibit a mixed pattern (mp) of thoracic (ventral pits) and abdominal (denticles) elements. PS14 is very similar to that in (**a**), presenting sclerotic plates (sp). (**c**) This phenotype is like that of *Df109* (Morata *et al.*, 1983). PS5–PS9 develop as PS4 just as they do in the triple combination or in *DfP9*. PS10–PS13 develop mixed patterns (mp), different from that of (**b**) but also composed of a mixture of thoracic and abdominal pattern elements. PS14 appears normal in these embryos; it does not present sclerotic plates. These embryos develop filzkorpers (f), which in the picture appear as white blots as they are located in the dorsal side of the embryo. Structures posterior to PS14 like the anal plates or the tuft (t) develop normally in the three genotypes.

define the area of activity of *abd-A* in the absence of *Ubx* and *Abd-B*. This extends from PS7 to PS13.

The phenotype of the double *Ubx*^{MX6}*abd-A*^{M1} (*Ubx*$^-$*abd-A*$^-$*Abd-B*$^+$) is the same as that of *Df109* (Morata *et al.*, 1983): PS5–PS9 develop as a chain of PS4s, just as they do in the triple combination or in *DfP9*. In the region PS10–PS13 the patterns found do not resemble any other metameric pattern. The denticle belts are formed by denticles of thoracic and of abdominal type. There are thoracic pattern elements like ventral pits and defective Keilin's organs. Unlike the previous triple and double combinations, these embryos do not show sclerotic plates which are part of PS14 (Casanova *et al.*, 1986). The area of activity of *Abd-B* is then PS10–PS14.

Adult phenotype. This was studied (i) by analysing the *trans* combinations of the triple mutant chromosome with a number of viable mutations of the BX-C and (ii) by generating cell clones of marked tissue made hemizygous for the triple and one double combination.

For the *trans* study we have made combinations of *Ubx*$^-$*abd-A*$^-$*Abd-B*$^-$ with the following mutations: *abx*^{2}, *bx*^{3}, *bx*^{34e}, *bxd*^{1}, *pbx*^{1}, *pbx*^{2}, *iab-2*^{k}, *iab-3*^{277}, *iab-4*^{302}, *abd-A*^{M3}, *iab-4,5*^{DB}, *iab-5*^{C7}, *x23-1 and iab-8*. (The phenotypes of all these mutations are known in detail; Lewis, 1963; Karch *et al.*, 1985; Sánchez-Herrero *et al.*, 1985a; Casanova *et al.*, 1985, 1986). The triple combination failed to complement any of these mutations and produced in every case a phenotype similar to that of their *trans*

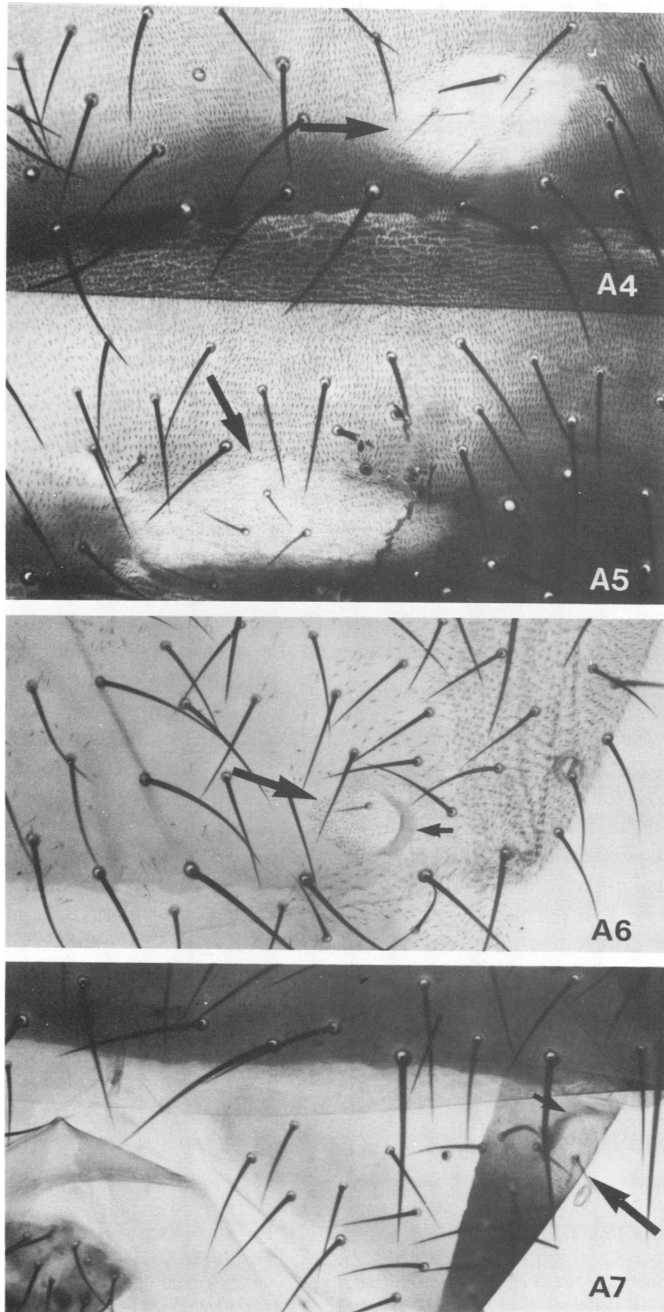

Fig. 5. Cell clones (arrows) hemizygous for the *abd-A*^{M1}*Abd-B*^{M8} double mutant combination in abdominal segments 4, 5, 6 and 7. All the clones develop the characteristic pattern of the Ala compartment (the posterior compartments of abdominal segments cannot be discerned in the adult cuticle). Distinctive features are the small, fine bristles and also the arch-like cuticular engrossment (small arrows).

combination with *DfP9*. Thus, the area of the body in which the chromosome *Ubx*$^-$*abd-A*$^-$*Abd-B*$^-$ produces a transformation spans from PS5 (anterior limit defined by *abx*) to PS14 (posterior end of BX-C function defined by *x23-1* and *iab-8*).

Analysis of mosaics was performed by X-ray induced mitotic recombination. This produces cell clones of marked tissue (see Materials and methods) which are mutant for the combination under study. These clones may appear in all body segments so that one can build up a piecemeal description of the adult phenotype.

Clones hemizygous for the triple combination, produced during the larval period, develop normally in the head (data not shown), in the thoracic part of PS3 (T1a, six clones) and in PS4 (T1p-T2a, 22 clones). For PS5 (T2p-T3a) the clones (n = 4) in T2p develop normally. They do not show the *postprothorax* (*ppx*, T2p→T1p) transformation because this appears only when the clones are initiated in early embryogenesis (Morata and Kerridge, 1981). Since the flies *Ubx*$^-$*abd-A*$^-$*Abd-B*$^-$/*abx*^{2} show the *ppx* phenotype, the tedious process of making clones in early development was judged unnecessary. In T3a (n = 19) the clones differentiate T2a structures. For example in the anterior haltere they form anterior wing. In PS6 (T3p-Ala), clones at T3p (n = 9) develop as T2p. Those in Ala fail to differentiate, probably because the development of thoracic structures cannot proceed in abdominal environment (Morata and García-Bellido, 1976; Kerridge and Morata, 1982). Probably for the same reason the clones failed to appear in the rest of the abdominal metameres. However, we sometimes see small patches of marked cuticle associated with invaginations and abnormal differentiations that we interpret as abortive clones showing thoracic transformation.

Clones hemizygous for the double combination *Ubx*$^+$*abd-A*$^-$*Abd-B*$^-$ were also produced. The mutant clones found in the body parts corresponding to all anterior parasegments until PS6 are all normal. In the metameres PS7–PS12 (that in the adult are visualized as the anterior compartments of A2–A7 segments) we found a total of 349 clones, more or less equally distributed in all segments, except in A7 where we detected fewer (n = 16) because of the difficulty in scoring clones in A7 of males. These clones are always homeotically transformed, differentiating Ala structures (Figure 5). This is the pattern corresponding to the total absence of abdominal functions of the BX-C (except of A1) and to the full expression of *Ubx*$^+$ activity. We do not find clones in the adult terminalia (genitalia and analia), indicating a requirement there. Thus, the entire adult abdomen (except A1), including terminalia, requires the functions encoded by the *abd-A* and *Abd-B* genes.

Discussion

The number of genes in the BX-C and their mode of action

Our results clearly argue that the three genes *Ubx, abd-A* and *Abd-B* code for the totality of the BX-C functions. The elimination by successive mutagenesis of the activity of the three genes results in a set of homeotic transformations identical to that described for *DfP9* (Lewis, 1978; Struhl, 1981), in which all the BX-C DNA, >300 kb (Karch *et al.*, 1985), is lacking. This conclusion is further supported by the phenotype of *esc*$^-$ embryos hemizygous for the triple mutant combination. In these embryos there is a derepression of any BX-C function present (Struhl, 1981, 1983) and we find that their phenotype (Figure 3) is indistinguishable from that of *esc*$^-$; *DfP9* embryos.

The results obtained for the adult structures also support the same view. The phenotypes of mutant clones both in the triple and the double *Ubx*$^+$*abd-A*$^-$*Abd-B*$^-$ combination are entirely consistent with the embryonic phenotypes. The results obtained with the cell clones of the double combination are particularly conclusive since in this combination we observe the same phenotype, in larval and adult cells, as with the physical deletion of all the abdominal BX-C genes (*DpP10DfP9*).

We have not studied the effect of these combinations in nonectodermal tissues like the mesoderm. However, BX-C mutations affect these tissues in the same manner they affect the epidermis (Lawrence, 1985). Therefore we presume that their effect

in other tissues is homologous to that seen in the ectoderm.

The phenotype of the double mutant combinations in which there is only one active BX-C gene allows us to demarcate the areas of activity of each gene in the absence of the other two. *Ubx* is active in PS5−PS13, *abd-A* in PS7−PS13 and *Abd-B* in PS10−PS14. These areas coincide with those reported in studies of distribution of BX-C gene products (Akam, 1983; Akam and Martínez-Arias, 1985; Harding *et al.*, 1985). The only discrepancy is that Harding *et al.* (1985) have reported that transcripts from *abd-A* are present in PS14. We believe that if these transcripts have a role there, it is a minor one. These results indicate that the presence or absence of one or two BX-C genes does not affect the area of activity of the others and suggest that each BX-C gene reacts autonomously to exogenous signals determining their spatial expression.

It is of interest that *Abd-B* is the only BX-C gene which by morphological criteria appears to be active in PS14, although its role there is special (Casanova *et al.*, 1986); the presence or absence of *abd-A* (or of *Ubx*) does not detectably affect the development of PS14. Consider for example that the triple combination (Figure 2a) or *DfP9* have the same phenotype in PS14 as a strong *Abd-B* mutation (Figure 4b).

The distribution of the *Abd-B* homeoproduct (Harding *et al.*, 1985) is also of interest in connection with the one metamere/one gene models as the *Abd-B* homeobox is located in the DNA region labelled *iab-7* by Karch *et al.* (1985). Yet it is expressed in the A5−A9 segments (PS10−PS14). This contradicts any one metamere/one gene hypothesis, in which the *iab-7* gene product should not be present in regions anterior to A7 (Lewis, 1978, 1982).

One relevant functional feature of the BX-C genes is that, although they are normally contiguous, there is no need of *cis* linkage for normal function. A chromosome fragment containing Ubx^{+} alone and another one carrying $abd\text{-}A^{+}$ and $Abd\text{-}B^{+}$ can be combined to produce a normal fly (Struhl, 1984). Similarly, flies containing separate Ubx^{+}–$abd\text{-}A^{+}$ and $Abd\text{-}B^{+}$ fragments have normal pattern, viability and fertility (Tiong *et al.*, 1987). Furthermore, the chromosome *Df109*, in which the genes *Ubx* and *abd-A* are lacking, contains a fully active *Abd-B* gene which complements all known *Abd-B* mutations (Sánchez-Herrero *et al.*, 1985a).

Finally, the three BX-C genes appear to specify different patterns. As seen in the $Ubx^{+}abd\text{-}A^{-}Abd\text{-}B^{-}$ combination (Figure 4a) all parasegments posterior to PS6 develop as PS6. Therefore, *Ubx* activity can only generate PS5 and PS6 patterns. Only if *abd-A* is active will the normal pattern of PS7−PS9 appear (Sánchez-Herrero *et al.*, 1985a,b). Thus, all the morphological differences between PS7−PS9 and PS6 are solely attributable to *abd-A* activity. A similar argument can be made for the PS10−PS13 domain and *Abd-B* activity [the case of PS14 is special, Casanova *et al.* (1986)].

By all the criteria used; their mutability, their areas of activity and the specific patterns they promote, the three BX-C genes behave independently of each other. Thus, the clustering of the BX-C genes may be relevant as to the evolutionary history of the complex, but appears to be of little functional significance.

A functional model of BX-C organization

The preceding discussion indicates that the entire BX-C domain is divided into three subdomains PS5−PS6, PS7−PS9, and PS10−PS14 whose specific development is determined by *Ubx*, *abd-A* and *Abd-B*, respectively. However, each subdomain contains several parasegments which exhibit different developmental patterns. This implies that the activities of the BX-C genes are modulated within each domain.

In the case of *Ubx* there is direct evidence (Akam and Martínez-Arias, 1985) that the amount of product is very different in PS5 and PS6. Similarly, the amount of *Abd-B* transcripts varies in different parts of the domain (Harding *et al.*, 1985). In *Ubx*, the appropriate level and possibly nature of the product appears to depend on the function of *abx* and *bxd*, two *cis*-acting elements specific to PS5 and PS6, respectively (Casanova *et al.*, 1985; Beachy *et al.*, 1985; White and Wilcox, 1985; Cabrera *et al.*, 1985). Both elements act independently of each other, but they both affect the spatial expression of *Ubx* function (Casanova *et al.*, 1985).

We know virtually nothing of the controlling elements of *abd-A* and *Abd-B*, although there is a mutation at *abd-A*, $iab\text{-}2^{k}$ (Kuhn *et al.*, 1981) which behaves as a mutation in a genetic element similar to *abx* or *bxd*. There is no evidence as to whether the *abd-A* and *Abd-B* are modulated like *Ubx* on a parasegmental basis. However, the observation (Casanova *et al.*, 1986) that the repeat pattern unit of esc^{-} and Pc^{-} embryos, in which *abd-A* and *Abd-B* are maximally expressed, is PS13 (A7p-A8a), not A8 (A8a-A8p) clearly suggests a parasegmental modulation. For this pattern could not be achieved if *Abd-B* were modulated in units other than parasegmental ones. This observation supports the hypothesis of Martínez-Arias and Lawrence (1985) that the parasegment, not the segment, is the fundamental unit of insect design.

Given the similar structure of the BX-C genes molecularly (i.e. the presence of a homeobox) and genetically in the way they are affected by mutations like *Pc* or *esc* (Casanova *et al.*, 1986; Struhl, 1981) we suggest they work in ways similar to *Ubx*: each gene codes a family of related homeobox-containing proteins and also contains controlling elements. These elements modulate the level and perhaps the nature of the gene product parasegment by parasegment. In this view the classical genetic elements of Lewis (*abx, bxd, iab-2, ... iab-8*) would be control regions of the expression of the three proper BX-C genes. The controlling elements may function through specific products or by acting directly on the transcription of *Ubx, abd-A* and *Abd-B*.

Materials and methods

Mutations, rearrangements and crosses

We have used a large number of mutations at the BX-C. They are referred to in the main text when appropriate, but a full description is not necessary as they have been described in recent papers (Lewis, 1978, 1982; Bender *et al.*, 1983; Sánchez-Herrero *et al.*, 1985a,b; Karch *et al.*, 1985). A full description of the BX-C deletions used here (*DfP9, Df109, DpP10 Df109, DpP10 DfP9*) can be found in Lewis (1978) and Morata *et al.* (1983).

As a rule, all the phenotypes have been defined in hemizygous combinations, using the appropriate deletions. To synthesize esc^{-} embryos hemizygous for the triple combinations, we used the same crosses as in Casanova *et al.* (1986).

Southern analysis

DNA was extracted basically as described in Henikoff (1983). Twenty flies from each relevant genotype were homogenized in 0.1 M Tris (pH 8.0), 0.01 M EDTA, 0.35 M NaCl, 2% SDS, 7 M urea. The homogenates were extracted twice with phenol:chloroform (1:1) and the nucleic acids precipitated twice with ethanol and finally resuspended in 10 mM Tris, 1 mM EDTA with 50 μg/ml boiled RNase A. Southern blots and nick translation were made as described in Maniatis *et al.* (1982). Exposures were at −70°C with intensifying screens for 3 days.

Production of genetic mosaics

Marked mutant clones hemizygous for the double $abd\text{-}A^{M1}Abd\text{-}B^{M8}$ combination were generated by X-irradiation of larvae of genotype *y; Dp(1;3)sc*^{J4} *Dp(3;3)146 M(3)i*^{55} *Df(3R)P115/mwh jv* $abd\text{-}A^{M1}Abd\text{-}B^{M8}$. Flies of this genotype have normal segment pattern because of the presence of the *Dp(3;3)146* which contains a full dose of the BX-C. After radiation-induced mitotic recombination in the III-L arm, clones *mwh jv* $abd\text{-}A^{M1}Abd\text{-}B^{M8}$*/mwh jv Df(3R)P115* are produced which are defective in *abd-A* and *Abd-B* functions. The clones are labelled with the cell markers *y, mwh* and *jv* [see Sánchez-Herrero *et al.* (1985b) for

a scheme and details of a similar mitotic recombination method]. Clones hemizygous for the triple combination *Ubx*^{MX12}*abd-A*^{M1}*Abd-B*^{M8} were produced by the same method substituting the chromosome bearing the double combination by another carrying the triple combination and appropriate marker mutants.

Preparation of the larval and adult cuticle

For the larval epidermis, late embryos were dechorionated with dilute hypochlorite and the vitelline membrane removed using a glass needle. They were mounted in Hoyers medium according to van der Meer (1977). After incubation at 60°C, slides were studied under bright-field phase or dark-field optics.

The adult cuticle was prepared by cutting the appropriate pieces under the dissecting microscope. The internal organs were digested with hot 10% KOH, washed in alcohol and mounted in Euparal for examination.

Acknowledgements

We thank Michael Akam and Peter Lawrence for critical reading of the manuscript, L.Crosby, I.Dawson, A.Rowe, E.Reoyo, J.Lince and R.Weinzierl for help and C.Hermoso for typing the manuscript. E.S.-H. is supported by an EMBO fellowship. The work in Spain is supported by the Comisión Asesora de Investigación Científica y Técnica and by the Fondo de Investigaciones Sanitarias.

References

Akam,M.E. (1983) *EMBO J.*, **2**, 2075–2084.
Akam,M.E. and Martínez-Arias,A. (1985) *EMBO J.*, **4**, 1689–1700.
Beachy,P.A., Helfand,S.L. and Hogness,D.S. (1985) *Nature*, **313**, 545–551.
Bender,W., Akam,M., Karch,F., Beachy,P.A. Pfeifer,M., Spierer,P., Lewis,E.B. and Hogness,D.S. (1983) *Science*, **221**, 23–29.
Cabrera,C., Botas,J. and García-Bellido,A. (1985) *Nature*, **318**, 369–371.
Casanova,J., Sánchez-Herrero,E. and Morata,G. (1985) *Cell*, **42**, 663–669.
Casanova,J., Sánchez-Herrero,E. and Morata,G. (1986) *Cell*, **47**, 627–636.
Harding,K., Wedeen,C., McGinnis,W. and Levine,M. (1985) *Science*, **229**, 1236–1242.
Hayes,P.H., Sato,T. and Denell,R.E. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 545–549, 2273.
Henikoff,S. (1983) *Nucleic Acids Res.*, **11**, 4735–4752.
Karch,F., Weiffenbach,B., Pfeifer,M., Bender,W., Duncan,I., Celniker,S., Crosby,M. and Lewis,E.B. (1985) *Cell*, **43**, 81–96.
Kerridge,S. and Morata,G. (1982) *J. Embryol. Exp. Morphol.*, **68**, 211–234.
Kuhn,D.T., Woods,D.F. and Cook,J.L. (1981) *Mol. Gen. Genet.*, **181**, 82–86.
Lawrence,P.A. (1985) *Cold Spring Harbor Symp. Quant. Biol.*, **50**, 113–117.
Lewis,E.B. (1963) *Am. Zool.*, **3**, 33–56.
Lewis,E.B. (1978) *Nature*, **276**, 565–570.
Lewis,E.B. (1982) In Burger,M. (ed.), *Embryonic Development: Genes and Cells*. Alan R.Liss Inc., NY, pp. 1–22.
Maniatis,T., Fritsch,E.F. and Sambrook,T. (1982) *Molecular Cloning. A Laboratory Manual.*. Cold Spring Harbor Laboratory Press, NY.
Martínez-Arias,A. and Lawrence,P. (1985) *Nature*, **313**, 639–642.
Morata,G. and García-Bellido,A. (1976) *Wilhelm Roux's Arch.*, **179**, 125–143.
Morata,G. and Kerridge,S. (1981) *Nature*, **290**, 778–781.
Morata,G., Botas,J., Kerridge,S. and Struhl,G. (1983) *J. Embryol. Exp. Morphol.*, **78**, 319–341.
Sánchez-Herrero,E., Vernos,I., Marco,R. and Morata,G. (1985a) *Nature*, **313**, 108–113.
Sánchez-Herrero,E., Casanova,J., Kerridge,S. and Morata,G. (1985b) *Cold Spring Harbor Symp. Quant. Biol.*, **50**, 165–172.
Struhl,G. (1981) *Nature*, **293**, 36–41.
Struhl,G. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 7380–7384.
Struhl,G. (1983) *J. Embryol. Exp. Morphol.*, **76**, 297–331.
Struhl,G. (1984) *Nature*, **308**, 454–457.
Tiong,S., Bone,L.M. and Whittle,J.R.S. (1985) *Mol. Gen. Genet.*, **200**, 335–342.
Tiong,S.Y.K., Whittle,J.R.S. and Gibbin,M.L. (1987) *Development*, in press.
Van der Meer,J.M. (1977) *Drosophila Inf. Serv.*, **52**, 160.
White,R.A.H. and Wilcox,M. (1984) *Cell*, **39**, 163–179.
White,R.A.H. and Wilcox,M. (1985) *Nature*, **318**, 563–567.

Received on May 20, 1987; revised on June 29, 1987