Development 117, 917-923 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993



# Normal and ectopic domains of the homeotic gene *Sex combs reduced* of *Drosophila*

**Soraya Pelaz, Nuria Urquía and Ginés Morata**

Centro de Biología Molecular, Universidad Autónoma de Madrid, 28049 Madrid, Spain

## SUMMARY

**The normal expression of the homeotic gene *Sex combs reduced (Scr)* is initially restricted to parasegment 2, later extends to 3, and by germ band retraction extends further to part of parasegment 4 (T1p). We find that in the absence of the bithorax complex (BX-C) genes there is *Scr* expression in the epidermis of the posterior compartments of the thoracic and abdominal parasegments. This ectopic expression appears at the same time as the normal one in T1p and requires the normal functions of the genes *Antennapedia (Antp)* and *engrailed (en)*. In particular, *en* appears to play an important role in the activation of *Scr* because the expansion of *en* expression in *naked* mutants produces a corresponding expansion of the ectopic *Scr* stripes. We also find that in the epidermis *Antp* can have opposite effects on *Scr* expression; moderate levels of Antp product enhance *Scr* expression, whereas high levels suppress it. We propose the existence of a secondary wave of *Scr* activation, which takes place during germ band retraction, is triggered by *en* and requires *Antp* expression. It is repressed by the BX-C genes in the meso-, metathoracic and the abdominal segments.**

Key words: homeotic genes, *Scr* expression, interactions between homeoproducts, *Drosophila*

## INTRODUCTION

The major part of the body of *Drosophila* originates from the parasegmental trunk, consisting of 14 parasegments, (Martinez-Arias and Lawrence, 1985), which have characteristic identities determined by the function of the homeotic genes of the ANT-C and BX-C genes (Kaufman et al., 1990; Lewis, 1978; Sanchez-Herrero et al., 1985).

*Scr* is a member of the ANT-C which specifies the development of part of the head and thorax (Kaufman et al., 1990; Wakimoto and Kaufman, 1981; Mahaffey and Kaufman, 1988). In $Scr^-$ mutant embryos the labial segment develops like the maxillary segment and the first thoracic (T1) like the second (T2) segment. Similar transformations are observed in imaginal cells (Struhl, 1982; Kaufman and Abbott, 1984); the normal identity of the prothoracic (T1) leg requires $Scr^+$ function. When the gene is defective this leg develops like the mesothoracic (T2) one.

The expression pattern of *Scr* during the embryonic and larval development has been described (Kuroiwa et al., 1985; Martinez-Arias et al., 1987; Riley et al., 1987; LeMotte et al., 1989). Like the rest of the homeotic genes, *Scr* is probably subjected to several levels of regulation involving segmentation as well as other homeotic genes. In this paper we are primarily concerned with regulatory interactions with other homeotic genes.

It was observed some time ago (Lewis, 1978) that embryos lacking the BX-C genes develop larval epidermal patterns of thoracic identity. It was subsequently shown (Hayes et al., 1984) that this pattern consists of a series of posterior prothoracic (T1p) and anterior mesothoracic (T2a) compartment units. This unit was later identified as parasegment 4 (T1p-T2a). Similarly, imaginal cells deficient in *Ubx* function show the same transformation towards T1p-T2a in the thoracic segments (Morata and Kerridge, 1981; Casanova et al., 1985). The developmental role of *Scr* suggested that the prothoracic transformation of the posterior compartments is due to ectopic expression of *Scr* in those compartments. Indeed, it was shown by Struhl, (1982) that this transformation is dependent on normal *Scr* function. Moreover, mutant leg discs lacking *Ubx* function in T2p contain Scr protein (Little et al., 1990) which is normally absent in this disc.

However, it has been reported (Riley et al., 1987), that $BX\text{-}C^-$ embryos do not show ectopic expression of *Scr*, in apparent contradiction with the genetic prediction. As it was pointed out by Riley et al., 1987, there was the possibility that the original antibody used for these studies was not sufficiently sensitive.

In this paper we examine the presence of Scr product in mutant embryos for different combinations of ANT-C and BX-C genes. We find that in the absence of the BX-C genes, *Scr* is expressed in an ectopic domain spanning the posterior compartments of the thoracic and abdominal segments. The establishment of this domain requires *engrailed* and *Antennapedia* functions. We also show that *Antp* can both activate and repress *Scr* activity depending on the concentration of Antp product.

## MATERIALS AND METHODS

### Mutant stocks

The following mutant stocks have been used: *Df(3R)P9*, referred to in the main text as *P9*, is a deletion of all the BX-C genes (Lewis, 1978). *Df(3R)109* is a deletion of *Ubx* and *abd-A* (Lewis, 1978; Casanova et al., 1987). $Ubx^{1}$ is a null mutation bearing an insert in the first exon (Bender et al., 1983). The chromosome $Dp(3R)bxd^{100}$ *Df(3R)P9* is defective for all the BX-C genes except that it carries a mutant form of *Ubx* in which the transcription unit is normal but lacks most of the upstream *bxd* element. As a consequence, the Ubx product is only expressed at the low level of parasegment 5 (Beachy et al., 1985; our own results). $Antp^{Ns+RC3}$ *Df(3R)P9* is a chromosome defective for *Antp* and the three BX-C genes. $nkd^{7E}$ $Ubx^{1}$ is a double mutant chromosome for the polarity gene *naked* (Martinez-Arias et al., 1988) and *Ubx*. It was a gift of Dr Phil Ingham. The *hsp70-Ubx* gene (called HSU, Gonzalez-Reyes and Morata, 1990) was recombined with *Df(3R)109* to overexpress the Ubx product in the absence of the endogenous *Ubx* and *abd-A* genes.

To study the effect of high levels of Antp product on *Scr* ectopic expression, we recombined the heat shock gene *hsp70-Antp* (called HSA, Gonzalez-Reyes et al., 1990) located on the third chromosome, with the $Ubx^{1}$ mutation.

### Heat shock treatments

Embryos of the stock *HSU Df(3R)109* were collected after a short egg-laying period of 2 hours and allowed to develop for 5 hours before they were heat shocked for 30 minutes. In the case of the *HSA* $Ubx^{-}$ experiment, to ensure a high level of Antp product, a stronger heat shock treatment of two 1 hour pulses was used (see main text for the details).

### Antibody staining

We have used the standard antibody protocol for single and double labelling (Lawrence et al., 1987; Macias et al., 1990). the anti-Scr antibody was a gift of Peter LeMotte and Walter Gehring. The anti-Ubx antibody is the monoclonal one developed by Robert White (White and Wilcox, 1984). The *en* expression was studied using the monoclonal antibody developed by Patel et al. (1989) and obtained from Peter Lawrence.

## RESULTS

### The normal expression of the *Scr* gene

The wild-type expression of *Scr* during embryogenesis and in the imaginal cells has been described (Riley et al., 1987; LeMotte et al., 1989). Scr product is initially detected in parasegment 2, later extending to parasegment 3 when the germ band elongates. At this time the most posterior limit of *Scr* expression corresponds to cells of the anterior compartment of the T1 segment. However, when the germ band is retracting, *Scr* label can also be seen in the posterior cells of the T1 segment (Carroll et al., 1988; LeMotte et al., 1989; see Fig. 1B), indicating that part of parasegment 4 (T1p-T2a) acquires *Scr* expression. As at this time (approximately 8-9 hours of development) cells of different parasegments already have specific lineages (Lawrence and Morata, 1977; Vincent and O'Farrell, 1992), the expression of *Scr* in T1p cells cannot be inherited from its predecessors but must result from a specific phenomenon of activation that occurs in parasegment 4.

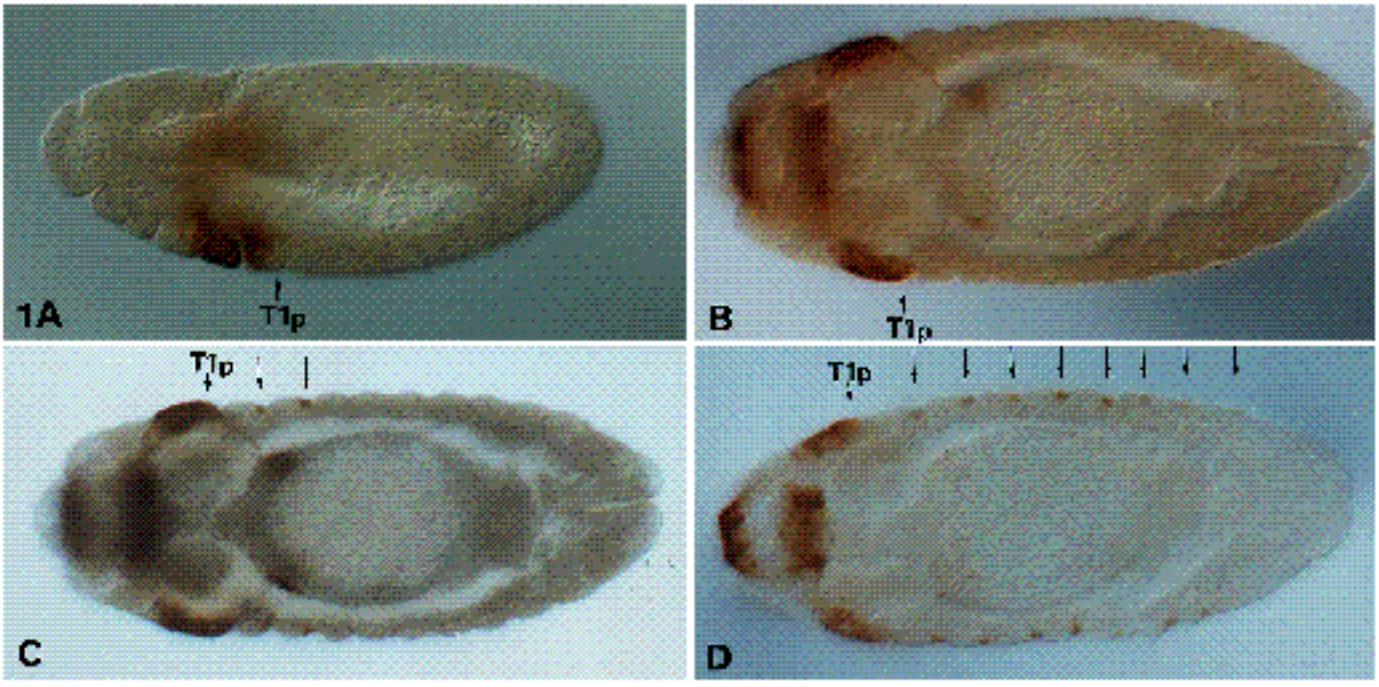


**Fig. 1.** Scr expression in wild type and in embryos defective in BX-C genes. (A) Wild-type embryo at stage 11 showing Scr antigen in parasegments 2 and 3. Note that T1p (arrow) has no label. (B) Wild-type embryo in stage 14 showing that Scr label has expanded to parasegment 4 (T1p, arrow). (C) $Ubx^{-}$ embryo in stage exhibiting Scr product in T2p and T3p (arrows). (D) *Df 109* embryo, defective for both *Ubx* and *abd-A*, in which *Scr* expression expands to A6p (arrows).

### In the absence of the BX-C genes *Scr* is derepressed in the posterior compartments of the thorax and abdomen, but only in the epidermis

Embryos of the stock *Df(3R)P9/DpP5* (where the *P9* chromosome is a deletion of all the BX-C genes) were doubly stained with Scr and Ubx antibodies. This allows the unambiguous identification of homozygous *P9* embryos, which lack Ubx. In these embryos *Scr* is expressed normally until germ band elongation, but when the germ band is retracting there appears an ectopic expression domain of *Scr* extending to the posterior compartments of the thoracic and abdominal segments (Fig. 3A). It consists of a string of *Scr*-expressing cells extending laterally and ventrally around the embryo. Double labelling with en and Scr antibodies indicates that the *en* domain and the ectopic *Scr* domain are largely coextensive. The most posterior *Scr* stripe appears in the posterior compartment of A8, which corresponds to parasegment 14. It is noteworthy that we find this ectopic expression of *Scr* restricted to the epidermis, for the mature nerve cord only exhibits Scr label in the normal domain. Other non-ectodermal tissues, like the visceral mesoderm, also fail to show any ectopic *Scr* expression.

We then examined the effect of individual BX-C mutations on *Scr* expression. Only embryos containing *Abd-B* function (Fig. 1D) exhibit ectopic *Scr* expression in the thoracic and abdominal segments, except in A7p and A8p, indicating a suppressing role of Abd-B product, which is expressed at high levels in this region (DeLorenzi et al., 1988). The addition of the *abd-A* gene (*Ubx*$^{-}$ embryos) further reduces the ectopic *Scr* domain, which now extends only to T2p and T3p (Fig. 1C).

The previous results indicate that each BX-C gene is able to suppress *Scr* expression in its domain, although *Abd-B* does it only in the region of its highest expression. In the case of *Ubx* we have tested the effect of the amount of *Ubx* product comparing the ectopic *Scr* expression in embryos of two different genotypes: (1) *Ubx*$^{+}$*abd-A*$^{-}$*Abd-B*$^{-}$ embryos containing a normal *Ubx* gene. In the absence of *abd-A* and *Abd-B, Ubx* is expressed at high level in parasegments 6-13, and (2) embryos of genotype *Dpbxd*$^{100}$ *DfP9* which contain a defective *Ubx* gene that can only express a low level of product (Fig. 2A), due to the lack of most of the *bxd* regulatory element. The *abd-A* and *Abd-B* genes are also absent. In these embryos parasegments 5-12 develop as parasegment 5.

The results are that in genotype 1 there is ectopic *Scr* expression in A8p exclusively, which is the only compartment of the parasegmental trunk lacking BX-C activity in these embryos. In genotype 2, there is some Scr protein in A7p and a greater amount in A8p (Fig. 2B). This indicates that the low level of Ubx product characteristic of parasegment 5 also represses *Scr* in most of the abdomen. The reason for its inability to suppress Scr in A7p is probably because there is almost no *Ubx* product in the epidermal cells of parasegment 13.

The repressing role of *Ubx* was further tested in an experiment in which, using a *hsp70-Ubx* gene (HSU), the Ubx product was expressed under heat shock control. HSU *Df109* embryos were given two pulses of heat shock, each of 30 minutes, at 5 and 7 hours of development and subsequently stained with Scr and abd-A antibodies. We observe that the ectopic *Scr* expression disappears in the

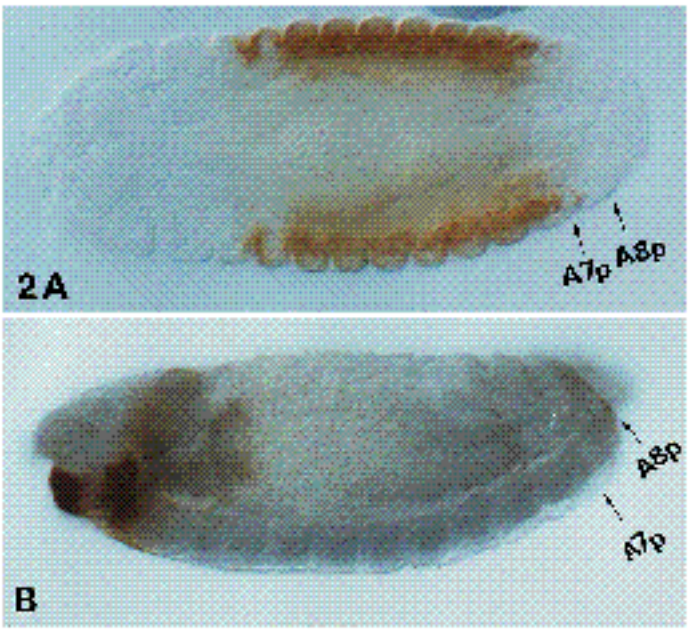


**Fig. 2.** The effect of low level expression of *Ubx* on the ectopic expression of *Scr*. (A) Embryo of genotype *Dp bxd*$^{100}$ *Df P9* showing a low and approximately uniform level of Ubx product. Note, however, that there is very little, if any, Ubx product in A7p and A8p (arrows). (B) Embryo of the same genotype but stained with Scr antibody. Only A7p and A8p show *Scr* label (arrows).

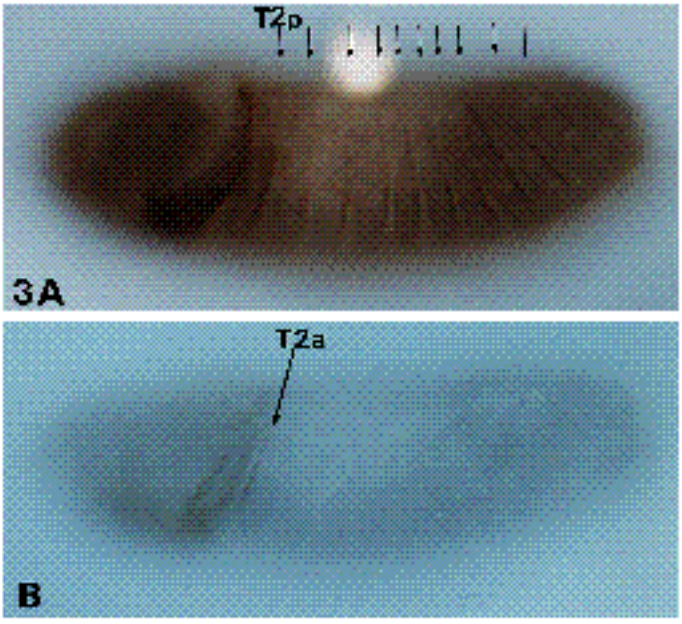


**Fig. 3.** The effect of *Antp* on the ectopic expression of *Scr*. (A) *A BX-C*$^{-}$ embryo of stage 14 exhibiting the ectopic bands of Scr protein in the posterior compartments of the thorax and abdomen (arrows) as well as the normal domain. (B) Embryo of genotype *Antp*$^{-}$*BX-C*$^{-}$ demonstrating the expansion of the normal *Scr* domain to T2a (arrow) as well as the elimination of the ectopic *Scr* domain.

treated embryos, but the normal *Scr* domain remains unaltered.

In conclusion, we find that the BX-C genes act as repressors of *Scr* in the thoracic and abdominal segments.

### Moderate levels of *Antp* are necessary for *Scr* activation in the posterior compartments of the thorax and abdomen, but high levels suppress it

We have tested the effect of *Antp* on the ectopic activation of *Scr*, for *Antp* has been shown to repress *Scr* in the vicinity of the domain (Riley et al., 1987). However, we were intrigued that in our previous experiments the expansion of the *Antp* expression domain in embryos lacking the BX-C genes (Hafen et al., 1984; Carroll et al., 1986; Wirz et al., 1986) still allows for *Scr* expression in posterior compartments. We used embryos of genotype $Antp^{-}$ $BX\text{-}C^{-}$ that were stained with Scr and Ubx antibodies. To our surprise, we found that these embryos failed to express *Scr* ectopically in the posterior compartments (Fig. 3B), even though we observed the expansion of the normal *Scr* domain to T2a, which normally does not possess Scr product.

This result indicated an activating or enhancing role of *Antp* on *Scr* in the posterior thoracic and abdominal compartments of the epidermis. It also indicated that there must be *Antp* expression in those compartments in $BX\text{-}C^{-}$ embryos. Indeed we found Antp product extending along the body axis. The *Antp* label is not uniform, but shows a stereotyped pattern; within the anterior compartments there are large differences in the amount of antigen in different cells, but in a reiterated pattern (Fig. 4). In the posterior compartments there is a moderate and homogenous amount of Antp product. In contrast, the level of *Antp* expression in the ventral cord is very high, in agreement with previous results (Hafen et al., 1984; Wirz et al., 1986).

Thus, the Antp product can have opposite roles in the epidermis; a repressing function in the regions near the normal domain, and an activating or enhancing one in the rest of the thorax and abdomen. Since in $BX\text{-}C^{-}$ embryos we only observe ectopic Scr product in the epidermis, where *Antp* is expressed at moderate level, but not in the CNS, where *Antp* is highly expressed, we hypothesized that the deciding factor might be the concentration of Antp product. To test this idea, we synthesized an *hsp70-Antp (HSA)* $Ubx^{1}$ line in which *Antp* can be expressed at high level all over the embryo (Gibson and Gehring, 1988; Gonzalez-Reyes et al., 1990). 3- to 5-hour-old embryos of this stock were heat shocked for 1 hour, recovered for 2 hours, then heat shocked again for 1 hour and fixed 3 hours after. They were subsequently doubly stained for Ubx and Scr antigens. Out of 196 *Ubx* homozygous embryos (which can be in all cases distinguished by the lack of Ubx antigen), 90 failed to show the two ectopic *Scr* stripes in T2p and T3p, and 106 still exhibited some, but much reduced, ectopic *Scr* expression. In addition, the normal *Scr* domain was, to a variable extent, reduced. By contrast, virtually all (118 out of 120) untreated embryos of the same stock showed the two *Scr* stripes, just like the regular $Ubx^{-}$ mutant embryos. This result strongly argues that the direction (positive or negative) of the regulation of *Scr* by *Antp* depends on the concentration of the Antp protein.

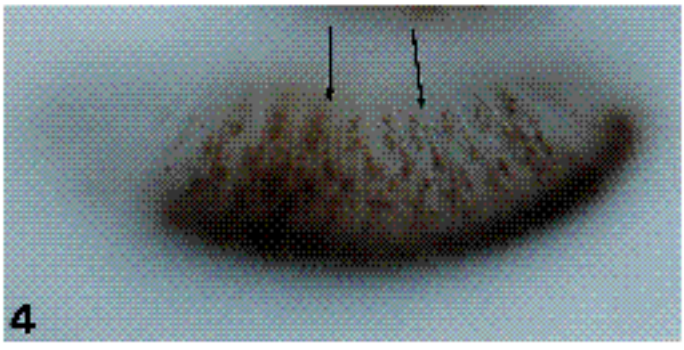


**Fig. 4.** *Antp* expression in the epidermis of a $BX\text{-}C^{-}$ embryo. There is a low but noticeable amount of Antp antigen in the posterior compartments (arrows), while the anterior compartments show a more heterogeneous but stereotyped pattern.

### The role of engrailed in the ectopic activation of *Scr*

Unlike the normal domain, the ectopic *Scr* expression in $BX\text{-}C^{-}$ embryos shows a segmental periodicity which may be indicative of control by polarity genes. This, and the observation that the normal expression of *engrailed* and the ectopic one of *Scr* are coextensive in the thorax and in the abdomen, suggested a possible role of *en* in the activation of *Scr*.

The effect of the elimination of the *en* gene was tested in $en^{-}Ubx^{-}$ embryos. We find that they lack the two stripes of *Scr* label that appear in T2p and T3p of $en^{+}Ubx^{-}$ embryos. However, the morphology of $en^{-}$ embryos is already very abnormal at the time of germ band retraction, when the Scr stripes are best observed and there is the possibility that these may be missing because of cell death or degeneration of posterior compartments.

We then decided to examine *Scr* expression in $Ubx^{-}$ embryos which are also deficient for *naked (nkd)* function. In these embryos the morphology at the stage of germ band retraction is not much altered, and they show a expansion of *en* activity which results in engrailed stripes about twice the normal width (Martinez-Arias et al., 1988). We reasoned that if *en* plays a positive role in the late activation of *Scr*, the *Scr* stripes in T2p and T3p of $nkd^{-}Ubx^{-}$ embryos should follow the *en* expansion and become broader than in $nkd^{+}$ embryos. As shown in Fig. 5, we find that this is the case; in comparison with $nkd^{+}Ubx^{-}$ embryos, the stripes of *Scr* in T2p and T3p are clearly wider.

## DISCUSSION

### Interactions of *Scr* with *Antp* and with the BX-C genes

Our results clearly demonstrate a developmentally significant interaction between *Scr* and the BX-C genes; in the absence of the latter, *Scr* becomes derepressed in the posterior compartment of the thoracic and abdominal epidermis. It had been previously shown that *Ubx* represses *Scr* function in the imaginal cells (Struhl, 1982; Little et al., 1990). Here we extend this observation to the embryonic cells and also show that the BX-C genes have the same

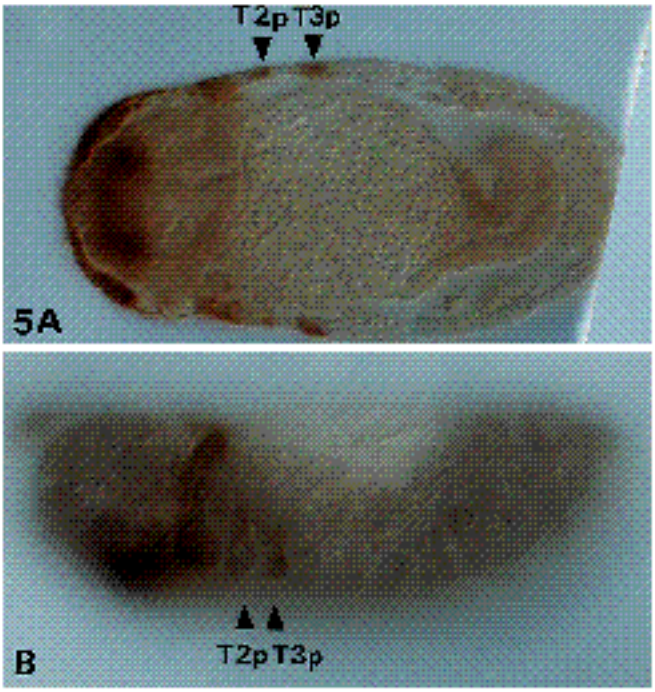


**Fig. 5.** Ventral (A) and lateral (B) views of *Scr* expression in an embryo defective in *Ubx* and *nkd* functions. The *Scr* ectopic stripes (arrowheads) are wider than $Ubx^{-}$ $nkd^{+}$ embryos (compare with Fig. 1).

property. However, the *Abd-B* gene can suppress *Scr* expression only in A7p and A8p, while the Abd-B domain extends from A4p to A8p (Sánchez-Herrero et al., 1985) We believe that this is due to the low level of Abd-B product in the anterior part of its domain (Celniker and Lewis, 1989; DeLorenci and Bienz, 1990).

Previous studies (Riley et al., 1987) have failed to observe the interaction between the BX-C genes and *Scr*, probably due to a lack of sensitivity of the original Scr antibody. Our results also provide an explanation for the phenotype of *P9* embryos, in which the body region made by parasegments 5-12 develops with the identity of parasegment 4, that is, compartments T1p and T2a. The identity of T1p is specified by the ectopic expression of *Scr* that we demonstrate in this paper. The identity of T2a is clearly dictated by *Antp*, which also becomes derepressed in the absence of BX-C genes, not only in the CNS (Hafen et al., 1984; Wirz et al., 1986), but also in the epidermis (Fig. 4).

One significant aspect of our results is the differential behavior of the epidermis and the ventral cord with respect to *Scr* expression, for we do not observe ectopic Scr product in the ventral cord. This cannot be due to lack of activating factors such as the *en* product, because it is present in the embryonic central nervous system (Patel et al., 1989; DiNardo et al., 1985). A clear difference that we can see in the two tissues is the level of *Antp* expression, which is low in the epidermis and high in the ventral cord. The experiments overexpressing *Antp* (see below) indicate that this is probably the cause for the differential *Scr* expression.

The interactions between *Scr* and *Antp* are more complex, because *Antp* can have opposite effects on *Scr* expression. It has been reported (Reuter and Scott, 1990) that *Antp* positively regulates *Scr* in the visceral mesoderm. In contrast, in the epidermis of $Antp^{-}$ embryos, the normal domain of *Scr* expands to T2a (Riley et al., 1987; our own results) indicating a repressing role of $Antp^{+}$, at least in the T2a compartment. We find that in $Antp^{-}BX\text{-}C^{-}$ embryos the ectopic *Scr* activity is eliminated, indicating an enhancing role of $Antp^{+}$. The reason for these apparently paradoxical results appears to be the different concentration of Antp product in the two regions: T2a is part of parasegment 4, the region of high *Antp* expression (Wirz et al., 1986; Carroll et al., 1986), which is also high in the CNS of $BX\text{-}C^{-}$ embryos, and these do not show ectopic *Scr* expression. By contrast, the amount of Antp product in the epidermis of $BX\text{-}C^{-}$ embryos is much lower (see Fig. 4) and allows for *Scr* expression.

The concentration hypothesis is very strongly supported

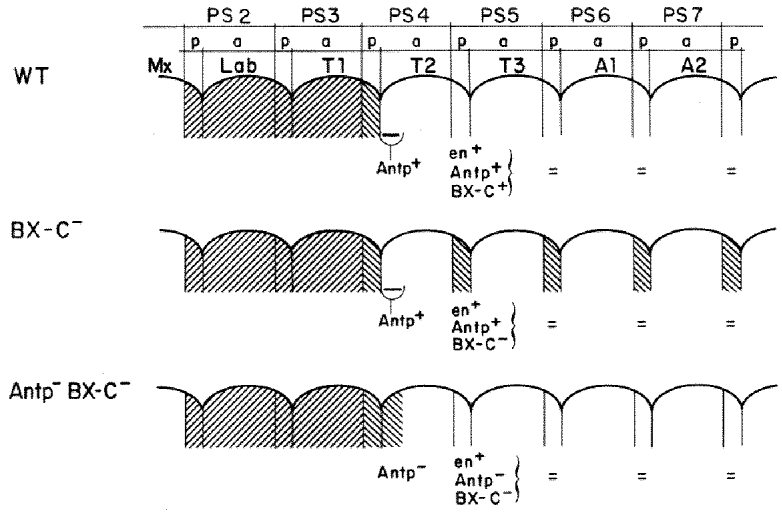


**Fig. 6.** Tentative model of the factors involved in the late activation of *Scr*. Only part of the parasegmental trunk (PS2-7) is considered. The normal and ectopic expression domains of *Scr* are indicated by the shaded areas and the regions of early and late activation by a change in the direction of the shading. The model is based on: (1) a dual function of *Antp*, which can both repress and activate *Scr*, (2) an activating role of *en*, and (3) a repressing role of the BX-C genes. In the WT *Scr* is activated late only in T1p and the repressing function of *Antp* prevents its expansion to T2a. In the posterior compartments of the thoracic and abdominal metameres late activation of *Scr* is prevented by the repressing function of the BX-C genes. In $BX\text{-}C^{-}$ embryos the lack of BX-C products allows the combined activity of *en* and *Antp* to activate *Scr*, but the presence of the normal dose of Antp product in T2a blocks *Scr* expansion to T2a. In $Antp^{-}$ $BX\text{-}C^{-}$ embryos the lack of Antp product permits the expansion of Scr to T2a, but at the same time prevents *Scr* activation in the thoracic and abdominal metameres.

by the heat shock experiment in which by overexpressing *Antp* in *Ubx*⁻ embryos we eliminate the ectopic Scr bands in T2p and T3p, as well as reduce the expression in the normal domain. As far as we are aware, this is the first case of an interaction between homeotic genes in which the outcome depends on the concentration of product. It may be of importance in the cases of genes that have overlapping domains of activity. This concentration-dependent effect may explain the enhancing role of *Antp* on *Scr* in the visceral mesoderm (Reuter and Scott, 1990), just assuming that in this tissue there is the corresponding enhancing level of Antp product.

In the gap genes there are comparable situations of interactions leading to opposite effects; *hunchback*, for example, can both activate and repress *Kruppel* depending on the concentration of product (Hülskamp et al., 1990; Struhl et al., 1992).

### The activation of *Scr* in the ectopic domain probably reflects a second wave of *Scr* activation in the normal domain

The ectopic *Scr* domain in *BX-C*⁻ embryos differs from the normal domain in several important features. The first one is that it appears when the germ band is retracting, approximately at 8-9 hours of development. At this time the original activating machinery, i.e. maternal, gap, pair-rule genes, has disappeared from the embryo and therefore cannot play a role. The second feature is that the *Scr* ectopic domain is discontinuous, a very unusual feature for homeotic gene expression. There is only one similar case reported, corresponding to the ectopic autocatalytic *Dfd* domain (Kuziora and McGinnis, 1988). In this case the process of *Dfd* activation also differs from the normal one in some fundamental features (Kuziora and McDinnis, 1988; González-Reyes et al., 1992). The third factor is that unlike the normal *Scr* domain, the ectopic one depends on *en* function. Not only do the *en* and ectopic *Scr* expressions coincide but also the broadening of the *en* stripe in *nkd* embryos is paralleled by *Scr*. This latter result is significant, for it strongly suggests that *en* plays an activating role for *Scr* in the posterior compartments of thorax and abdomen.

In our view, the ectopic *Scr* domain in the posterior compartments of *BX-C*⁻ embryos probably reflects a second wave of *Scr* activation that in wild-type embryos results in the late expression of *Scr* in T1p cells. As schematized in Fig. 6, at about 8-9 hours of development the presence of *en* activates *Scr* in the series of posterior compartments T1p-T2p-T3p-A1p- - - - A8p. The process requires *Antp* function, present in T1p-T2p, but is repressed by *Ubx*, present in T2p. As a consequence, *Scr* is activated in wild-type embryos only in T1p. The elimination of BX-C would eliminate the repressing factors and augment the amount of *Antp*, giving rise to *Scr* activity in all the posterior compartments (Fig. 6). We note the need of some early acting repressing factor(s) preventing the activating function of *en* before 8 hours of development.

The requirement of *engrailed* function for this second tier of regulation may be significant, for it suggests a role of some polarity genes in the late regulation of homeotic function. *en* also plays a role in the control of *abd-A* expression after the original activation (Macias et al., unpublished data). Another polarity gene, *wingless*, is necessary for the autoregulatory expression of *Deformed* (González-Reyes et al., 1992), and is also needed for *Ubx* expression in the visceral mesoderm (Thuringer and Bienz, personal communication). *wingless* is also required for the induction of *labial* in the endoderm (Immergluck et al., 1990).

We thank Ernesto Sánchez-Herrero, Jordi Casanova and Acaimo González-Reyes for their helpful comments on the manuscript. We also thank Peter LaMotte and Walter Gehring for their gift of Scr antibody and Phil Ingham for the *nkd*⁻ *Ubx*⁻ chromosome. This work has been supported by a grant of the Dirección General de Investigación Científica y Técnica and by an institutional grant from the Fundación Ramón Areces. S. Pelaz is supported by a fellowship of the Eusko Jaurlaritza (Autonomous Basque Government).

## REFERENCES

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

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

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

**Carroll, S., DiNardo, S., O'Farrell, P., White, R. and Scott, M**. (1988). Temporal and spatial relationships between segmentation and homeotic genes expression in *Drosophila* embryos: distribution of the *fushi tarazu, engrailed, Sex combs reduced, Antennapedia* and *Ultrabithorax* proteins. *Genes Dev.* **2**, 350-360.

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

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

**Celniker, S. E., Keelan, D. J. and Lewis, E. B.** (1989). The molecular genetics of the bithorax complex of *Drosophila*: characterization of the products of the *Abdominal-B* domain. *Genes Dev.* **3**, 1424-1436.

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

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

**DiNardo, S., Kuner, J. M., Theis, J. and O'Farrel, P. H**. (1985). Development of embryonic pattern in *D. melanogaster* as revealed by accumulation of the nuclear engrailed protein. *Cell* **43**, 59-69.

**Gibson, G. and Gehring, W. J.** (1988). Head and thoracic transformations caused by ectopic expression of *Antennapedia* during *Drosophila* development. *Development* **103**, 657-675.

**González-Reyes, A., Macías, A. and Morata, G.** (1992). Autocatalysis and phenotypic expression of *Drosophila* homeotic gene *Deformed* : its dependence on polarity and homeotic gene function. *Development* **116**, 1059-1068.

**González-Reyes, A. and Morata, G**. (1990). The developmental effect of overexpressing a Ubx product in *Drosophila* embryos is dependent on its interactions with other homeotic products. *Cell* **61**, 515-522.

**González-Reyes, A., Urquía, N. Gehring, W. J., Struhl, G. and Morata, G.** (1990). Are cross-regulatory interactions between homeotic genes functionally significant? *Nature* **344**, 78-80.

**Hafen, E., Levine, M. and Gehring, W**. (1984). Regulation of *Antennapedia* transcript distribution by the bithorax complex in *Drosophila*. *Nature* **307**, 287-289.

**Hülskamp, M., Pfeifle, C. and Tautz, D.** (1990). A morphogenetic gradient of hunchback protein organizes the expression of the gap genes *Kruppel* and *knirps* in the early *Drosophila* embryo. *Nature* **346**, 577-580.

**Immergluck, K., Lawrence, P. and Bienz, M**. (1990). Induction across germ layers in *Drosophila* mediated by a genetic cascade. *Cell* **62**, 261-268.

**Hayes, P. H., Sato, T. and Dennell, R. E.** (1984). Homeosis in *Drosophila*: The Ultrabithorax syndrome. *Proc. Natl. Acad. Sci. USA* **81**, 545-549.

**Kaufman, T. and Abbott, M.** (1984). Homeotic genes and the specification of segmental identity in the embryo and adult thorax of *Drosophila melanogaster*. *Molecular aspects of early development* (ed. G. M. Malacinsky and W. H. Klein), pp. 189-218. New York: Plenum Press.

**Kaufman, T., Seeger, M. and Olsen, G.** (1990). Molecular and genetic organization of the Antennapedia gene complex of *Drosophila melanogaster*. *Adv. Genet.* **27**, 309-361.

**Kuroiwa, A., Kloter, U., Baungartner, P. and Gehring, W**. (1985). Cloning of the homeotic *Sex Combs reduced gene* in *Drosophila* and *in situ* localization of its transcripts. *EMBO J.* **4**, 3757-3764.

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

**Lawrence, P., Johnston, P., Mcdonald, P. and Struhl, G.** (1987). Borders of parasegments in *Drosophila* embryos are delimited by the *fushi tarazu* and *even-skipped* genes. *Nature* **328**, 440-442.

**Lawrence, P. and Morata, G.** (1977). The early development of mesothoracic compartments in *Drosophila*. An analysis of cell lineage and fate mapping and an assessment of methods. *Dev. Biol.* **56**, 40-51.

**LeMotte, P., Kuroiwa, A., Fessler, L. and Gehring, W.** (1989). The homeotic gene *Sex combs reduced* of *Drosophila*: gene structure and embryonic expression. *EMBO J.* **8**, 219-227.

**Lewis, E**. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565-570.

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

**Macías, A., Casanova, J. and Morata, G**. (1990). Expression and regulation of the *abd-A* gene of *Drosophila*. *Development* **110**, 1197-1207.

**Mahaffey, J. and Kaufman, T.** (1988). The homeotic genes of the Antennapedia Complex and the Bithorax Complex of *Drosophila melanogaster*. In *Development of Higher Organisms* (ed. G. M. Malacinski), pp. 329-360. New York: MacMillan.

**Martínez-Arias, A., Ingham, P., Scott, M. and Akam, M**. (1987). The spatial and temporal deployment of *Dfd* and *Scr* transcripts throughout development of *Drosophila*. *Development* **100**, 673-683.

**Martínez-Arias, A., Baker, N. E. and Ingham, P. W**. (1988). The role of segment polarity genes in the definition and maintenance of cell states in the *Drosophila* embryo. *Development* **103**, 157-170.

**Martínez-Arias, A. and Lawrence, P**. (1985). Parasegments and compartments in the *Drosophila* embryo. *Nature* **313**, 639-642.

**Morata, G. and Kerridge, S.** (1981). Sequential functions of the bithorax complex of *Drosophila*. *Nature* **290**, 778-781.

**Reuter, R. and Scott, M**. (1990). Expression and function of the homeotic genes *Antennapedia* and *Sex combs reduced* in the embryonic midgut of *Drosophila*. *Development* **109**, 289-303.

**Riley, P., Carroll, S. and Scott, M**. (1987). The expression and regulation of Sex combs reduced protein in *Drosophila* embryos. *Genes Dev.* **1**, 716-730.

**Patel, N. H., Martín-Blanco, E., Coleman, K. G., Poole, S. J., Ellis, M. C., Kornberg, T. B. and Goodman, C. S.** (1989). Expression of engrailed proteins in arthropods, annelids and chordates. *Cell* **58**, 955-968.

**Sánchez-Herrero, E., Vernos, I., Marco, R. and Morata, G.** (1985). Genetic organization of the *Drosophila* bithorax complex. *Nature* **313**, 108-113.

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

**Struhl, G., Johnston, P. and Lawrence, P. A.** (1992). Control of *Drosophila* body pattern by the hunchback morphogen gradient. *Cell* **69**, 237-249.

**Vincent, J. and O'Farrell, P**. (1992). The state of *engrailed* expression is not clonally transmitted during early *Drosophila* development. *Cell* **68**, 933-931.

**Wakimoto, B. and Kaufman, T.** (1981). Analysis of larval segmentation in lethal phenotypes associated with the Antennapedia gene complex in *Drosophila melanogaster*. *Dev. Biol.* **81**, 51-64.

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

**Wirz, J., Fessler, L. and Gehring, W**. (1986). Localization of the Antennapedia protein in *Drosophila* embryos and imaginal discs. *EMBO J.* **5**, 3327-3334.

(Accepted 27 November 1992)