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

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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-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). Ubx1 is a null mutation bearing an insert in the first exon (Bender et al., 1983). The chromosome Dp(3R)bxd100 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^{Sc+RC3} Df(3R)P9 is a chromosome defective for Antp and the three BX-C genes. nkd^{7E} Ubx1 is a double mutant chromosome for the polarity gene nkd (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 Ubx1 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 HSU 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 Dpbxd100 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 bxd100 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\textsuperscript{−} BX-C\textsuperscript{−} 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-C\textsuperscript{−} 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-C\textsuperscript{−} 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\textsuperscript{L} 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\textsuperscript{−} 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.

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

Unlike the normal domain, the ectopic Scr expression in BX-C\textsuperscript{−} 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\textsuperscript{−}Ubx\textsuperscript{−}* embryos. We find that they lack the two stripes of Scr label that appear in T2p and T3p of *en\textsuperscript{−}Ubx\textsuperscript{−}* embryos. However, the morphology of *en\textsuperscript{−}* 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\textsuperscript{−} 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\textsuperscript{−}Ubx\textsuperscript{−}* embryos should follow the *en* expansion and become broader than in *nkd*\textsuperscript{−} embryos. As shown in Fig. 5, we find that this is the case; in comparison with *nkd\textsuperscript{−}Ubx\textsuperscript{−}* 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
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-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-C embryos, and these do not show ectopic Scr expression. By contrast, the amount of Antp product in the epidermis of BX-C embryos is much lower (see Fig. 4) and allows for Scr expression.

The concentration hypothesis is very strongly supported

**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).

**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-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-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 McGinnis, 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).

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