Distribution of the *Sex combs reduced* Gene Products in *Drosophila melanogaster*

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ABSTRACT

The spatial and temporal distribution of RNA and protein encoded by the homeotic *Sex combs reduced* (*Scr*) gene were examined during Drosophila development. The gene products are present in the epidermis of both the labial and first thoracic segments as would be predicted from prior genetic studies. However, the pattern in the central nervous system (CNS) and mesoderm is further restricted; the major expression located in the labial neuromere of the CNS and the mesoderm of the first thoracic segment. The spatial restriction within the CNS is correlated with and may be due to a differential timing of expression in the labial and first thoracic ectoderm. The labial ectoderm accumulates the *Scr* RNA prior to segregation of the neuroblasts while expression in the first thoracic ectoderm occurs after neuroblast segregation. The protein is also observed in the subesophageal ganglia of both larvae and adults, as well as in the labial and first thoracic imaginal discs. Surprisingly, the protein is also present to a lesser extent in second and third thoracic leg discs.

THE establishment of segmental identity in *Drosophila melanogaster* has been shown to be under the control of a group of selector genes (GARCIA-BELLIDO 1977). These genes, initially identified by homeotic mutations that transform the identity of one segment into that of another, act as switches which control segmental identity and therefore the developmental pathway a segment may follow (LEWIS 1978). There are two well known clusters of homeotic genes on the right arm of the third chromosome of *D. melanogaster*, the Bithorax-Complex (BX-C) and the Antennapedia-Complex (ANT-C). Within the ANT-C, there are five genes (*labial, proboscipedia, Deformed, Sex combs reduced* and *Antennapedia*) which are involved in establishing the identity of segments in the head and anterior thorax (KAUFMAN, LEWIS and WAKIMOTO 1980; HAZELRIGG and KAUFMAN 1983).

Recently, studies have begun to elucidate the function of the homeotic genes at the molecular level (BENDER, SPIERER and HOGNESS 1983; SCOTT *et al.* 1983; GARBER, KUROIWA and GEHRING 1983). The temporal and spatial distribution of RNAs encoded by several of these genes has been determined using the technique of *in situ* hybridization (LEVINE *et al.* 1983; AKAM 1983; HARDING *et al.* 1985; MARTINEZ-ARIAS 1986). The results show that the transcripts accumulate in the embryonic epidermis with a pattern that would be predicted from the results of prior morphogenetic studies. One intriguing observation is that the transcripts also accumulate in the central nervous system (CNS) of the developing embryo in a similar segmental pattern as that observed in the epidermis.

The pattern of protein accumulation has also been examined for two of the homeotic genes, *Ultrabithorax* (WHITE and WILCOX 1984; BEACHY, HELFAND and HOGNESS 1985; WHITE and WILCOX 1985) and *Antennapedia* (CARROLL *et al.* 1986; WIRZ, FESSLER and GEHRING 1986). The findings from these experiments agree with those obtained for RNA distribution; however, immunological techniques allow a more precise localization of the gene products than does *in situ* hybridization. These proteins, like other proteins which contain the homeobox, are localized to the nuclei of cells within the regions in which they are expressed. This, along with the finding that the homeobox protein domain is capable of binding DNA (DESPLAN, THEIS and O'FARRELL 1985) suggest that the homeotic gene products may be directly involved in the regulation of other genes in the pathway of segmental identity.

The homeotic *Sex combs reduced* (*Scr*) gene of the ANT-C has some characteristics which make it of particular interest when compared with other homeotic genes. Embryos which completely lack the *Scr* gene function die during late embryogenesis and show a divergent homeotic transformation. The labial segment acquires a more anterior identity, that of the maxillary segment, while the first thoracic segment is transformed posteriorly into a second thoracic identity (WAKIMOTO and KAUFMAN 1981; SATO, HAYES and DENELL 1985). This results in a partial duplication of the maxillary sense organ and loss of normal labial

derivatives, as well as the appearance of mesothoracic (second thoracic) denticles in place of the normal prothoracic (first thoracic) denticle belts. In contrast, similar loss of function mutations in other homeotic genes cause unidirectional transformations. Furthermore, since the *Scr* gene is required at the head-trunk boundary, a study of its expression pattern will allow us to compare segmental specification in the head relative to the trunk.

To understand further the role of the *Scr* gene during development, we have followed the expression of the *Scr* gene using both *in situ* hybridization to localize the transcripts and immunolocalization of the protein encoded by the *Scr* gene. Our results show that the *Scr* protein accumulates in the nuclei of cells in the epidermis of the labial and first thoracic segments as would be predicted from the *Scr* mutant phenotypes. However, the expression in the CNS and mesoderm only represents a subset of the epidermal pattern. We also show that the *Scr* protein is present in the subesophageal ganglia of the CNS throughout the life of the fly including adults. Furthermore, the pattern of protein distribution in third instar larval imaginal discs is broader than expected from morphogenetic studies. We discuss these results with respect to their implications on the role of homeotic gene function during Drosophila development.

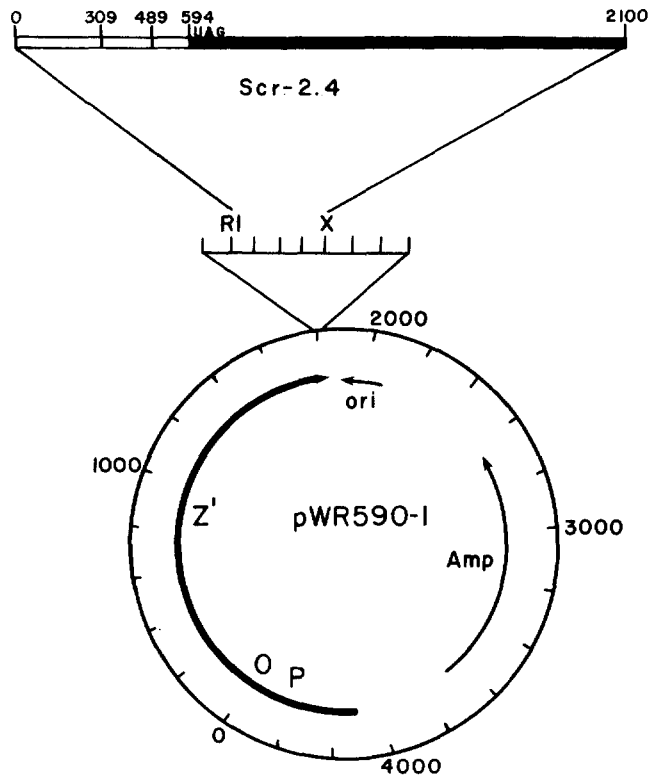

FIGURE 1.—Construction of β-gal-*Scr* fusion. A 2.1-kb *Eco*RI-*Xba*I fragment from a 2.4-kb *Scr* cDNA was cloned into the *Eco*RI-*Xba*I restriction sites within the polylinker of plasmid pWR590-1. This placed the *Scr* protein coding portion of the cDNA in frame with the *lacZ* coding portion of the plasmid. The solid line of *Scr*-2.4 is the 3′ untranslated portion of the cDNA.

MATERIALS AND METHODS

Drosophila stocks: The standard lab stock, Oregon R, was used for all of our wild-type experiments. Several different *Scr* null mutations were used for the controls; A68, A72 and P1. These mutations have been well characterized and will be described elsewhere (D. OTTESON, J. W. MAHAFFEY and T. C. KAUFMAN, unpublished data).

Isolation of *Scr* cDNA and construction of gene fusion: The *Scr* cDNA clone was isolated from a 3–12-hr embryonic cDNA library described by POOLE *et al.* (1985). A 7.0-kb genomic *Eco*RI fragment known to encode a portion of the *Scr* gene including the homeobox was labeled by nick translation and used as a probe to screen the library. In this manner we obtained several cDNAs homologous to the *Scr* gene, the largest being 2.4 kb. The coding portion of the cDNA was determined by DNA sequencing (MAXAM and GILBERT 1980). To construct the gene fusion, we isolated a 2.1-kb *Eco*RI-*Xba*I fragment from the cDNA that contained the translated region (Figure 1). This fragment was subcloned into the *Eco*RI-*Xba*I sites of the fusion plasmid pWR590-1 (GUO *et al.* 1984). This yielded the proper reading frame to align the homeobox with the β-galactosidase coding region of the plasmid.

Expression of the β-galactosidase-*Scr* fusion protein: The plasmid described above was transformed into several bacterial strains. Strain JM101 (YANISCH-PERRON, VIEIRA and MESSING 1985) gave the best yield of fusion protein. The cells were grown overnight in L-broth with no induction of the *lac* operon, pelleted and resuspended in one-fifth the original volume of 10 mM Tris (pH 8.0), 1 mM EDTA. Lysozyme was added to 10 μg/ml and the cells were incubated at 37° for 20 min. The cells were then lysed by passing through a series of at least six freeze thaw cycles (dry ice ethanol bath, then 37°). The lysate was cleared by centrifugation at 35,000 × *g* and the supernate was decanted. The resulting pellet was dissolved in one-tenth the initial culture volume in SDS lysis buffer (LAEMMLI 1970).

Purification of the fusion protein: A volume of the protein solution containing 200–400 μg of the fusion protein, as estimated by comparison to known standards, was applied to a 7.5% SDS polyacrylamide gel. After electrophoresis the fusion band was identified by staining the edges of the gel and the band was excised. When required, the fusion protein band was electroeluted from the gel slice in 0.5× Tris-acetate EDTA buffer (MANIATIS, FRITSCH and SAMBROOK 1982). The protein solution was then dialyzed against 0.1 M MOPS buffer (pH 7.0).

Production and purification of anti-*Scr* antibodies: Rabbits were injected subcutaneously with approximately 100 μg of the fusion protein. The excised gel band was homogenized with an equal volume of Freund's adjuvant (complete for the first injection and incomplete thereafter). Initial boosts were at 3-week intervals and monthly after *Scr* specific antibodies were detected. Anti-*Scr* antibodies were purified from the sera as follows. The sera were heated to 56° for 30 min to inactivate complement and then repeatedly passed over a β-galactosidase affinity column created by linking β-galactosidase (Sigma) to an Affigel 10 matrix (Bio-Rad). The flow-through material from this column was then applied to an *Scr* fusion protein column constructed in a similar manner using electroeluted *Scr*-fusion protein. Both anti-β-galactosidase antibodies and anti-*Scr* antibodies were eluted with either 0.1 M glycine (pH 2.5) followed by neutralization with one-tenth volume of 2.0 M Tris (pH 7.6), or with 4.0 M guanidine-HCl, 10 mM Tris (pH 8.0) followed

by dialysis against phosphate-buffered saline (PBS). Antibodies were stored at 4° after addition of sodium azide to 0.025%. The antibodies were tested for specificity to the fusion protein and β-galactosidase by immunoblotting techniques (Vector Labs).

Immunological staining: Embryos were collected on molasses agar plates containing a small amount of live yeast paste. For whole mounts, the embryos were dechorionated, fixed, and devitellinized by a simplification of the procedure of KARR and ALBERTS (1986). Endogenous peroxidases were inactivated by pretreating embryos in 0.3% H_2O_2 in methanol. After rehydration, the embryos were blocked to prevent nonspecific sticking of the antibodies by incubating them for 30 min in PBT (PBS containing 0.2% bovine serum albumin (BSA), 0.1% Triton X-100) followed by PBT + 5% normal goat serum for 30 min. All antibody incubations were done in the same solution and washes were done in PBT without the normal goat serum. Primary antibody reactions were incubated with gentle rocking overnight followed by washing with several changes of buffer for at least 1 hr. The secondary antibodies were affinity purified (Cappel or U.S. Biochemical) and were preincubated with fixed Drosophila embryos to reduce the background staining. Fluorescent slides were mounted in 90% glycerol, 2% propylgalate, and 1× PBS and were viewed under epifluorescence optics. Discs and CNS preparations were processed essentially as above except the dissected tissues were fixed in 3.7% formaldehyde for 5 min then washed in PBS and blocked and stained as above. For horseradish peroxidase staining, after secondary antibody treatment and wash, whole embryos were incubated in 0.1 mg/ml diaminobenzidine (Sigma) in PBT, after 15 min, H_2O_2 was added to 0.003% and color production was monitored until an appropriate level of staining was reached. Embryos were cleared and viewed in methyl salicylate. The use of either fluorescein (FITC) or horseradish peroxidase (hrp) labeled goat anti-rabbit IgG gave similar results, although each had its own advantages and problems. With FITC, it is not possible to optically section an embryo; also fluorescence time is quite short. As the hrp-stained embryos are "cleared" with the use of methyl salicylate, optical sectioning is possible and the staining is permanent. However, another problem became apparent, that of staining in the yolk mass after germ band extension. This is known to be an artifact in that no RNA is observed in this region, and antibodies made in several mice against the same fusion protein did not stain this region. Since the area in which we detect specific staining (staining not observed in *Scr* null embryos) is anterior to this gut staining, we have chosen to use the hrp technique in whole embryos due to its advantages over FITC. Recently, we have been able to remove this nonspecific staining.

***In situ* hybridization:** Embryos for *in situ* localization of *Scr* transcripts were dechorionated and fixed following a similar procedure to that used for immunological staining [see AKAM and MARTINEZ-ARIAS (1985) and INGHAM, HOWARD and ISH-HOROWICZ (1985) for description of *in situ* techniques]. After embedding and sectioning, the sections were hybridized with ^{35}S-labeled single-stranded RNA probes produced from the *Scr* cDNA cloned into plasmid pGEM2 (Promega Biotec). Hybridization conditions were those of INGHAM, HOWARD and ISH-HOROWICZ (1985).

RESULTS

Detection of RNA and protein during embryogenesis: A genetic analysis of null alleles has shown that the *Scr* locus is required during embryogenesis for proper segmental identity in the labial and first thoracic segments. In order to ascertain the pattern of expression of the *Scr* gene, we examined the spatial distribution of both the RNA and the protein it encodes during embryogenesis. After fertilization, the nuclei of a Drosophila embryo undergo several rounds of division, migrate to the surface forming a syncytial blastoderm. Shortly thereafter, the nuclei are enclosed in membranes, establishing the cellular blastoderm (2:50 hr) [see CAMPOS-ORTEGA and HARTENSTEIN (1985) for stages and timing of Drosophila development]. When embryos at this stage were examined for hybridization to the labeled cDNA probe, or binding of the antibodies directed against the fusion protein, no specific signal was detected in either case (Figure 2A), even though the *in situ* hybridization was exposed for quite a long time (26 days) in order to detect any possible pattern.

As gastrulation begins (2:50–3:10 hr), characteristic folds are observed within the embryo: the cephalic furrow and ventral furrow. When embryos at this stage were examined by *in situ* hybridization, grains were observed in a 3–4-cell wide band just posterior to the cephalic furrow (Figure 2B, *center* and *right*). This region will give rise to portions of the gnathocephalic segments at a later stage (TECHNAU and CAMPOS-ORTEGA 1985). Despite the obvious accumulation of *Scr* transcript, no staining was observed with the antibodies in the corresponding position of a similarly staged embryo (Figure 2B, *left*).

Once the germ band has completely extended, but before contraction has begun (about 6:20–7:20 hr) the first antibody staining is detected (Figure 2C, *left*). Nuclei within the labial segment are stained as is the gut region (yolk mass) mentioned above. No antibody staining was observed in the labial segment in 26.7% out of 132 embryos from a cross of balanced *Scr* null parents (data not shown). When sections from similarly staged embryos are examined by *in situ* hybridization, grains can be observed over a larger area than the antibody staining (Figure 2C, *center* and *right*). In the labial segment, grains are present within both layers of the ectoderm; the outer layer which will give rise to the epidermis, and over the neuroblasts which have previously segregated from the ectodermal germ layer. Grains are also observed over the mesodermal layer associated with the first thoracic segment and the anterior portion of the outer layer of the ectoderm, but apparently not within the neuroblasts of the first thoracic segment nor within the mesoderm of the labial segment.

As the germ band begins to contract, (7:20 hr) staining with the *Scr*-antibodies is first observed within the first thoracic segment (Figure 3A, *left*). A band of nuclei along the anterior portion of the first thoracic segment stain with the antibodies as do the nuclei in

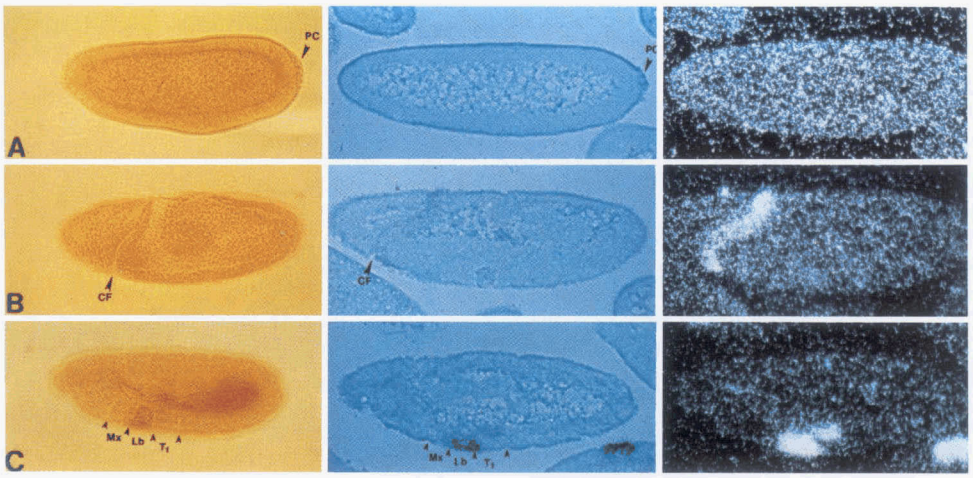

FIGURE 2.—Accumulation of *Scr* gene products during early embryogenesis. In all cases anterior is to the left and dorsal is up. The left panel shows whole embryos stained with the *Scr*-antibodies, the middle panel is a phase contrast view of an *in situ* hybridization tissue section and the right panel is a darkfield view of the same section. Serial sections were used to determine segmental identity in the *in situ* sections. Line A is an embryo at the cellular blastoderm stage (PC, pole cells). Line B shows an embryo during late gastrulation to early germ band extension (CF, cephalic furrow). Line C, an embryo just prior to the onset of germ band contraction (Mx, maxillary segment; Lb, labial segment; T_1, first thoracic segment).

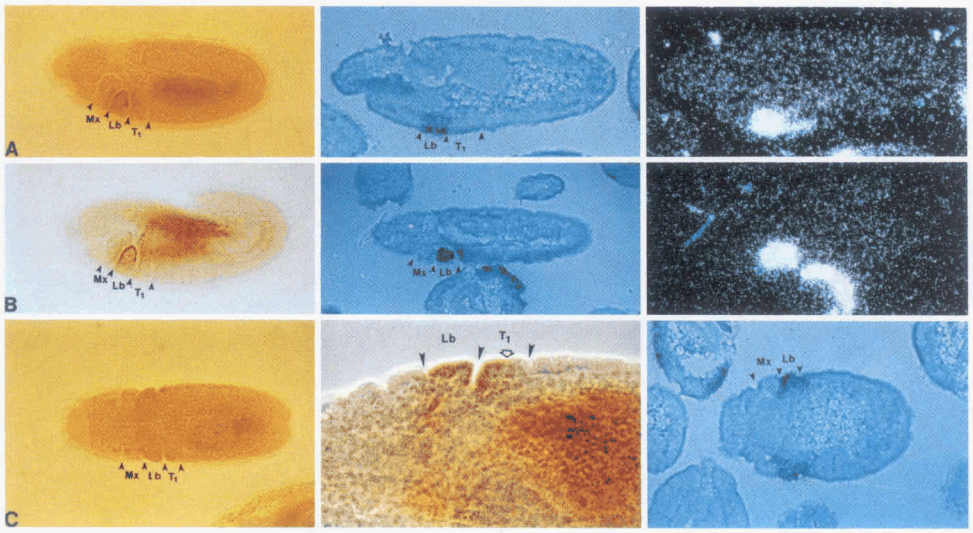

FIGURE 3.—Accumulation of *Scr* gene products during germ band contraction. The orientation and abbreviations are the same as in Figure 2 except for line C. Line A shows an embryo just beginning germ band contraction. Line B, an embryo at mid germ band contraction. Note the increase in the width of the band of stained nuclei in T_1 especially toward the dorsal edge, in the left panel of line B. Line C shows a contracting germ band stage embryo from the ventral surface. Anterior is still to the left. The middle panel is a higher magnification of an embryo at the same stage. Stained nuclei are observed in the labial and anterior half of the T_1 segments. The open arrowhead demarks the boundary of staining and nonstaining nuclei. The right panel shows a phase contrast view of an oblique transverse section through an embryo at the germ band contraction stage. Grains are observed only over the labial segment.

the labial segment. At this stage, grains from *in situ* hybridization in the labial segment are distributed as they were earlier at complete germ band elongation, but now, in the first thoracic segment, grains are detected throughout the outer ectodermal layer (see Figure 3A, *right*). Although not shown here, grains can still be observed over the mesoderm of the first thoracic segment.

As contraction of the germ band continues and dorsal closure initiates, (Figure 3B, *left*), more nuclei within the anterior half of the first thoracic segment begin to stain with the *Scr* antibodies. This can also be seen from the ventral surface (Figure 3C, *left*, and at higher magnification, *center*). Note there is no evidence of protein within the maxillary segment. Furthermore, *in situ* hybridization (Figure 3, B, *center* and *right*, and C, *right*) shows no indication of grains within the maxillary segment.

When germ band contraction is completed, the labial segments migrate to the ventral midline and fuse in preparation for head involution (9:20–10:20 hr). At this stage, the *Scr*-antibodies stain nuclei throughout the epidermis of both the labial (Figure 4A), and first thoracic (Figure 4B) segments.

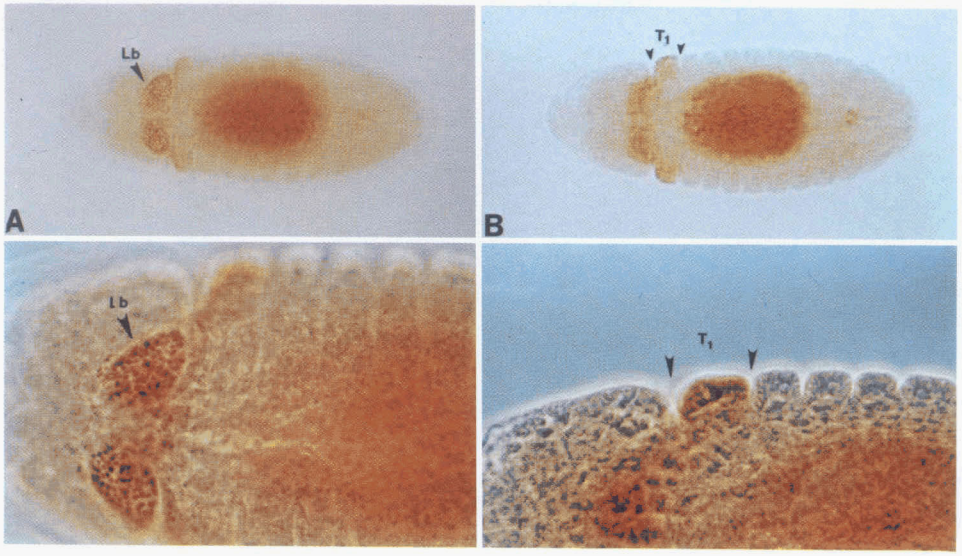

FIGURE 4. *Scr* antibody staining of embryos at complete germ band contraction. All views are from the ventral surface, anterior is to the left. The lower panel is a higher magnification of the upper panel. Panel A is focused at a level which shows stained nuclei in the labial segment. The labial segments have migrated toward the ventral midline where they will fuse prior to head involution. Panel B is the same embryo focused at a more dorsal level to view nuclei in the first thoracic segment. Nuclei stain throughout the first thoracic epidermis.

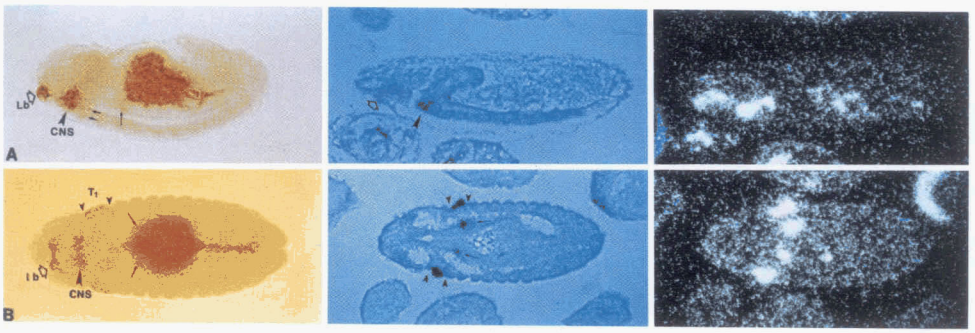

FIGURE 5.—*Scr* gene product distribution during head involution stage. The panels are the same organization as in Figure 2. Line A, dorsal is up and anterior is to the left. Staining with the *Scr* antibody is observed within the labial segment which has migrated anteriorly and is just about to be internalized (Lb, *clear arrowhead*), in the subesophageal region of the CNS as well as two nuclei within the next most posterior neuromere (*small horizontal arrows*), and within a few nuclei in the anterior wall of the midgut (*longer arrows*). In the center and right panels, grains are observed over the same regions as the antibody staining. Line B shows a ventral view of an animal at the same stage as that in A. *Scr* antibody staining is observed in the labial segment, CNS and midgut (designated as in line A) as well as throughout the epidermis of the first thoracic segment (T_1).

After fusion, the labial segments begin to migrate anteriorly as the process of head involution begins (10:20–11:20 hr). It is at this stage that *Scr*-antibody staining of the ventral nerve cord of the CNS is first detected (Figure 5, A and B, *left* panels). This staining is detected in two regions; a large group of nuclei within the posterior portion of the subesophageal ganglia and two to six nuclei in the next more posterior segment. Also at this stage, several nuclei in the wall of the anterior midgut stain with the antibody as well as the previously detected epidermal staining in the labial and first thoracic segments. Sections of embryos from *in situ* hybridization experiments have grains in comparable locations (Figure 5, A, *center* and *right*, and B, *center* and *right*).

Upon completion of head involution, secretion of the cuticular structures of the mouthparts begins. Nuclei which stain with the *Scr* antibody are observed in a group, internally, just ventral to the H-piece of the mouthparts (data not shown). This corresponds to the region where structures derived from the labial segment appear in the larvae (JURGENS *et al.* 1986). Staining is also observed in nuclei within the ventral nerve cord as before.

***Scr* antibody staining within the CNS:** As mentioned above, the most intense staining with the *Scr* antibodies was observed within the subesophageal ganglia of the ventral nerve cord. In order to precisely identify the position of the nuclei stained by the *Scr* antibodies, a double immunofluorescent staining technique was employed. Embryos were opened from the dorsal surface and the yolk mass was removed exposing the ventral nerve cord. The resulting "fillet" was stained with mouse anti-β-tubulin monoclonal antibodies (detected using rhodamine labeled goat anti-mouse IgG) as well as with the rabbit anti-*Scr* antibodies (detected with FITC-labeled goat anti-rabbit IgG). When illuminated with filters appropriate for rho-

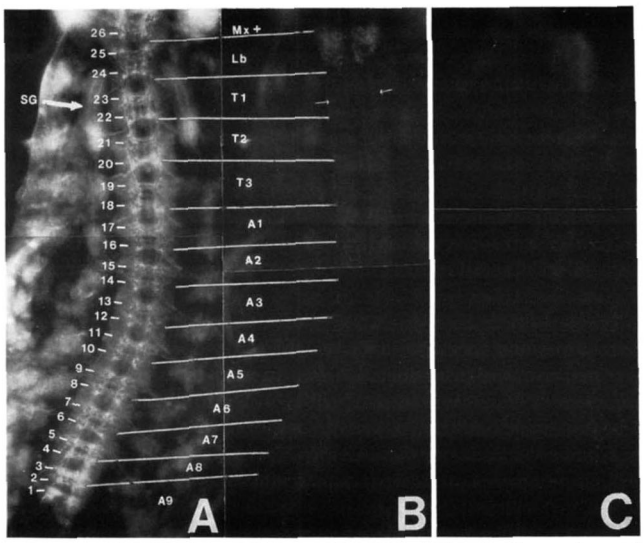

FIGURE 6.—Position of *Scr* antibody staining in the embryonic CNS. Panel A shows an exposed CNS illuminated to detect a mouse β-tubulin antibody which stains the repeated commissure pattern. The commissures are numbered beginning with the single commissure in the ninth abdominal segment (the remaining segments have two, the anterior or A/B commissure of each neuromere are denoted by odd numbers, and the posterior or C commissure of each neuromere by even numbers). Segmental identities are as described in the text (see DISCUSSION) (SG, salivary gland). Panel B shows the same CNS preparation illuminated to detect the rabbit *Scr*-antibody. Comparison to panel A shows the *Scr*-antibody staining accumulates between the 24th and 26th commissures (C commissure to next C commissure). *Arrows* show the location of two positively stained nuclei within the first thoracic neuromere which were consistently detected during microscopic examination. Note the absence of staining in the salivary glands which are thought to be labial derivatives. Panel C shows an *Scr* null CNS. No specific staining was detected although the brain hemisphere fluorescence is observed. The salivary glands are absent in the *Scr* null prep.

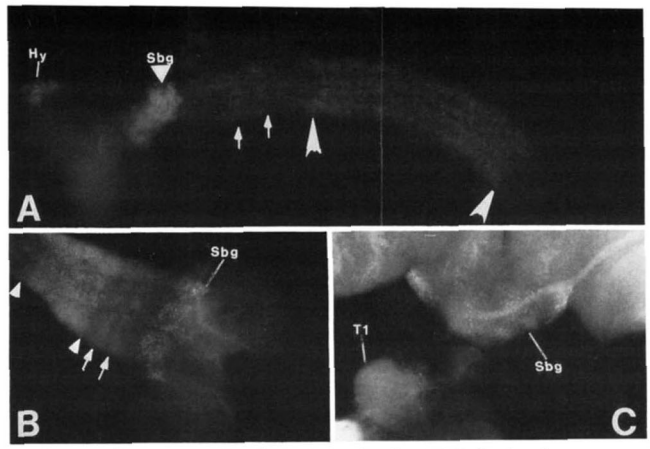

FIGURE 7.—*Scr* antibody staining in the CNS during later stages of development. Panel A shows the CNS dissected during late embryogenesis after CNS contraction has begun. *Scr* antibody staining is still observed in subesophageal ganglia (Sbg) and faintly in more posterior regions of the CNS, between the small arrows is a faint region which follows a gap one segment wide where no nuclei are stained. This is followed by a gap of very low intensity fluorescence and then a region of moderate staining about six or seven segments wide (between *large arrowheads*). There is no staining at the caudal end of the CNS. Panel B shows the CNS dissected from a third instar larvae. The pattern of staining is the same as in the late embryo. Panel C shows the CNS dissected from a 3-week-old adult. Staining was observed only within the subesophageal ganglia (Sbg).

damine detection, the β-tubulin antibody staining showed the characteristic "ladder" of commissures (Figure 6A) (THOMAS *et al.* 1984). Each segment of the CNS contains two commissure "rungs" (A/B and C) except for the ninth abdominal segment which contains only one (CAMPOS-ORTEGA and HARTENSTEIN 1985). Therefore, by counting the commissures anteriorly from the ninth abdominal segment it is possible to determine over which commissures the *Scr* protein accumulates. However, there is a controversy about what defines a segmental boundary within the CNS (THOMAS *et al.* 1984; CAMPOS-ORTEGA and HARTENSTEIN 1985). We have used the interpretation of the latter group (CAMPOS-ORTEGA and HARTENSTEIN 1985) for reasons we will discuss below. By next illuminating the same CNS with filters to detect the FITC staining, the position of nuclei that stain with the *Scr*-antibodies can be determined (Figure 6B). These nuclei lie between the 24th and 26th commissures, both of which are C commissures. This would place these nuclei within the labial neuromere. Two nuclei within the first thoracic neuromere, one in each hemisegment, also appear to stain. This agrees with the overall pattern observed in whole embryos. There is no staining within the ventral cord of an *Scr* null embryo (Figure 6C).

During the late stages of embryogenesis the ventral nerve cord contracts. When the pattern of *Scr* antibody staining was examined in the CNS from late embryos (Figure 7A), staining within the subesophageal ganglia is still observed. However, in addition to this, a fainter staining is seen in more posterior portions of the ventral cord. Just posterior to the strong staining in the subesophageal ganglia is a gap about one segment in width where no stained nuclei were observed. This is followed posteriorly by a region about two segments in width of faint but clear staining followed by another gap of very faint or no staining. Following this is a broad band six to seven segments long of uniform staining. The very posterior end, in what appears to be one to one and a half segments of the nerve cord, does not stain. A similar pattern was observed in the CNS from third instar larvae (Figure 7B).

We have also examined the *Scr* antibody staining pattern within the adult CNS. The CNS from 3-week-old adult Drosophila was dissected from the head and thorax. This was stained with the *Scr* antibodies which were detected using fluorescein labeled secondary antibodies. As can be seen in Figure 7C, nuclei within the subesophageal region stain positively with the antibody. No other region was found to stain.

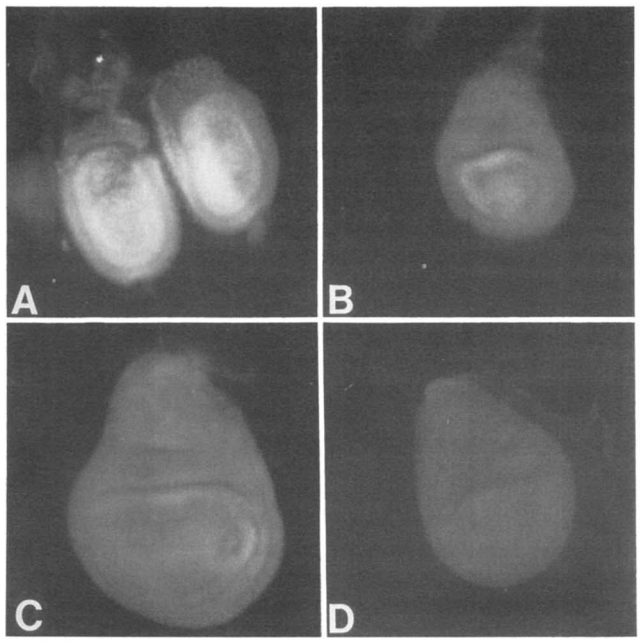

FIGURE 8.—*Scr* antibody staining within imaginal discs dissected from third instar larvae. (A) Staining within the first thoracic leg discs, (B) a second or third leg disc and (C) a wing and panel D haltere discs. Note the faint but definite staining within nuclei of the posterior leg disc shown in (B) but the lack of nuclei staining within dorsal discs (C and D) of the same segment.

***Scr* antibody staining in imaginal discs:** Results from genetic analysis of the affects of *Scr* mutations on development of the adult fly indicate that the *Scr* gene product is required for normal development of the labial palps of the mouthparts and within the first thoracic segment. In order to determine whether the protein distribution followed the prediction of the genetic analysis, we stained imaginal discs dissected from third instar larvae with the *Scr* antibodies. As expected, first thoracic leg discs showed intense staining (Figure 8A), as did the dorsal first thoracic discs (around the anterior spiracle) and the labial discs (data not shown). Unexpectedly, second and third thoracic leg discs also show faint but detectable staining (Figure 8B). The dorsal discs of these two posterior thoracic segments (wing Figure 8C and haltere Figure 8D) did not stain with the *Scr* antibody. No other discs were observed to stain above background.

DISCUSSION

An examination of the distribution of the gene products, both RNA and protein, encoded by the *Sex combs reduced* gene at various stages of Drosophila development has, in general, followed the predictions of previous genetic analysis (WAKIMOTO and KAUFMAN 1981; STRUHL 1983; SATO, HAYES and DENELL 1985). However, there are some striking exceptions to the predicted pattern.

Within the embryonic epidermis, where genetic analysis has demonstrated a role for the *Scr* gene product in the labial and first thoracic segments, the RNA and protein are indeed present throughout the epidermis of both of these segments. Interestingly, there appears to be a difference of about 2–3 hr between the detection of the RNA by *in situ* hybridization and the staining of the protein by immunological techniques. This may suggest posttranscriptional regulation or may be caused by the extensive time required to transcribe and process RNA from this gene. In either case, subsequent to this initial lag, the protein staining consistently follows the pattern of RNA; that is, wherever we detected the RNA, we could later detect the protein.

Using the cDNA probe which lacks the 5′ most portion of the *Scr* transcript, homologous RNA was first detected in embryos during gastrulation, posterior to the cephalic furrow. This region is just posterior to the position of the *Deformed* RNA at the same stage (CHADWICK and MCGINNIS 1987). Another study (INGHAM and MARTINEZ-ARIAS 1986) has reported that using a 5′ probe from the *Scr* transcribed region for *in situ* analysis of blastoderm stage embryos, a striped pattern similar to that observed with *fushi tarazu* (*ftz*) probes was seen. This pattern was absent in mutant embryos lacking the *ftz* gene product. Using a probe which covered almost two thirds of the *Scr* transcript, we cannot duplicate this result. We have also used a 5′ probe and still find the first evidence of the *Scr* transcript just posterior to the cephalic furrow during gastrulation. Furthermore, we have determined that the *Scr* protein does accumulate in embryos lacking the *ftz* gene, suggesting that the lack of the wild-type *ftz* gene product does not block expression of the *Scr* gene (J. W. MAHAFFEY and T. C. KAUFMAN, unpublished data). Future experiments will be necessary to explain the discrepancy between these two contrasting results.

The timing of *Scr* transcript accumulation differs between the labial and first thoracic segments. In the labial segment, the transcript appears in the ectoderm before the neuroblasts have segregated. Once the labial neuroblasts have segregated, they continue to express the *Scr* transcript. In contrast to this, the ectoderm of the first thoracic segment does not accumulate transcripts homologous to the *Scr* cDNA until after the neuroblasts have segregated from the ectodermal germ layer. Since the pattern of expression of *Scr* in the CNS is primarily in the labial neuromere (see below) it is possible that timing of *Scr* gene expression in the ectoderm could be the factor that specifies the degree to which the *Scr* gene product accumulates within the two different neuromeres (labial *vs.* prothoracic) of the CNS.

This result has several implications on development of the CNS in Drosophila. As mentioned above, there are two views as to what defines a segment within the

CNS. The one which has been used in the analysis of other homeotic gene products, that of THOMAS *et al.* (1984) places the segmental boundary just anterior to the A/B commissure which are the odd numbered commissures in this report (Figure 6A) [see WHITE and WILCOX (1985), BEACHY, HELFAND and HOGNESS (1985) and WIRZ, FESSLER and GEHRING (1986) for examples of the localization of proteins in the CNS from other homeotic genes]. Therefore, using this assignment, a segment or neuromere would extend from one A/B commissure to the next. The second model (CAMPOS-ORTEGA and HARTENSTEIN 1985) places the boundary at the C commissure which are the even numbered commissures in this report (Figure 6A) so that a neuromere would extend from the posterior of one C commissure through to the next. These views differ in that the commissures are either in the anterior portion (THOMAS *et al.* 1984) or in the posterior portion (CAMPOS-ORTEGA and HARTENSTEIN 1985) of a neuromere. Our results appear to support the boundaries set by the latter investigators.

The CAMPOS-ORTEGA/HARTENSTEIN model would place the observed *Scr* protein in the CNS in the labial neuromere (see RESULTS) while the THOMAS model would place the signal in the posterior maxillary and anterior labial neuromeres. When we examined embryos at the extended germ band stage for the distribution of *Scr* transcript, grains were localized over the labial epidermis and neuroblasts. Grains were never observed over the neuroblasts (or epidermis) of the maxillary segment. Additionally *Scr* protein is only observed in the epidermis of the labial segment not in the maxillary. The simplest interpretation of these observations is that the *Scr* gene product is present in the labial neuromere alone and is not expressed more anteriorly. However we cannot rule out the possibility that the accumulation of *Scr* transcripts within the labial neuroblasts does not lead to protein production in all of these cells and that *Scr* gene product is accumulated in the posterior maxillary neuronal derivatives subsequent to head involution when the CNS is no longer aligned with the epidermis. This later restructuring of the spatial pattern of *Scr* expression would yield a parasegmental pattern of expression in the CNS as compared to an apparent segmental pattern in the epidermis. As there is no direct evidence for this restructuring at present, we prefer the simpler explanation that a segment within the ventral nerve cord extends from one C commissure to the next. If this is correct and holds true for more posterior neuromere boundaries, then the position of other homeotic proteins in the CNS may need to be reassessed.

Regardless of the boundaries of the segments within the ventral nerve cord, the near absence of *Scr* protein in the first thoracic neuromere (except for possibly 1–3 nuclei per hemisegment), which lies between commissures 22 through 24, creates a gap of one segmental (or parasegmental) width in which no identified homeotic gene product accumulates to any great extent. The region of high accumulation of the *Antennapedia* (*Antp*) protein has, as its anterior boundary, the C commissure we have numbered 22 (WIRZ, FESSLER and GEHRING 1986). As with the *Scr* protein, there may be a few nuclei which express the *Antp* protein which are in the more anterior (first thoracic) neuromere between commissures 22 and 24. Therefore, for the known homeotic gene products (*Scr* and *Antp*) which one would predict to accumulate in the nuclei of this region, neither do to any great extent. What implications this may have on the role of homeotic genes in the CNS is only speculation at this time. Is there another as yet unidentified gene expressed in this region of the CNS, or is this the "ground state" and no homeotic gene is required here? Only further information as to the role of homeotic genes in the development of the CNS will answer these questions. Interestingly, the domain of expression of *Scr* and *Antp* overlap in the first thoracic epidermis.

A further question as to the function of homeotic genes in the CNS arises from the observation that the *Scr* gene product (protein) is present in the subesophageal region of the adult CNS. Since morphogenesis and the establishment of segmental identity is certainly complete (except for the aging process) in 3-week-old adults, this observation suggests that there may be other function(s) for the homeotic genes within the CNS which requires their continuous expression for proper function and/or maintenance of these cells. It is also possible that the protein is present only as a reflection of the cells origin, the labial ectoderm, and that the protein no longer has a function in the CNS. This seems unlikely however when one considers the energetics required to maintain this level of the *Scr* protein. A similar observation, that of homeobox containing transcripts being present in the adult CNS, have been obtained in vertebrates (CARASCO and MALACINSKI 1987).

Not only do the labial and first thoracic segments make different choices as to the expression of the *Scr* gene within the CNS but also in the mesoderm. In this case, however, the *Scr* protein accumulates in the mesoderm of the first thoracic segment but not labial mesoderm. At this time, we have not observed an effect upon mesodermal development in embryos which lack the *Scr* gene, so we do not know of a function for the gene product in the mesoderm.

Another unexpected observation was that there is a low level of *Scr* protein accumulation in second and third thoracic leg discs. Clonal analysis (STRUHL 1982) indicates that the absence of the *Scr* gene product does not have an affect on second and/or third leg

development. Therefore, the reason for the presence of the *Scr* protein in these discs is unknown. However, it is suggestive of a mechanism of action for several of the proposed *trans*-acting regulators of the homeotic genes. Many of these genes, *Polycomb* (DENELL 1978), *Enhancer of Polycomb* (SATO, RUSSELL and DENELL 1983), *extra sex combs* (STRUHL 1983), *Polycomblike* (DUNCAN 1982), *Sex combs on midleg*, *Additional sex combs* and *Posterior sex combs* (JURGENS 1985), all have the common property that at least some mutations at each locus result in a transformation of second (and sometimes third) thoracic legs toward partial first thoracic leg identity. This homeosis manifests itself most clearly by the appearance of sex comb teeth on posterior legs of adult males. It would appear that the low level of *Scr* protein present within the posterior leg discs does not affect normal development, but possibly, a slight shift in either the amount of *Scr* protein or the ability of this protein to function, can have dramatic results on segmental identity of the legs. Mutations at these *trans*-acting regulators may allow a higher expression of the *Scr* gene product within at least the second and third leg discs which results in a partial transformation of their segmental identity. Experiments are in progress which will test this hypothesis.

Previous reports on the localization of the *Scr* transcripts during embryogenesis have presented conflicting results. The study of HARDING *et al.* (1985) places the *Scr* transcript within the epidermis and mesoderm of the labial segment and in the posterior maxillary-anterior labial parasegment of the CNS. The results of KUROIWA *et al.* (1985) place the major localization of the *Scr* transcript in the epidermis of the labial and first thoracic segments and within the subesophageal ganglion and anterior portion of the first thoracic neuromere of the CNS. Our localization of the *Scr* gene products does not completely agree with either of the previous reports. Although we detect *Scr* expression within the labial and prothoracic epidermis as did KUROIWA *et al.* (1985), the localization of *Scr* transcripts in the labial neuroblast and not in neuroblast derived from the anterior first thoracic ectoderm leads us to assign the major region of *Scr* expression in the CNS only to the labial neuromere. Our ability to localize both the RNA and protein encoded by the *Scr* gene as well as a more complete examination of their distribution at various stages of embryonic development has allowed us to make a more precise localization of the *Scr* gene products.

As a final comment, we have noted some differences in the pattern of expression of the *Scr* gene and other homeotic genes which are expressed in trunk segments; a segmental *vs.* parasegmental pattern of expression in the epidermis, a shift in segmental expression pattern in the CNS, and the morphogenetic observation that mutations at the *Scr* locus result in a divergent transformation. This and other observations (J. W. MAHAFFEY and T. C. KAUFMAN, unpublished data) suggest there may be a difference in developmental pattern of head segments when compared to those of the trunk. Therefore, models of gene expression and development based solely on observations and patterns in trunk segments may not be entirely adequate for describing the developmental process in Drosophila. The *Scr* gene allows us to compare and contrast these differences since it functions within both domains. It is the differences, not the similarities, of gene action which allow us to understand how genes regulate morphogenesis.

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