Development 105, 167–174 (1989)
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Novel patterns of homeotic protein accumulation in the head of the *Drosophila* embryo

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Summary

Antibodies that specifically recognize proteins encoded by the homeotic genes: *Sex combs reduced*, *Deformed*, *labial* and *proboscipedia*, were used to follow the distribution of these gene products during embryogenesis. The position of *engrailed*-expressing cells was used as a reference and staining conditions were established that could distinguish, among cells expressing *engrailed*, one of the homeotic proteins or both. Our observations demonstrate two important facts about establishing identity in the head segments. First, in contrast to the overlapping pattern of homeotic gene expression in the trunk segments, we observe a non-overlapping pattern in the head for those homeotic proteins required during embryogenesis. In contrast, the spatial accumulation of the protein product of the non-vital *proboscipedia* locus overlaps partially with the distribution of the *Deformed* and *Sex combs reduced* proteins in the maxillary and labial segments, respectively. Second, two of the proteins, *Sex combs reduced* and *Deformed*, have different dorsal and ventral patterns of accumulation. Dorsally, these proteins are expressed in segmental domains while, within the ventral region, a parasegmental register is observed. The boundary where this change in pattern occurs coincides with the junction between the ventral neurogenic region and the dorsal epidermis. After contraction of the germ band, when the nerve cord has completely separated from the epidermis, the parasegmental pattern is observed only within the ventral nerve cord while a segmental register is maintained throughout the epidermis.

Key words: *Drosophila*, homeotic gene, protein, head segmentation, *invected*, *Sex combs reduced*, *Deformed*, pattern.

Introduction

The morphogenic pathways that lead to the segmented insect body plan involve elaborate networks of gene action. Nowhere is this more apparent than in our current understanding of the genetic hierarchy governing *Drosophila* embryogenesis (Akam, 1987). The initial step, establishment of the embryonic axes, is under the control of numerous genes, many of which are required during oogenesis while others are zygotically active (Perrimon & Mahowald, 1988; Nüsslein-Volhard *et al.* 1987; Anderson, 1987). Once the embryonic axes have been established, other zygotic genes direct the subdivision of the embryo into repeating metameric units (Nüsslein-Volhard & Wieschaus, 1980; Nüsslein-Volhard *et al.* 1985). Finally, the homeotic or selector genes impart specific identities to each segment (Garcia-Bellido, 1977; Lewis, 1978).

In *Drosophila melanogaster*, these homeotic genes reside in two clusters on the right arm of the third chromosome. The three genes of the Bithorax-Complex (BX-C) are required for the establishment of proper segmental identity in the trunk, including the second thoracic through the eighth abdominal segments (Lewis, 1978; Bender *et al.* 1983; Sanchez-Herrero *et al.* 1985; Karch *et al.* 1985; Hogness *et al.* 1985). The Antennapedia-Complex (ANT-C) contains five homeotic loci: *Antennapedia* (*Antp*), *Sex combs reduced* (*Scr*), *Deformed* (*Dfd*), *proboscipedia* (*pb*), and *labial* (*lab*) (Kaufman *et al.* 1980; Mahaffey & Kaufman, 1988). All of these genes except *Antp* and *Scr* are required solely within the head segments. The *Antp* locus is required within the first and second thoracic segments, while the *Scr* domain spans the boundary between the head and the trunk in that it is required in the labial head segment as well as in the adjacent first thoracic segment (Sato *et al.* 1985).

As an initial step toward understanding the mechanisms responsible for establishing segmental identity, the spatial expression pattern has been examined for a number of the homeotic genes (White & Wilcox, 1984, 1985; Beachy *et al.* 1985; Harding *et al.* 1985; Carroll *et al.* 1986; Wirz *et al.* 1986; Martinez-Arias, 1986; Mahaffey & Kaufman, 1987; Riley *et al.* 1987; Martinez-Arias

et al. 1987; Chadwick & McGinnis, 1987; Pultz *et al.* 1988). However, while establishing the overall pattern of homeotic gene expression, few of these studies obtained the high resolution required to determine the pattern at the cellular level. This is mainly due to the absence of clear and precise morphological landmarks whereby the position of homeotic gene products can be readily determined in individual cells; a problem that is particularly acute in the embryonic head. A solution to this problem has been devised in the study of homeotic gene expression in the trunk segments by Carroll *et al.* (1988). They have determined the position of *Scr*, *Antp* and *Ubx* protein accumulation using *engrailed* expression as a reference.

In order to assess accurately the pattern of homeotic protein distribution in the head of the *Drosophila* embryo, we have employed a similar double immunolocalization technique which is capable of simultaneously defining the position of homeotic proteins with respect to a reference marker (*engrailed*). The analysis has been restricted to the ectodermal tissues of the *Drosophila* embryo (epidermis and central nervous system (CNS)) although all of these gene products also accumulate in the mesoderm or endoderm (midgut). The results of this study demonstrate a novel pattern of homeotic protein accumulation in the head as compared to the trunk. In the trunk, all segments express multiple homeotic gene products (Harding *et al.* 1985; Akam, 1987; Carroll *et al.* 1988). However, in the head, we observe, with one exception, a non-overlapping pattern of accumulation of the protein products of the homeotic loci. Furthermore, we observe a shift in the spatial pattern of accumulation between the ventral (neurogenic) and lateral (dorsal epidermal) portions of the embryo. The shift occurs at the junction of the ventral neurogenic region and the dorsal epidermis.

Materials and methods

Construction of the homeotic fusion proteins and the manner of production and purification of the antibodies that recognize the homeotic proteins were essentially as described in Mahaffey & Kaufman (1987), as were the techniques for collection and fixation of the embryos. Binding of the primary antibodies to the embryos was carried out overnight at room temperature in PBT with 5% normal goat serum (Karr & Alberts, 1986). This was followed by washing the embryos in at least five changes of PBT over a period of 3 h. The embryos were again placed in PBT with 5% normal goat serum for 30 min followed by addition of both secondary antibodies (alkaline-phosphatase-labelled goat anti-rabbit and horseradish peroxidase labelled goat anti-mouse, BioRad). After binding of the secondary antibodies, the embryos were washed for one hour in five changes of PBT followed by three to four washes in 0·1 M-Tris, pH 8·2. Both enzyme staining reactions were performed in this Tris buffer. To detect the horseradish peroxidase (mouse antibodies), diaminobenzidine was added to 0·1 mg ml^{-1} followed by addition of hydrogen peroxide to 0·003%. After the appropriate density of stain was deposited, the embryos were washed with four changes of 0·1 M-Tris, pH 8·2 and then stained using the Vector Labs ABC alkaline phosphatase kit III and blocker (levamisole) as directed to detect the rabbit antibodies. After appropriate staining, the embryos were washed several times in 0·1 M-Tris pH 8·2 followed by two brief washes in 95% ethanol and finally two washes in water. The stained embryos were mounted in Gurr's water mounting media. Dissections were performed in the mounting media using sharpened tungsten needles. In most cases, the embryos were opened on their dorsal surface and as much of the yolk and gut removed as was possible. For CNS preparations, the ventral nerve cord was completely dissected away from other tissues. In all cases, after dissection, the tissues were mounted in the same Gurr's media, covered with a cover slip and observed under Nomarski interference optics on a Zeiss Photoscope III. Photographs were taken using Kodak VRG 100 colour print film with the blue filter in place for colour correction with a tungsten light source.

Results

In order to establish precisely the position of cells expressing the homeotic proteins, a double immunolocalization protocol was developed using a mouse monoclonal antibody directed against the *invected* (*inv*) protein as a reference and rabbit polyclonal antisera to detect the position of homeotic protein accumulation. The *invected* protein has extensive regions of homology with the *engrailed* (*en*) protein and both loci are expressed within the same cells, those that define the posterior compartment of each segment (Coleman *et al.* 1987). The *inv* antibody used in our study is directed against one of the conserved regions and will therefore detect both *inv* and *en* proteins (C. Goodman, personal communication). Therefore, *en* and *inv* could be used interchangeably in our description of pattern as, indeed, the antibody will bind both proteins. We have chosen to use *inv* in our description.

invected

Before presenting the pattern of homeotic protein accumulation, the position of cells that express *inv* in the embryonic head will be reviewed. The pattern will be described with reference to Figs 2, 3 and 4. In the early embryo, the *inv* protein accumulates within 15 nearly equidistant stripes of cells which encircle the early gastrula stage embryo (Kornberg *et al.* 1985; DiNardo *et al.* 1985). The most-anterior three of these bands define the posterior compartments of the mandibular, maxillary and labial (anterior to posterior) head segments. The first *inv* band to appear, at the beginning of gastrulation, corresponds to the posterior compartment of the maxillary segment (see Fig. 2A and DiNardo *et al.* 1985). In addition to these 15 bands, there are at least five other regions that stain with the *inv* antibody in each lateral half of the head of the *Drosophila* embryo. After the initial 15 bands appear, at least two (and possibly three) groups of *inv* staining cells are observed on the lateral portion of the procephalon. The more ventral group (below *PC* in Fig. 3A and 3a′) runs parallel to and about five cells removed from the ventral edge of the procephalon. Along the mid-lateral aspect of the procephalon lies another group of *inv*-positive cells which appear more as spots than bands (to the left of *PC* outside of the

square in Fig. 3A). At about the same time that these procephalic staining regions appear, *inv*-positive cells also appear behind the stomatodeal invagination but anterior to the mandibular *inv* band (labelled *Hy* in Figs 2B, 3a′, 3B and 3b′). The location of these cells suggests that they define the posterior portion of the hypopharyngeal lobes. A large block of *inv*-positive cells also is observed within the clypeolabrum (labelled *Cly* in Figs 2B and 3B). Finally, after segmentation is evident in the head, *inv*-positive cells appear along the dorsal edge of the labial segment. These cells form an arc that extends from the posterior of the labial segment anteriorly to the posterior edge of the maxillary segment (above *Lb* in Fig. 2B). These cells appear to migrate dorsally where they come to lie between the dorsal anterior edge of the first thoracic segment and the dorsal posterior edge of the procephalon (labelled DR in Figs 3D and 4B). They will form the posterior component of the dorsal ridge.

The *inv*-staining pattern is not static during development. Changes appear to occur by either: (1) change in the accumulation of the *inv* protein, or (2) change in the position of *inv*-positive cells. It is not always possible to distinguish between these two alternatives. The first change in pattern is observed during germ band extension. Along the ventral midline of each of the initial 15 *inv* stripes there is an increase in the number of *inv*-positive cells (data not shown). This leads to a broadening of each stripe within the ventral neurogenic region (as defined by Campos-Ortega & Hartenstein, 1985). Many or all of these *inv*-positive cells will delaminate as neurogenesis proceeds. The origin of these cells is uncertain. Whether they are daughter cells of those initially expressing the *inv* antigen, or represent cells newly expressing *inv* is not known. Finally, a change in the *inv* pattern occurs through the alteration in position of the gnathal segments during head involution.

Homeotic protein patterns

The pattern of the homeotic proteins will be described beginning with the most posteriorly expressed member (*Scr*) and will proceed anteriorly with the exception that the *pb* pattern will be described last. The patterns of protein accumulation in the CNS are covered together at the end.

Sex combs reduced

The pattern of *Scr* protein accumulation has previously been described (Mahaffey & Kaufman, 1987; Riley *et al.* 1987; and Carroll *et al.* 1988). However, use of (a) *inv* as a reference and (b) the more-sensitive alkaline phosphatase detection system has allowed further refinement of the *Scr* pattern. The *Scr* protein can first be detected when the embryo is at the end of fast germ band extension (3:40 h, stage 8 of Campos-Ortega & Hartenstein, 1985). Fig. 1A shows the distribution of *Scr* protein in an embryo undergoing slow germ band extension. Laterally, a segmental pattern of accumulation is observed. The anterior boundary of *Scr* protein abuts, but does not overlap with, the *inv*-positive cells of the maxillary segment. The expression of *Scr* continues through the posterior labial segment into the anterior portion of the first thoracic segment. A parasegmental pattern is observed in the ventral portion of the embryo. Here, the *Scr* protein overlaps the *inv* cells of the maxillary segment and extends posteriorly only through the anterior portion of the labial segment. Staining does not extend into the posterior labial *inv* stripe or into the anterior first thoracic segment. Additionally, a few cells within the ventral posterior maxillary region stain only with *inv* antibodies and not with those directed against the *Scr* protein (long arrow in Fig. 1A). The boundary between the lateral and ventral patterns of staining is quite distinct (small arrows).

After segmentation, the lateral (segmental) pattern of *Scr* protein accumulation is observed in the epidermis. The protein is found throughout the labial segment and in the anterior portion of the first thoracic segment. A few *Scr*-positive cells are observed transiently at the posterior edge of the maxillary segment near the ventral–lateral boundary (data not shown, see Carroll *et al.* 1988). However, after completion of germ band contraction this staining is no longer observed so that there is no overlap of *Scr* staining with cells in the maxillary epidermis (Fig. 1B). The ventral (parasegmental) *Scr*-staining pattern is now restricted to the CNS (see Fig. 1B and below).

Deformed

The *Dfd* protein is the earliest expressed homeotic protein, beginning at cellular blastoderm simultaneous with or just prior to the accumulation of *inv* protein. Fig. 2A shows an embryo just beginning gastrulation stained to detect both *Dfd* and *inv* proteins. Examination of numerous embryos, both whole mounts and dissections, has demonstrated that the posterior limit of *Dfd* protein accumulation coincides with the *inv*-positive cells of the maxillary segment (note the black appearance of these cells labelled *pMx* in Fig. 2A as compared to the brown appearance of *inv* alone). The *Dfd* staining extends about four to five cells anterior of the maxillary *inv* band. The cephalic furrow (*CF*) can be seen forming between the maxillary and mandibular *inv* bands. Cells staining positively for *Dfd* extend about one or two cells anterior of the mandibular *inv* band.

The *Dfd* pattern is very dynamic during development. As germ band extension begins, the *Dfd* protein accumulates in the posterior portion of the hypopharynx where it overlaps with *inv* cells of the hypopharyngeal lobe. In addition, as the number of *inv*-positive cells increases within the ventral neurogenic region, many of those cells within the ventral portion of the posterior compartment of the maxillary segment do not stain positively for the *Dfd* protein. Instead, as described above, these cells will stain with the *Scr* antibodies. When a mouse *Scr* monoclonal antibody is used in conjunction with the rabbit *Dfd* antisera, there appears to be no extensive overlap between cells expressing *Dfd* and *Scr* (data not shown).

As the germ band reaches full extension, segmental

boundaries appear in the head. At this point in embryogenesis, the *Dfd* staining is reduced in intensity within many cells of the maxillary and mandibular segments. Specifically, staining is diminished in the lateral/anterior portion of both the maxillary and mandibular segments as well as the mid-ventral portion of the mandibular segment (Fig. 2B and C). Strong staining is still observed in the *inv*-positive cells of the hypopharyngeal lobes (small arrows in Fig. 2C and D). Also at this time, a small patch of *Dfd*-positive cells is observed at the anterior edge of the dorsal ridge (data not shown, see Chadwick & McGinnis, 1987 for RNA localization and Jack *et al.* 1988 for protein localization). During head involution the *Dfd*-positive portions of the maxillary, mandibular and hypopharyngeal lobes fuse to form a continuous *Dfd*-positive region which will come to lie on the lateral edges of the stomatodeal opening (Fig. 2D).

labial

The *lab* protein is first detected during the fast phase of germ band extension. Unlike the homeotic proteins described earlier, the *lab* protein does not accumulate in the postoral segments. Instead, *lab*-positive cells are observed along the ventrolateral edge of the procephalon (Fig. 3A and in higher magnification in 3a′). They are bounded laterally by the ventral *inv*-positive cells of the procephalon, and ventrally by the dorsal edges of the postoral lobes (*Hy*, *Mn* and *Mx* in Fig. 3a′). Staining embryos with both the *Dfd* and *lab* antibodies indicates that, prior to the retraction of *Dfd* staining in the mandibular and maxillary lobes, there is no gap between the cells that accumulate *lab* and those that accumulate *Dfd* (Fig. 3C, the arrow marks the separation between the procephalon and the postoral region). Furthermore, the positions of *Dfd*- and *lab*-expressing cells with respect to *inv* suggest there is no overlap between cells accumulating these two proteins. As the hypopharyngeal lobes involute into the stomodeum (Fig. 3B and b′) the *lab*-positive cells are found lateral to the invagination.

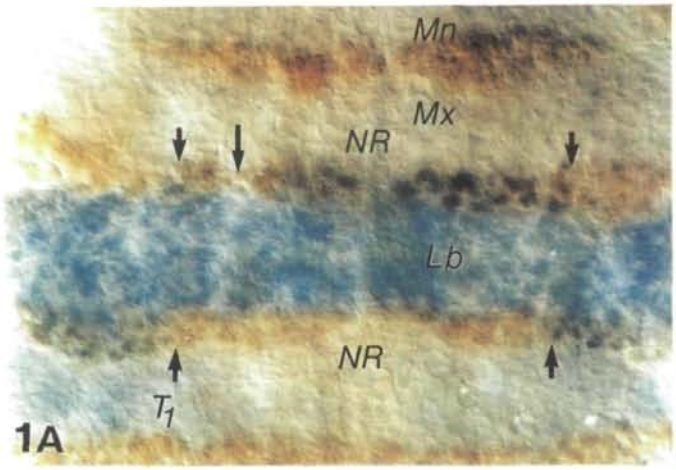
During germ band contraction, a change is observed in the pattern of *lab* protein accumulation. All of the cells that form the dorsal ridge, except possibly the most posterior row, stain positively for both *inv* and *lab* (Fig. 3D, arrows indicate cells that may only stain with *inv* antibodies). At the same time, the continuity of the *lab* staining on the procephalic lobe becomes broken. As shown in Fig. 3D, a group of cells dorsal and apparently interior to the maxillary lobe express the *lab* protein as well as a large block of cells within the anterior/ventral portion of the procephalon. This procephalic staining appears to be composed of two groups of cells, one that will form a portion of the CNS while the other will form epidermal derivatives which internalize during head involution.

As the anterior migration of the head segments continues in preparation for head involution, the three regions of epidermal *lab* expression – dorsal ridge, supramaxillary and procephalon – come together along the dorsal edge of the postoral segments. They will

Fig. 1. The distribution of *Scr* with respect to *inv*. In all figures, cells expressing the *Scr* (or other homeotic protein) are blue while those of *inv* are brown. Cells that accumulate both proteins appear dark brown to black. Dissected embryos are viewed from the ventral surface unless otherwise indicated. (A) An embryo during germ band extension that has been stained then cut along the dorsal surface to remove the yolk mass. This will be referred to as a 'pelt' in future figures (a name coined by W. Bender). Anterior is to the top so that the uppermost *inv* stripe is the posterior mandibular compartment. The long arrow denotes a cell within the ventral neurogenic region that only stains with the *inv* antibody. The small arrows indicate the position where the stain accumulation shifts from the ventral to the dorsal pattern. (B) Pelt from an embryo after segmentation and contraction of the germ band. Dorsal closure was not complete in this animal. The labial segments have begun to migrate ventrally and are near the point of fusion. The cells that will form the CNS have delaminated and lie below the epidermis. *Mn*, mandibular; *Mx*, maxillary; *Lb*, labial; T_1, first thoracic segment; *NR*, neurogenic region.

Fig. 2. The expression of *Dfd* with respect to *inv*. (A) Whole embryo stained to detect both *Dfd* (blue) and *inv* (brown). Anterior is to the left. The posteriormost extent of cells staining positive for *Dfd* is within the posterior maxillary (*pMx*) as defined by *inv* staining and continues anteriorly about four to five cells. The faint blue, which appears behind the *inv* positive cells, is due to the more diffuse cellular pattern of staining with alkaline phosphatase as compared to the nuclear staining pattern with horseradish peroxidase. The alkaline phosphatase product diffuses more readily than does the horseradish peroxidase product. *CF*, cephalic furrow. (B) Pelt of an embryo after complete germ band extension which has begun segmentation within the head. Anterior is to the left, ventral view. Staining with *Dfd* is observed within the ventral portion of the maxillary segment and in the lateral portion of the mandibular lobes. Faint staining can also be seen in the region of the hypopharyngeal (*Hy*) segments where *Dfd* appears to overlap with *inv*. *Cl*, clypeolabrum; *PC*, procephalon. (C) Higher magnification of an embryo pelt slightly older than that in B. The labial segments have begun to migrate ventrally and the CNS has delaminated. Staining is as in B. The small arrow points to the *inv*/*Dfd*-positive region of the hypopharyngeal segment. (D) Pelt from an embryo undergoing head involution. The maxillary segments have rotated and fused to the lateral portion of the mandibular segments and both are near the anterior of the embryo. The small arrow points out the *inv*-positive cells of the hypopharyngeal segment. The labial segments are overtop of the CNS.

Fig. 4. Accumulation of *pb* and *inv*. (A) Pelt of an embryo during the early stages of germ band contraction. *pb* is in blue, anterior up, ventral view. The CNS has delaminated from the epidermis. Staining for *pb* is observed within the dorsal portions of the labial and maxillary lobes, and within the ventral posterior region of the mandibular segment extending into the ventral anterior of the maxillary segment. Slight staining is also visible at the lateral edge of the mandibular segment. The staining is actually in cells one or two layers under the surface. (B) Pelt of an embryo undergoing head involution. The labial segments are about to fuse and are now juxtaposed next to the ventral portion of the mandibular segment. The stomatodeal opening is visible as a curve just anterior to the ventral portion of the mandibular segment.



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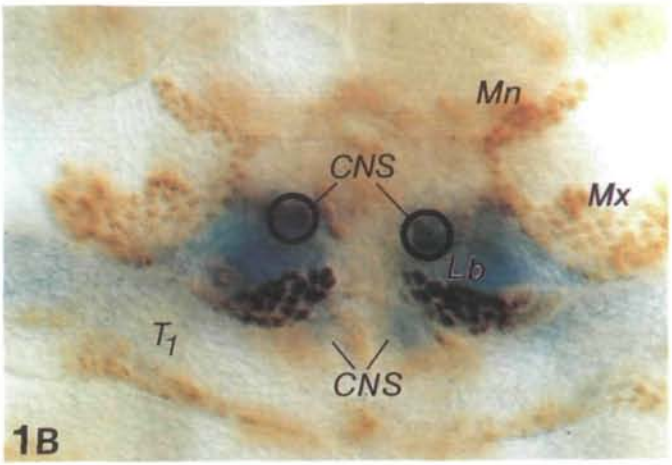

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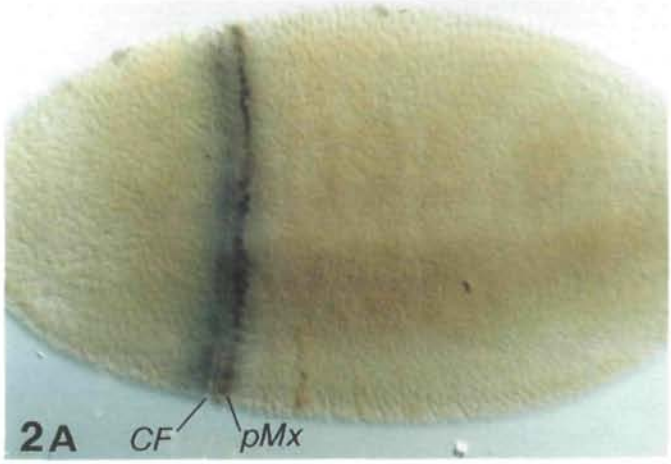

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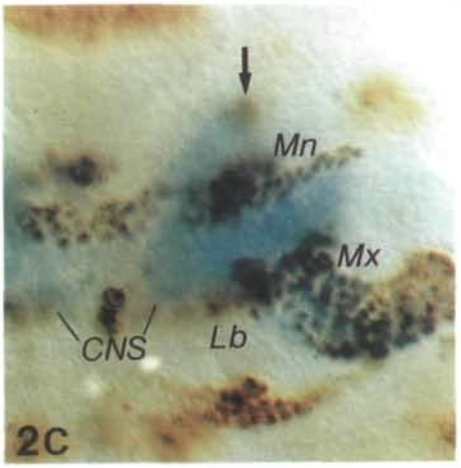

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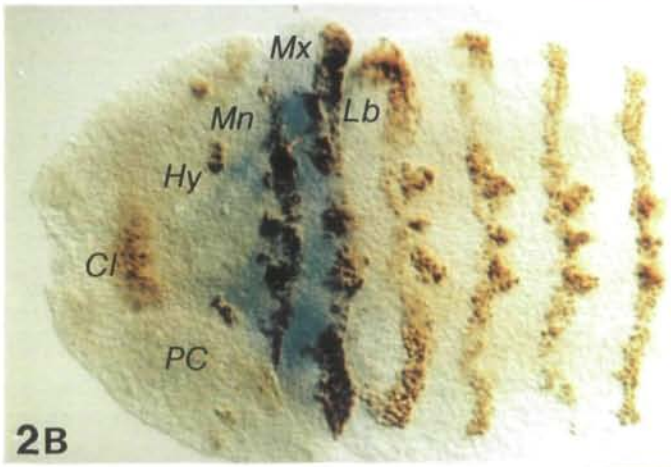
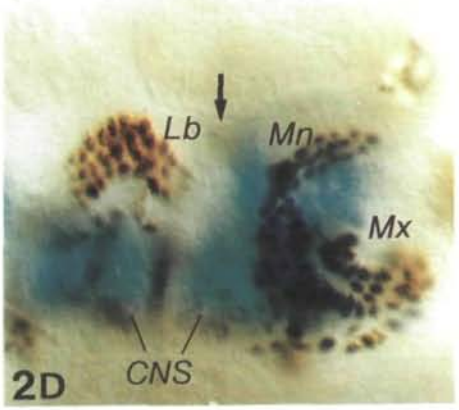


2B



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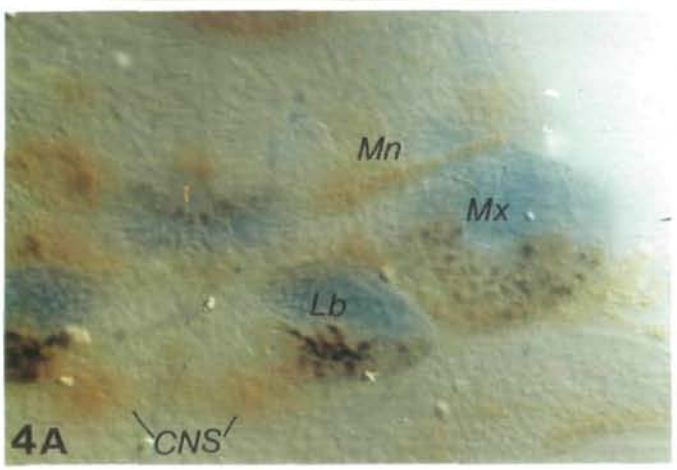

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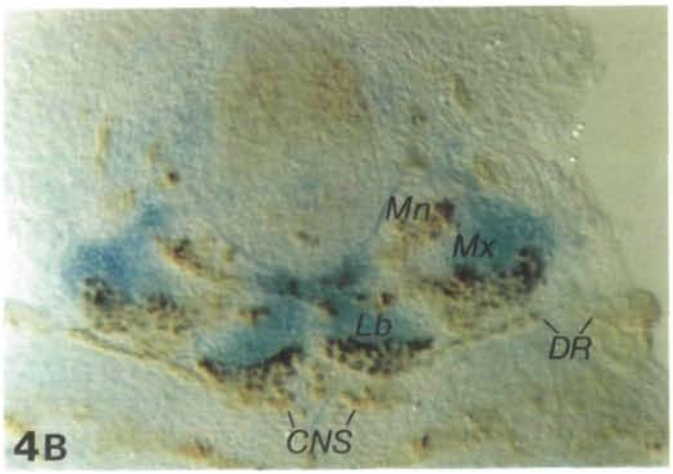

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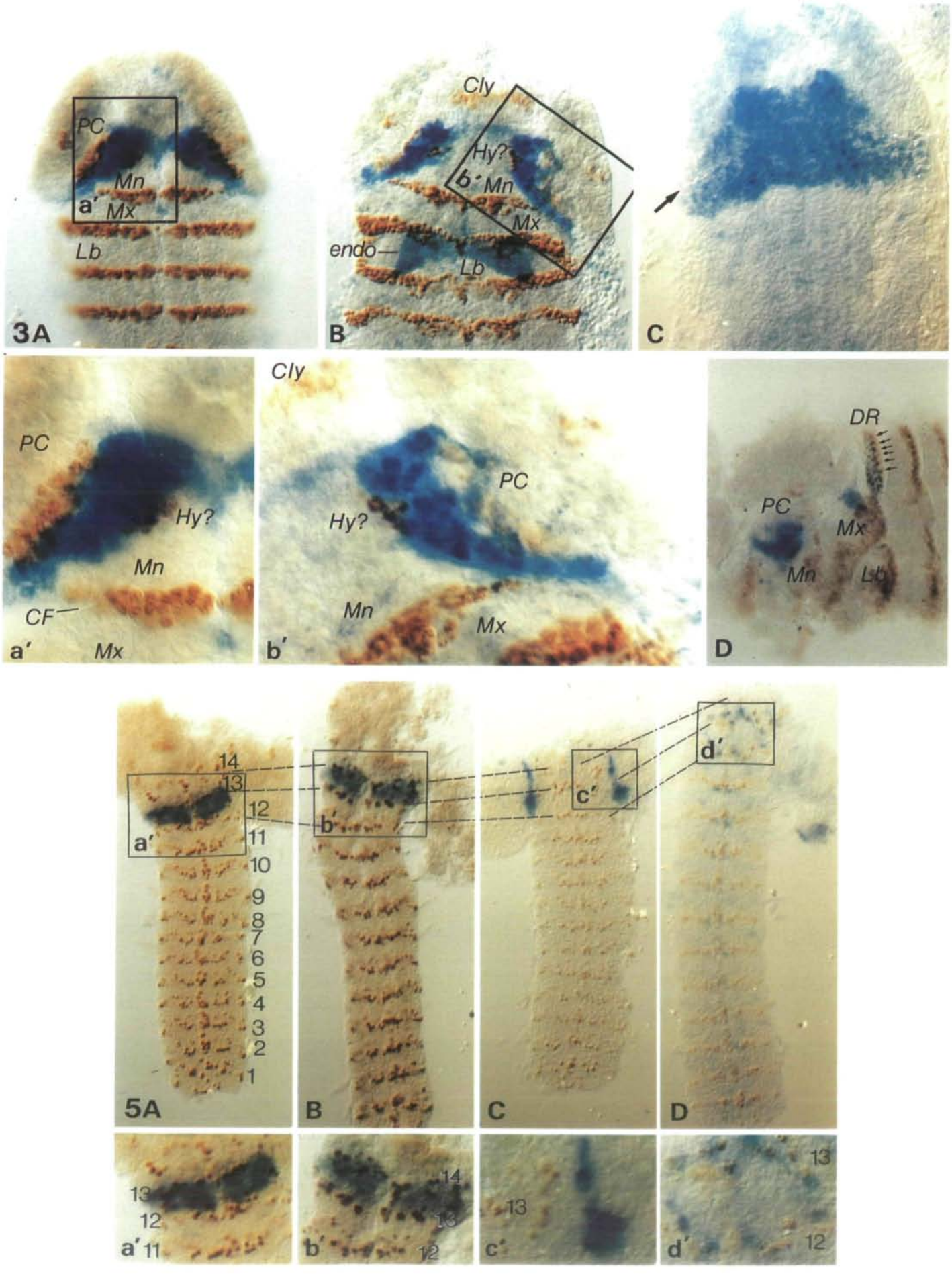
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Fig. 3. The *lab* pattern with respect to *inv*. (A and a′) The early pattern of *lab* (blue) staining in pelts of embryos during germ band extension. Anterior is up, ventral view. The stained cells are located on the procephalon (*PC*) just ventral to the *inv* band and extend to the edge of the postoral segments defined by the mandibular and hypopharyngeal regions. a′ is a closeup of the region outlined in A. (B and b′) Pelts of embryos after germband extension and the onset of segmentation. Anterior is up. Breaks in the continuity of *lab* staining are becoming apparent. b′ is a higher magnification of the region outlined in B. (C) Pelt of an embryo during germ band extension stained with both *Dfd* and *lab* antibodies, both in blue. There is no gap between the two regions of stain and, by comparing to individual staining with *inv*, no overlap is apparent. The arrow indicates the position of separation between the procephalon and the postoral segments. (D) Bright-field photograph of a lateral pelt of an embryo after contraction of the germ band. Bright-field optics were used due to the three-dimensional aspect of the sample. Note the three discontinuous regions of *lab* protein accumulation. Anterior is to the left, dorsal up. Staining is observed in the anterior ventral portion of the procephalon, above the maxillary segment, and within cells of the posterior dorsal ridge (*DR*). Note that the cells at the posterior edge of the dorsal ridge stain only with *inv* (arrows).

Fig. 5. Expression pattern of gene products in the CNS. The CNS was dissected out of late-stage embryos after staining. Anterior is up. The *inv* bands have been numbered beginning with the posterior most band. This allows a direct comparison of the various positions of the homeotic gene products. (A) *Scr* accumulates between *inv* bands 12 and 13, ovelapping only with 13 (see higher magnification in a′). (B) The pattern of *Dfd* staining between bands 13 and 14, overlapping only with band 14 (higher mag. in b′). (C) The position of *lab* protein accumulation. There are few or no *inv* staining cells in this region of the CNS. Double staining with *Dfd* indicates that the two proteins abut (or nearly so) only within a small region midway through the *lab* band (approximately at the position of the number 13 in c′) and the two stained regions appear perpendicular to one another. (D) The pattern of *pb* accumulation within the CNS. Note that it is unlike the other homeotic proteins in that it accumulates in several cells of all segments and not within a segment-wide block. d′ shows a higher magnification of the region of highest number and strongest intensity of *pb*-stained cells. This coincides with the area between the *inv* bands delimiting the position of *Scr* accumulation.

eventually internalize and be found parallel to the pharynx and contiguous with the frontal sac. The cells of the dorsal fold become continuous with the *lab*-expressing cells of the procephalic lobe and after head involution are found at the roof of the pharynx associated with the dorsal pouch (data not shown, Diederich *et al.* unpublished data).

proboscipedia

The *pb* gene is enigmatic in that, although the gene product accumulates in many embryonic cells, there appears to be no effect on embryogenesis caused by a complete deletion of the gene. However, the gene is required during pupal development (Pultz *et al.* 1988). The use of the double-labelling system has allowed us to refine the position of *pb* protein accumulation. We first observe the *pb* protein at the extended germ band stage, just prior to the onset of segmentation. Protein is detected in the lateral portions of the labial and maxillary epidermis as well as in the mesoderm of the mandibular segment. As the mesodermal cells separate from their close attachment to the epidermis, one observes that cells in the ventral portion of the mandibular segment also stain positively for the *pb* protein (data not shown).

Fig. 4A shows the pattern of *pb* staining in an embryo after contraction of the germ band and Fig. 4B shows the *pb* pattern as the head segments migrate anteriorly in preparation for head involution. The mesodermal tissue has been removed. Within the labial segment (*Lb*), staining is observed throughout most of the lateral regions but stops one or two cells posterior to the *inv*-positive cells of the maxillary lobe (see Fig. 4B, the gap is not readily visible in Fig. 4A as it is located within the fold that separates the lobes). This gap in the *pb* expression has been further demonstrated by double staining for *pb* and *Scr* proteins. The anterior labial segment cells accumulate *Scr* but not *pb* (data not shown). Staining is also observed within the lateral portions of the maxillary lobe (*Mx*). However, as in the labial segment, there are regions of the maxillary segment that do not stain. Many of the cells that accumulate the *pb* protein in the maxillary and mandibular lobes correspond to the cells in which *Dfd* expression is reduced, but there is not a complete correlation. The predominant staining in the mandibular lobe (*Ma*) is observed in the ventral portion beginning in the *inv*-positive cells of mandibular identity and extending into the anterior/ventral maxillary epidermis. A small cluster of *pb*-positive cells is observed on the lateral edge and one or two cells below the surface of the mandibular lobes.

Central nervous system

As has been previously documented, both the homeotic as well as *en* antigens are present within specific cells of the CNS. We have examined the pattern of accumulation in the CNS of the five antigens used in this study (Fig. 5). The proteins encoded by *Scr*, *Dfd* and *lab* accumulate in non-overlapping succession. Unlike what was observed in the lateral epidermis in the early embryo or in the entire epidermis after germ band contraction, *Scr* and *Dfd* accumulate in parasegmental patterns in the CNS (Fig. 5A and B; note that, although the *inv* staining is quite dark in 5B, there is no blue staining anterior of the 13th (see below) *inv* band). A few *inv*-positive cells in the CNS are internal to each block of homeotic expression. Within these internal regions, it is often difficult to determine whether cells are expressing both antigens or are singly stained but overlie each other.

In order to present the positions of homeotic protein accumulation in the simplest manner, we have numbered the *inv* band in each neuromere, beginning the numbering with the posteriormost band. Following this nomenclature, the *Scr* antigen accumulates just anterior

to the 12th *inv* band and extends to, and includes, most of the cells that make up the 13th (Fig. 5A and a'). Examination of many dissections indicates that the *Dfd* protein begins anterior to the 13th *inv* band and extends through the last, the 14th (Fig. 5B and 5b', again, note the absence of blue around the *inv*-positive cells in the 13th band). Furthermore, double staining with *Scr* and *Dfd* indicates there is no overlap between cells expressing these two antigens (data not shown). The *lab* protein accumulates at what appears to be the junction of the supra- and subesophageal ganglia. Staining is observed along the base of both brain hemispheres (Fig. 5C and c'). Double staining with *lab* and *Dfd* antibodies indicates that there is at most only a short region where cells expressing the two antigens abut (data not shown).

The pattern of accumulation of the *pb* protein in the CNS deviates from that of the other homeotics in that the *pb* protein accumulates within several cells of each neuromere (Fig. 5D). This pattern of accumulation more closely resembles that observed for the pair-rule genes *fushi tarazu* and *even skipped* than the homeotics (Doe *et al.* 1988*a*). The region with the largest number of *pb*-positive cells lies between the 12th and the 13th *inv* bands, the position of *Scr* accumulation (Fig. 5d'). Double staining with the *Scr* antibody indicates that only a few of the *pb*-positive cells also accumulate the *Scr* antigen. In fact, the *pb*-stained cells appear to encircle the *Scr*-positive cells. Furthermore, there is little coincidence between *pb* and *inv* staining. As one observes the more posterior portions of the CNS, the number of *pb*-positive cells decreases and there is less (or no) coincidental expression with *inv*.

Discussion

The above observations demonstrate two important facts about the expression of the homeotic genes of the ANT-C within the head of the *Drosophila* embryo. First, the spatial expression pattern of these loci appears to be exclusive and non-overlapping. This non-overlapping pattern of accumulation of the homeotic gene products in the head segments stands in contrast to the distribution of proteins expressed in the trunk segments. In the trunk, both molecular and genetic evidence indicate that the identity of each segment is controlled by multiple homeotic genes (Lewis, 1978; Bender *et al.* 1983; Harding *et al.* 1985; Akam, 1987; Carroll *et al.* 1988). Therefore, the two contrasting patterns of gene expression suggest a different paradigm for the establishment of segmental identity between these two regions of the embryo. Whether this indicates a higher specialization of the head segments relative to a greater similarity between trunk segments, or the evolution of mechanisms that allow stricter control and/or flexibility in the establishment of segmental identity in the head and trunk, respectively, is at present uncertain.

The preceding discussion of course assumes that all of the homeotic genes that affect head development have been identified, an assumption that may prove to be untrue. Unfortunately, the identification of additional homeotic loci that affect this region of the embryo is clouded by the fact that many mutations disrupt head formation and it is often difficult to ascribe a homeotic phenotype to these lesions. Two cases in point are the affects of mutations at the *Dfd* and *lab* loci. These homeobox-containing members of the ANT-C show clear disruptions of the embryonic head but do not produce the obvious segmental transformations seen in $Antp^{-}$ or Ubx^{-} embryos (Merrill *et al.* 1987; Merrill, Diederich, Turner and Kaufman, unpublished data; although see also Regulski *et al.* 1987). The homeotic nature of these loci, however, is revealed when clonal patches of mutant tissue are examined in adult flies (ibid.). A further complication is that several mutations that do cause homeotic transformation in the head are likely regulators of the homeotic loci and not selector genes themselves (e.g. *Polycomb*, Denell, 1978; Wedeen *et al.* 1986; Riley *et al.* 1987; or *spalt*, Jurgens, 1988; Frei *et al.* 1988). These genes are expressed more globally than are the members of the ANT-C and presumably regulate the expression of homeotic loci in both the head and trunk. The resolution to this difficulty will be provided if and when additional head homeotics are discovered.

An additional puzzle is presented by the observation that the *Dfd* protein accumulates within two adjacent head segments (maxillary and mandibular) in the early embryo and that these segments develop independent distinguishing characteristics. Since no essential ANT-C gene products are accumulated in this region, it is likely that other cues are necessary to separate these segments into their respective identities. Although this conclusion is consistent with the hypothesis that all of the genes responsible for head segment specification have not been identified, it is also possible that other mechanisms may create these segmental distinctions. For example, the combinatorial action of the *Dfd* protein with other segmentation gene products (such as the gap or pair-rule genes, or genes involved in the establishment of the embryonic axes) may contribute to segmental identity. The early expression of *Dfd* would coincide with a simultaneous expression of these gene products and evidence has recently been obtained that *Dfd* expression is influenced by mutations in the pair-rule genes (Jack *et al.* , 1988). Alternatively, the number and position of the *Dfd*-expressing cells within the individual segments or even the level of protein accumulation may affect identity decisions. At present we do not have definitive evidence to demonstrate which, if either, of these possibilities is correct.

The second major point derived from these observations is that within a specific postoral segment, a different pattern of homeotic protein accumulation exists between the dorsal and ventral domains. Dorsally, a segmental register of accumulation is observed while ventrally the pattern appears parasegmental (Martinez-Arias & Lawrence, 1985; Ingham *et al.* 1985). Cell counts have indicated the position where this switch in register coincides with the junction of the

dorsal epidermis and the ventral neurogenic region (Campos-Ortega & Hartenstein, 1985). The different spatial registers suggest different modes of regulation of these homeotic genes in cells possessing different positional values: one for epidermal cells and another for cells destined to a neural fate.

Although the late pattern of protein accumulation agrees with previously reported patterns of mRNA and protein distribution (Martinez-Arias *et al.* 1987; Carroll *et al.* 1988; Jack *et al.* 1988), our interpretation of how these patterns arise (that is, whether the segmental or parasegmental pattern is established first), do not agree. As shown in Fig. 2A, the posterior extent of the earliest *Dfd* protein accumulation coincides with the single row of cells expressing the *inv* antigen. This would place these cells in the blastoderm within the posterior compartment of the maxillary segment. Therefore, the earliest pattern of *Dfd* protein accumulation would be segmental. After gastrulation begins and the post-blastoderm cell divisions have been initiated, a larger number of cells can be seen to accumulate *Dfd* protein. Also during this period, the ventral neurogenic region and the dorsal epidermis can be distinguished with respect to the relative patterns of accumulation of the *Dfd*, *Scr* and *inv* proteins. In the posterior portion of the maxillary ventral neurogenic region, *inv*-positive cells appear which do not stain with *Dfd* antibodies. The inv^+/Dfd^- cells do however later accumulate *Scr* protein. It is this subsequent ventral shift in register that generates the parasegmental pattern observed here and in the CNS. No such change is observed in the dorsal epidermis. The cells at the posterior border of the maxillary lobe begin as being both *inv* and *Dfd* positive and remain that way. Additionally, the cells at the anterior of the labial lobe accumulate only the *Scr* protein.

There are two possible ways that the shifted ventral pattern could be generated. (1) The new *inv* cells are related by descent to the initial single row of cells of posterior maxillary identity and express early both *Dfd* and *inv* but, subsequent to cell division, the daughters cease to accumulate the *Dfd* protein and begin to express *Scr* or (2) The *inv* cells are not related to the *Dfd*/*inv*-positive cells in the posterior compartment of the maxillary segment, rather they represent cells showing new accumulation of *inv* protein in cells that are within the realm of *Scr* expression. Parsimony would seem to favour the later of the two possibilities in that it requires only the turning on of a single locus. In either case, however, these cells (and the parasegmental pattern of homeotic gene expression they maintain) eventually are found only within the ventral nerve cord, and not in the epidermis of the embryo. This suggests that the parasegmental pattern of homeotic protein accumulation may be restricted to the CNS from an early point in *Drosophila* development at least with respect to the patterns in the head segments.

The observation that the epidermal and neurogenic expression patterns are different for genes important to the process of pattern formation is not unique to the homeotic head genes. Other segmentation genes have different patterns and modes of regulation within the nervous system as compared to the epidermis (Doe *et al.* 1988*a*,*b*). Furthermore, recent experiments by DiNardo *et al.* (1988) clearly demonstrate different *cis*-regulatory elements that control ventral and dorsal/lateral expression of *en*. Unfortunately, in our study it is not possible to determine whether the alternate dorsal/ventral register is manifested through changes in the pattern of *inv* or the homeotic genes, or both.

This study demonstrates that the homeotic genes expressed in the head of *Drosophila* may have different modes of expression within cells of neural fate as compared to those destined to form epidermis. Furthermore, our observations indicate that the manner of establishing segmental identities in the head is apparently different from that in the trunk. Future experiments directed at a comparison of modes of regulation between the homeotic genes of the head and those of the trunk should facilitate our understanding of the mechanisms involved in the establishment of segmental identity.

We would like to thank the many people who have contributed to this study. In particular, we thank Drs C. Goodman and M. Muskavitch for supplying us with the cells producing the *invected* antibody, Suman Olivelle and the Indiana University Molecular Institute Monoclonal Antibody Facility for supplying the *inv* ascites fluid, Dr D. Brower for the *Scr* monoclonal antibodies, Dr W. McGinnis for the *Dfd* cDNA used to produce the *Dfd* fusion protein. Finally, we would like to acknowledge the help of our lab colleagues, especially Drs M. A. Pultz and V. Merrill for supplying the *pb* antibodies, and Dr D. Cribbs for his comments on the manuscript.

This work was supported by NIH Grant PHS R01GM2499 to TCK, NIH Postdoctoral Fellowship F32 GM10036 to JWM, and NIH Predoctoral training grant T32 GM07447 to RJD.

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(Accepted 29 September 1988)