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, AniP and Ubx protein accumulation using engrafted 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 (engrafted). 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 horse-radish 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 engrafted (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
of the maxillary segment. The expression of 
Scr

con-al. 1987; and Carroll et al. The expression of the anterior boundary of extension. Laterally, a segmental pattern of accumulation has previously been described (Mahaffey & Kaufman, 1987; Riley 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 (3:40h, 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).

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 'pel' 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) Pel 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; T1, 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) and mandibular (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) Pel from 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. CI, clypeolabrum; PC, procephalon. (C) Higher magnification of an embryo pel 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) Pel 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) Pel 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) Pel of an embryo undergoing head involution. The labial segments are about to fuse and are now juxtaposed 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.

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

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
The use of the double-labelling system has allowed us to complete deletion of the gene. However, the gene is enigmatic in that, although the gene pb appears to be no effect on embryogenesis caused by a product accumulates in many embryonic cells, 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. 4. 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, overlapping 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.

Fig. 3. The lab pattern with respect to inv. (A and a’) The early pattern of lab (blue) staining in pels 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’) Pels 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, overlapping 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.

Head homeotic patterns

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 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).

proboscidopia

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 (Mx) 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.
to the 12th \textit{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 \textit{Dfd} protein begins anterior to the 13th \textit{inv} band and extends through the last, the 14th (Fig. 5B and 5b', again, note the absence of blue around the \textit{inv}-positive cells in the 13th band). Furthermore, double staining with \textit{Scr} and \textit{Dfd} indicates there is no overlap between cells expressing these two antigens (data not shown). The \textit{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 \textit{lab} and \textit{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 \textit{pb} protein in the CNS deviates from that of the other homeotics in that the \textit{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 \textit{fushi tarazu} and even \textit{skipped} than the homeotics (Doe et al. 1988a). The region with the largest number of \textit{pb}-positive cells lies between the 12th and the 13th \textit{inv} bands, the position of \textit{Scr} accumulation (Fig. 5d'). Double staining with the \textit{Scr} antibody indicates that only a few of the \textit{pb}-positive cells also accumulate the \textit{Scr} antigen. In fact, the \textit{pb}-stained cells appear to encircle the \textit{Scr}-positive cells. Furthermore, there is little coincidence between \textit{pb} and \textit{inv} staining. As one observes the more posterior portions of the CNS, the number of \textit{pb}-positive cells decreases and there is less (or no) coincidental expression with \textit{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 \textit{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 effects of mutations at the \textit{Dfd} and \textit{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 \textit{Anup} or \textit{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. \textit{Polycomb}, Denell, 1978; Wedeen et al. 1986; Riley et al. 1987; or \textit{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 \textit{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 \textit{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 \textit{Dfd} would coincide with a simultaneous expression of these gene products and evidence has recently been obtained that \textit{Dfd} expression is influenced by mutations in the pair-rule genes (Jack et al., 1988). Alternatively, the number and position of the \textit{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. Dor-sally, 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. 1988a,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.

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