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Distribution of *Ultrabithorax* proteins in *Drosophila*

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We have used a monoclonal antibody to examine the distribution of *Ultrabithorax* (*Ubx*) proteins in *Drosophila* embryos and imaginal discs by immunofluorescence. *Ubx* proteins are nuclear and show a spatially restricted distribution in the nervous system, epidermis and mesoderm. Labelling extends from the first thoracic segment (T1) to the eighth abdominal segment (A8) in the midline cells, from T2 to A8 in the ventral nervous system and epidermis and from A1 to A8 in the somatic mesoderm. In the nervous system and epidermis the patterns of labelling exhibit a repeat unit, the *Ubx* metamere, that is out of phase with the segmental repeat unit. At least in the epidermis this repeat unit appears to extend between anterior-posterior compartment boundaries and consists of a posterior compartment together with the succeeding anterior compartment. The most prominently labelled metamere in the nervous system and epidermis is that comprising the posterior region of T3 and the anterior region of A1. Within each metamere the nuclei are heterogeneously labelled. Clear heterogeneity of labelling is also seen amongst the nuclei of the T3 imaginal discs.

Key words: bithorax complex/*Drosophila*/homeotic genes

Introduction

Little is known of the molecular events that lie on the developmental pathways followed by cells as they divide, organize and differentiate during morphogenesis. Homeotic mutants in *Drosophila* have provided an entry point into these pathways. Homeotic mutations cause cells to follow a developmental pathway that is inappropriate for their position in the animal. For example, in the *bithorax* mutant, cells in the third thoracic segment that would normally give rise to anterior haltere structures instead produce the anterior part of the wing – a second thoracic segment structure. The effects of mutations in the homeotic gene cluster called the bithorax complex (BX-C) have been extensively studied by Lewis and this analysis has led to the formulation of a model for the role of products of the BX-C in directing the developmental pathways followed by cells in the thoracic and abdominal regions (Lewis, 1978). Lewis proposed that as one moves posteriorly from the third thoracic segment to the eighth abdominal segment a new gene is activated in each successive segment. Thus each segment would have a unique array of gene products. In the second thoracic segment (T2) all the genes would be inactive and in the eighth abdominal segment (A8) all the genes would be active. The particular array of gene products present in the cells of a given segment would determine the developmental pathway followed by those cells.

More recently it has become apparent that the genes of the BX-C act on units which are out of phase with the segments. The most anterior region of the animal affected by *Ubx* mutations is the posterior compartment of T2 (Morata and Kerridge, 1981). Animals which are Ubx^+ but which lack the more distal genes in the BX-C reiterate a pattern consisting of the posterior compartment of T3 plus the anterior compartment of A1 (Struhl, 1984). Models have been presented for the 'out of register' deployment of BX-C gene products (Hayes *et al.*, 1984; Struhl, 1984) and the units which extend between successive anterior-posterior (A/P) compartment boundaries have been named parasegments (Martinez-Arias and Lawrence, 1985).

The BX-C has recently been cloned (Bender *et al.*, 1983) thus opening the way to a molecular analysis of its role in development. Detailed molecular analysis has concentrated on the genes in the centromere proximal region of the BX-C (Beachy *et al.*, 1985; R. Saint, P. Beachy and D. Hogness, unpublished results). A transcription unit has been defined that spans ~70 kb. This *Ubx* domain gives rise to several transcripts most of which share common sequences in the 5′ exon. *In situ* hybridization analysis revealed that *Ubx* transcripts show a restricted spatial distribution which correlates well with that predicted by the Lewis model and further that the major band of *Ubx* transcript expression may be out of register with segments (Akam, 1983).

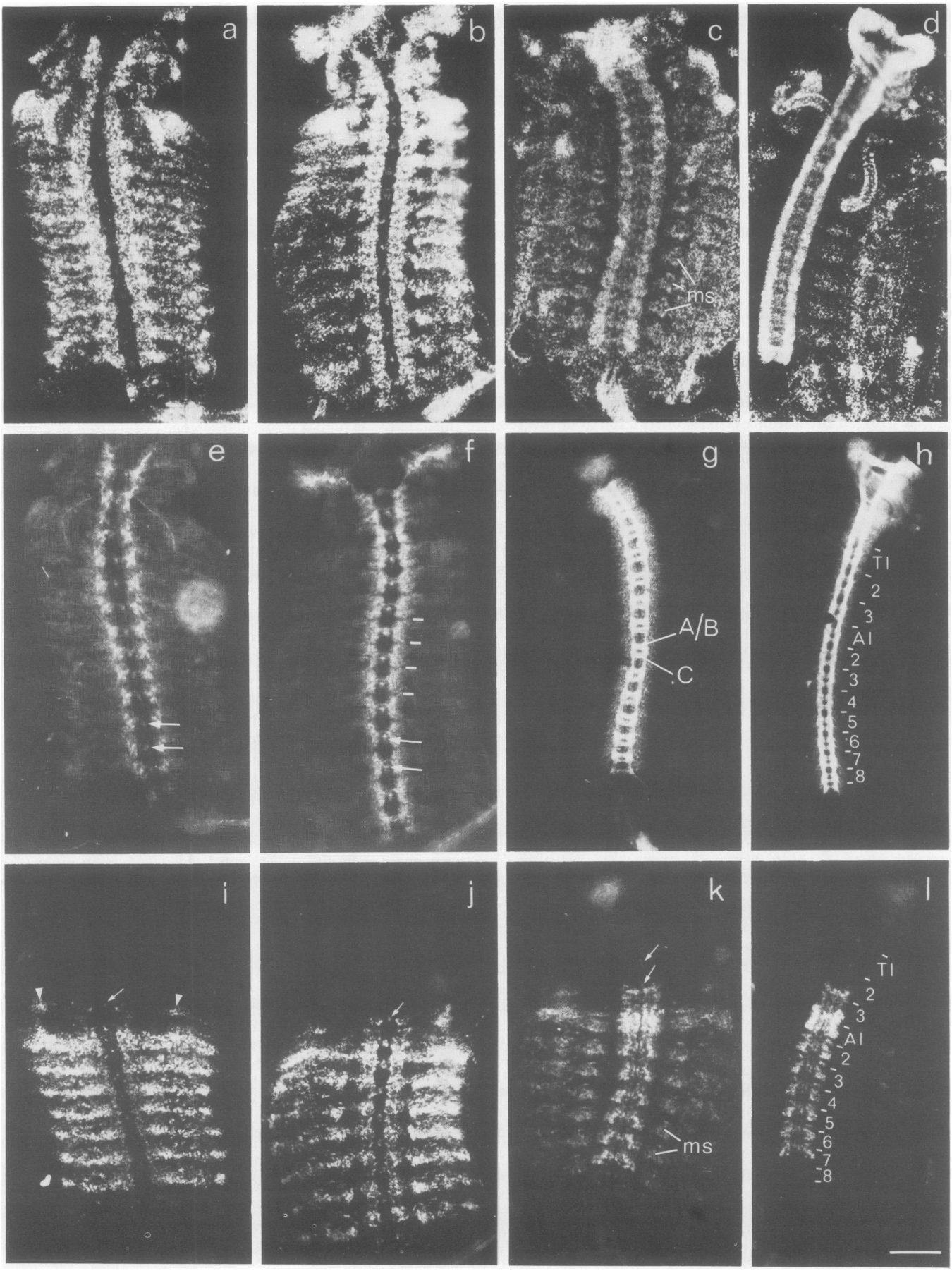
In order to study the role of products of the *Ubx* transcriptional domain we have made a monoclonal antibody which recognizes a protein sequence encoded in the *Ubx* 5′ exon (White and Wilcox 1984). This antibody detects at least three products on protein blots of third larval instar imaginal discs. Immunofluorescence analysis of embryonic and larval tissues reveals that the products recognized by the antibody are nuclear and show a restricted spatial distribution that correlates well with the transcript distribution. There is no labelling of embryos that are homozygous for a deletion covering the *Ubx* region. Similar distributions of *Ubx* proteins in embryos and imaginal discs have been reported by others (Beachy *et al.*, 1985). Here we present a more detailed analysis of the distribution of *Ubx* proteins in embryonic and larval tissues.

Results

Whole mounts of *Drosophila* embryos (Thomas *et al.*, 1984) provide particularly useful preparations for immunofluorescence analysis. Figure 1 shows four whole mount preparations covering the period from ~10 h to ~15 h of development [stages 11–13 (Bownes, 1975)]. The monoclonal antibody, FP.3.38, which recognizes the *Ubx* products, labels the ventral nervous system, the epidermis and the mesoderm. In all these tissues the labelling is confined to the cell nuclei and is spatially restricted in the anterior-posterior body axis. The tissue distribution of the *Ubx* products changes dramatically over this period. At 10 h the labelling extends fairly uniformly across the embryo although the mesoderm appears to be the most strongly labelled tissue. At later stages the ventral nervous system clearly predominates.

Landmarks in the ventral nervous system – the Ubx metamere

In the ventral nervous system *Ubx* protein expression is seen as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a pattern of blocks of labelling; this is most evident in Figure 1l. The boundaries of these blocks define a repeat unit that we shall call the *Ubx* metamere.

It has been reported that the boundaries of the *Ubx* metamere do not appear to coincide with the segment boundaries in the ventral nervous system. This observation is based on the comparison between the boundaries of blocks of *Ubx* transcript or protein expression with the boundaries of the segmental neuromere (Akam, 1983; White and Wilcox, 1984; Beachy *et al.*, 1985). The limits of the segmental neuromere have been defined with respect to the pattern of the commissures (Thomas *et al.*, 1984). The commissures are revealed by labelling with anti-horseradish peroxidase (HRP) antiserum (Jan and Jan, 1982). In *Drosophila* the commissures are called A/B and C by comparison with the pattern in the grasshopper and the segmental boundary has been suggested to lie about midway between the commissure C and the succeeding commissure A/B (Thomas *et al.*, 1984). Superimposition of the commissure pattern onto the *Ubx* protein pattern reveals that the boundaries of the *Ubx* metamere lie at the anterior edge of commissure C. This is seen, for example, at the anterior boundary of the labelling in T2, at the start of the strong block of labelling in T3 and at the posterior boundary of the labelling in A7. In Figure 1h and l the segmental 'reference frame' is indicated, based on the commissure pattern, and the *Ubx* labelling pattern is seen to be out of register with the segments.

How does the *Ubx* metamere correlate with other landmarks in the ventral nervous system? The clusters of cells that lie in the ventral midline between the two columns of the ventral nervous system provide a clear set of landmarks. We will call these cells the midline cells [they might be analogous to the midline precursor cells in the grasshopper nervous system (Bate and Grunewald, 1981)]. In Figure 1b and f they can be seen as a line of 14 clusters of cells. The strongest block of *Ubx* product expression appears to be precisely flanked by clusters of midline cells (Figure 1j). Also, in Figure 1,i,j and k, a cluster of midline cells lies on the anterior boundary of *Ubx* product expression in the ventral nervous system. This arrangement may be contrasted with the relationship between the midline cell clusters and the blocks of labelling in the anti-HRP pattern that are seen in Figure 1e and f. The anti-HRP pattern appears to reflect the segmental neuromere pattern and the midline cells lie close to the centres of these blocks of labelling.

In summary, in the ventral nervous system, we appear to have two repeat units, the neuromere and the *Ubx* metamere. Both have the same repeat lengths, i.e., they maintain a constant relationship along the animal, but they are clearly out of phase.

Landmarks in the epidermis

Ubx protein expression in the epidermis also exhibits a metameric pattern (Figures 2b and 7). The strongest block of labelling is again precisely flanked by the midline cells (Figures 1i and 2d). Thus the *Ubx* metamere in the epidermis coincides with that in the ventral nervous system.

Can we use landmarks in the epidermis to support the contention that the *Ubx* metamere is out of phase with segments? Figure 2e and f shows part of a whole mount preparation of an extended germ band stage embryo (<9 h) and represents the earliest stage in which we have thus far seen *Ubx* protein expression. The tracheal pits are indicated and lie in the middle of the band of *Ubx* protein labelling. The tracheal pits are believed to form at the segment boundaries (Keilin, 1944; Martinez-Arias and Lawrence, 1985). Thus the *Ubx* metamere in the epidermis also appears to be out of phase with the segmental unit. This conclusion is strongly supported by the pattern of *Ubx* protein labelling in sections of stage 12 and 13 embryos. In Figure 4f, the major band of *Ubx* protein expression is out of phase with the segmental folding pattern in the epidermis.

Do the boundaries of *Ubx* metameres coincide with A/P compartment boundaries? To approach this question we have made use of a monoclonal antibody DOV 4 which enables us to identify the Keilin's organs and other sensory structures in the thoracic segments of embryos of stage 13 and older (Figure 3). There is evidence that the A/P compartment boundary runs through the Keilin's organs (Struhl, 1984). (It is not clear whether the Keilin's organs and the other sensory structures lie on one plane across the anterior-posterior axis. In several preparations there appears to be a kink in the line of structures laterally. However, in the following discussion we will treat the line of sensory structures as a marker for the A/P compartment boundary.) The only unambiguous boundary in the *Ubx* protein pattern lies in A1 at the posterior limit of the major band of labelling. Unfortunately we cannot correlate this boundary directly with Keilin's organs as these organs are not found in A1. A more indirect correlation does however suggest that this discontinuity in the *Ubx* protein pattern in A1 lies close to the boundary of the metameric unit that is defined by the sensory organs. In Figure 2a and b, superimposition of the Hoechst pattern and the *Ubx* protein pattern reveals that the discontinuity in the *Ubx* pattern in A1 lies close to the middle of the mesodermal block. By comparison of the Hoechst pattern and the DOV 4 pattern it is clear that the line of sensory structures also lies close to the centre of the mesodermal block (Figure 2a and c). Thus the *Ubx* protein discontinuity in A1 lies in a position equivalent to that marked by the line of sensory

Fig. 1. A series of embryo whole mounts spanning stages 11 – 13 (Bownes, 1975) ~10 – 15 h after egg laying. The embryos are of increasing maturity from left to right. Anterior is at the top and ventral is central. Each preparation is labelled with Hoechst dye, anti-HRP and the monoclonal antibody FP.3.38 (top to bottom). **a – d:** Hoechst labelling. This reveals the total pattern of nuclei; **a:** the ventral nervous system (vns) consists of two columns of cells on either side of the ventral midline. The epidermis and mesoderm extend out laterally. **b:** clusters of midline cells can be seen between the two columns of the vns. **c:** the vns has fused. Blocks of mesoderm (ms) nuclei flank the vns. **e – h:** labelling with fluorescein-coupled goat anti-HRP. In **g** and **h** this reveals the commissure pattern. Each segmental neuromere contains an anterior (A/B) and a posterior (C) commissure. The segmental plan is indicated in **h**. In **e** and **f** the midline cells are visible (arrows). Fourteen groups of midline cells can be counted in **f**. In **e** and **f** the labelling in the vns is in a series of blocks (three of these blocks are indicated in **f**). Notice that the midline cells lie close to the centre of the blocks of labelling in the flanking columns of the vns. **i – l:** *Ubx* protein distribution. The whole mounts were labelled with the monoclonal antibody FP.3.38 followed by Texas Red-conjugated sheep anti-mouse immunoglobulin. **i:** the midline cells are visible mid-ventrally. A midline cell in T2 is arrowed. Laterally, just posterior to this level, brightly labelled clusters of nuclei surround the tracheal pits (arrow heads). Nuclei in the region extending from the posterior part of T3 to the anterior part of A7 are well labelled all across the embryo. Some labelling extends into A8. **j:** the midline cell cluster in T2 is arrowed. This marks the anterior boundary of labelled nuclei in the columns of the vns. The midline cells in T3 and Al flank the brighter band of labelling in the vns. **k:** the midline cell clusters in T1 and T2 are arrowed. Greater heterogeneity of labelling is now seen both within the vns and between tissues. The mesodermal blocks in A5 and 6 are indicated (ms). Notice there is no labelling of the blocks of mesodermal nuclei in T3. **l:** the heterogeneity of labelling within the vns is clearly seen. The pattern consists of a series of blocks of labelling the repeat unit of which is out of phase with the segmental repeat unit. The segmental assignment was derived by the superimposition of **h** onto **l**. The most intensely labelled block extends from posterior T3 into anterior A1. Bar equals 50μm.

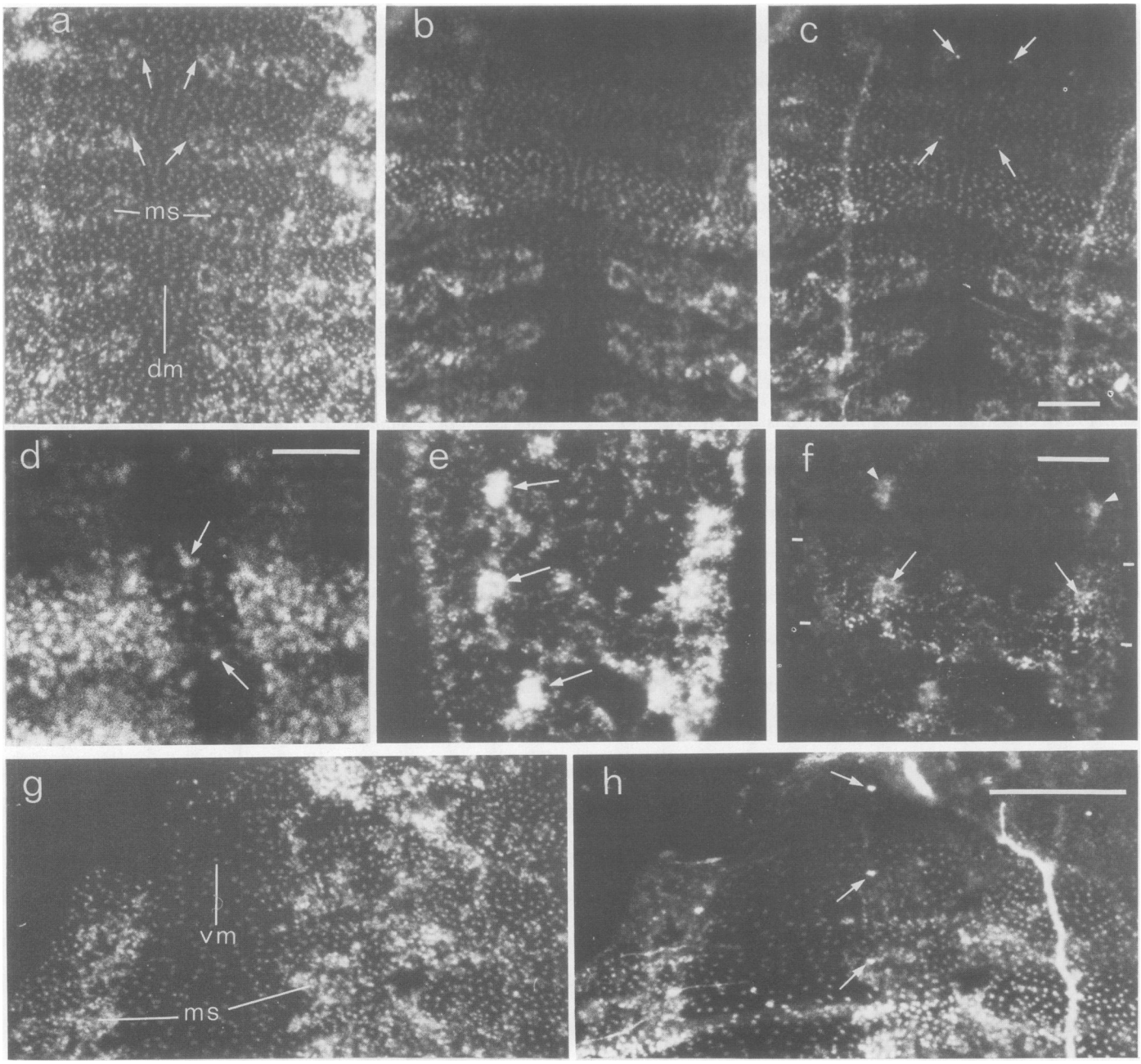

Fig. 2. *Ubx* protein distribution in the epidermis. **a–c:** registration of *Ubx* protein pattern with dorsal sensory organs. **a, b** and **c** are the same whole mount preparation of a stage 13 embryo. **a:** Hoechst labelling. On top of the background of epidermal nuclei, fingerlike bands of mesodermal nuclei extend towards the dorsal midline (indicated as ms in A1). Anterior is at the top of the figure. The arrows indicate the positions of dorsal sensory structures in T2 and T3 derived from superimposition of the DOV 4 labelling pattern (Figure 2c and see Figure 3) on the Hoechst pattern. **b:** FP.3.38 labelling showing the pattern of *Ubx* protein distribution in the epidermis. The strongest region of labelling is in A1. The mesoderm nuclei are weakly labelled in A1 and more strongly labelled posteriorly. **c:** double labelling with FP.3.38 and DOV 4. The arrows indicate the dorsal sensory organs in T2 and T3. Bar equals 25 μm. **d:** higher magnification of a part of Figure 1i. FP.3.38 labelling reveals that the midline cells (arrowed) of T3 and A1 flank the strong band of *Ubx* product expression in the epidermis (the sparse nuclei visible close to the ventral midline) and in the vns (the more heavily labelled densely packed nuclei situated more laterally). Anterior is top. Bar equals 20 μm. **e** and **f**: registration of *Ubx* protein pattern with tracheal pits. **e:** whole mount of germ band extended embryo (<9 h) labelled with Hoechst. Three tracheal pits are arrowed on the left side. Anterior is top. **f:** same preparation as in **e** labelled with FP.3.38. The arrowed tracheal pits (presumably marking the segment boundary between T3 and A1) lie in the middle of the major band of *Ubx* protein expression. The boundaries of this band are indicated laterally. The preceding pair of tracheal pits are also well labelled (arrow heads). Bar equals 20 μm. **g** and **h:** registration of epidermal *Ubx* protein expression with Keilin's organs. **g:** whole mount of stage 13 embryo. Ventral view. The ventral nervous system has been removed. Anterior is top. Hoechst labelling. Close to the ventral midline (vm) all the nuclei are epidermal whereas, more laterally, the overlying mesodermal nuclei (ms) are also seen. **h:** double labelling of same preparation with FP.3.38 and DOV 4. Keilin's organs of T1, T2 and T3 are arrowed on the right side. The *Ubx* protein distribution extends anterior to the Keilin's organs in T2. Bar equals 50 μm.

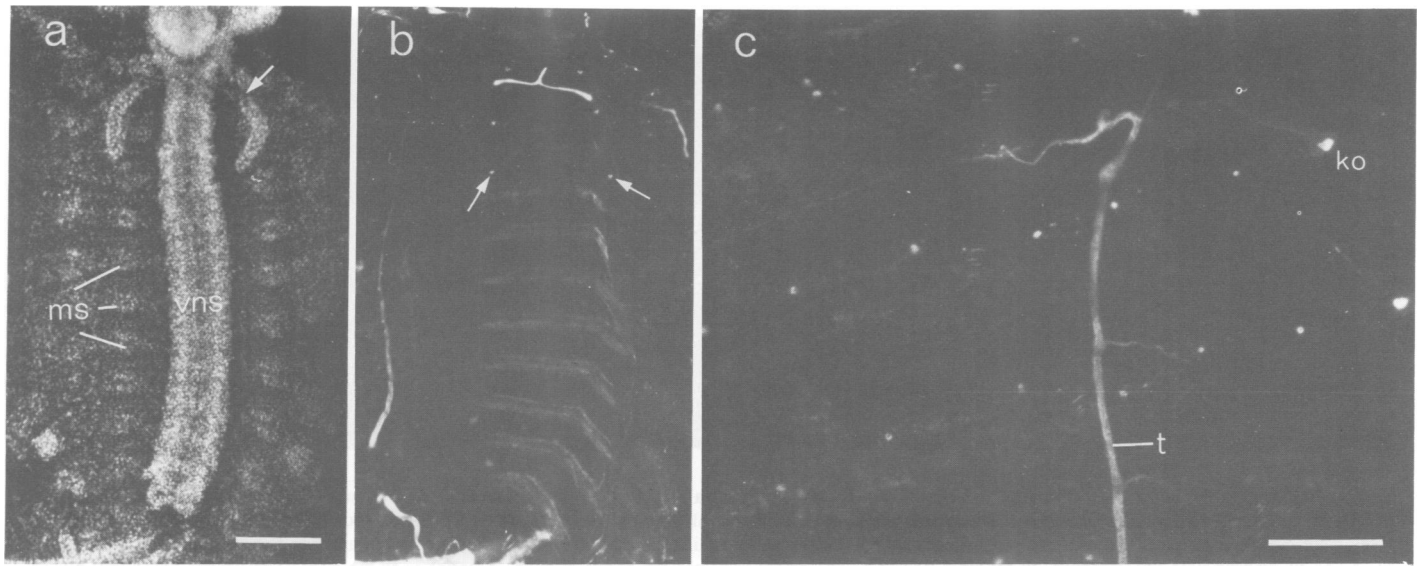

Fig. 3. The monoclonal antibody DOV 4 provides landmarks in the late embryo. **(a)** Stage 13 embryo labelled with Hoechst. A salivary gland is arrowed. Mesodermal blocks (ms) flank the ventral nervous system (vns). Bar equals 50 μm. **(b)** Same embryo labelled with DOV 4 followed by Texas Red-conjugated sheep anti-mouse immunoglobulin. The Keilin's organs are seen in the three thoracic segments. Arrows indicate the Keilin's organs in T3. The ducts of the salivary glands are seen between the Keilin's organs of T1 and T2. **(c)** A higher magnification view of DOV 4 labelling showing the lines of sensory organs in T1, T2 and T3. A Keilin's organ in T2 is indicated (ko). The tracheal trunk is also labelled (t). Bar equals 20 μm.

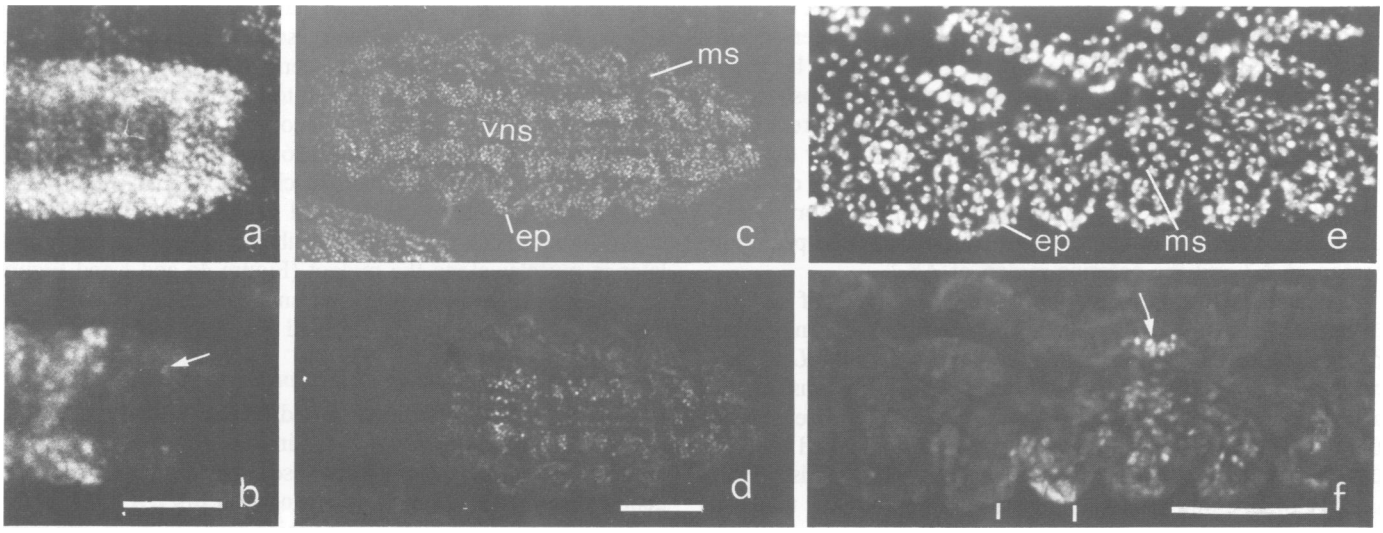

Fig. 4. Heterogeneity in the pattern of *Ubx* protein expression. **a** and **b:** higher magnification view of posterior end of vns of Figure 1d and l. **a:** Hoechst labelling. **b:** FP.3.38 labelling showing the predominant expression of *Ubx* products in two nuclei in A8 in this plane of focus. One of the nuclei is arrowed. Bar equals 20 μm. **c** and **d:** longitudinal section of a stage 13 embryo. Anterior is to the left. **c:** Hoechst labelling. The section passes through the vns. ms: mesoderm; ep: epidermis. **d:** FP.3.38 labelling revealing heterogeneity of the labelled nuclei. The major area of labelling corresponds to the posterior region of T3 and the anterior region of A1. Bar equals 50 μm. **e** and **f**: section through stage 13 embryo. Anterior is to the left and ventral is to the bottom of the figure. **e:** Hoechst labelling. **f:** FP.3.38 labelling. The boundaries of *Ubx* protein expression in the brightest *Ubx* metamere in the epidermis are indicated. Clearly this unit is out of phase with the segmental grooves. The boundaries of the *Ubx* metamere divide the segment into a larger anterior region and a smaller posterior region. The arrow indicates the labelling in the visceral mesoderm. Bar equals 50 μm.

structures in the thoracic segments. Although we would prefer a more direct correlation, this does suggest that the *Ubx* metamere may indeed extend between A/P compartment boundaries in the epidermis.

Patterns of expression of Ubx products

Having aligned the patterns of *Ubx* protein expression with landmarks we can now discuss the distribution of *Ubx* products in more detail. A schematic outline of these distributions is presented in Figure 7.

The midline cells

Of the 14 clusters of midline cells 10, those of T1 to A7, are clearly labelled. There is evidence too of weak labelling in the A8 cluster. The clusters are not equally labelled: the most strongly labelled are T3 and A1 and the labelling tails off both anteriorly and posteriorly (Figure 1).

The ventral nervous system

The labelling is clearly in blocks; the most prominent of these is the *Ubx* metamere containing the posterior region of T3 and

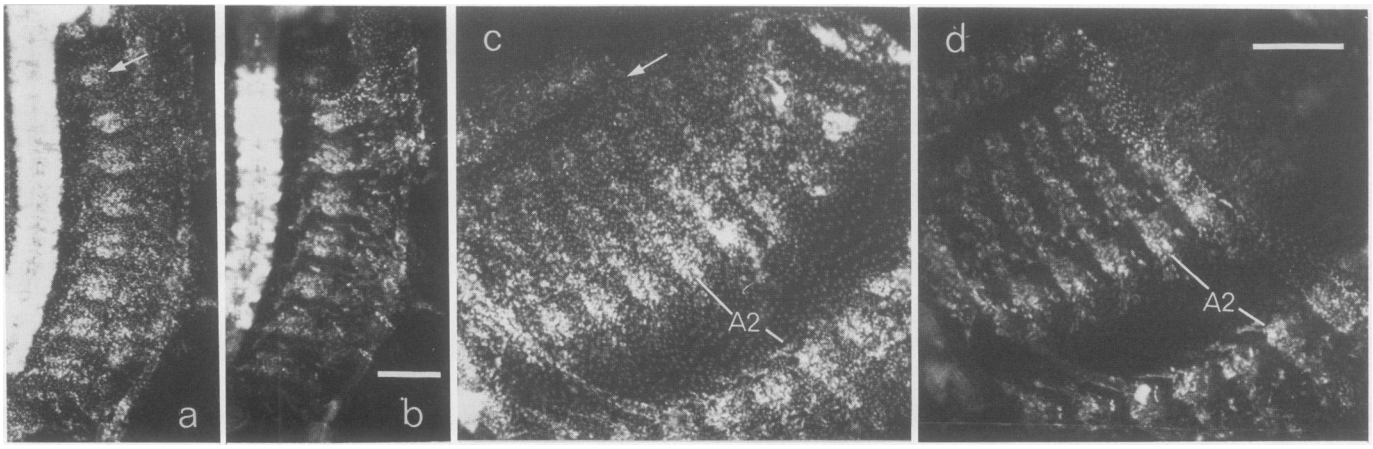

Fig. 5. *Ubx* protein distribution in the somatic mesoderm of stage 13 embryos. **a** and **b:** view of right-hand side of embryo. Anterior is top. The vns lies to the left. **a:** Hoechst labelling. The blocks of mesodermal nuclei are visible lateral to the vns. The block in T3 is arrowed. **b:** FP.3.38 labelling. The *Ubx* proteins are clearly expressed in the mesodermal blocks of A1-A7. There is a tail off in the intensity of labelling from anterior to posterior. Bar equals 50 μm. **c** and **d:** embryo with vns removed. **c:** Hoechst labelling. The arrow indicates the dorsal midline. Anterior is to the right. The ventral mesoderm blocks in A2 are indicated. **d:** FP.3.38 labelling. The mesoderm is clearly labelled in A1 and posteriorly. There is no labelling in T3. Notice the kink in the pattern of mesodermal nuclei as one proceeds from ventral to dorsal. Bar equals 50 μm.

the anterior region of A1 (Figure 1). Anteriorly the pattern starts abruptly and the posterior region of T2 is moderately well labelled. This is followed by weak labelling in the anterior region of T3. This pattern suggests that the *Ubx* metamere can be divided into two units. These subunits may correspond to anterior and posterior lineage compartments although these have not been defined by cell lineage studies in the nervous system. Posteriorly this subdivision is less evident although the posterior region of each metamere does seem to be labelled more strongly than the anterior. In general the labelling decreases gradually down to the anterior region of A7 and then drops off more abruptly in the last labelled metamere which corresponds to the posterior region of A7 and the anterior region of A8.

The nuclei within each anterior or posterior subunit of the *Ubx* metamere are not equally labelled. This heterogeneity can be seen by comparing the Hoechst patterns with those of the *Ubx* product expression (Figure 1). It is particularly obvious in the anterior A8 where the heterogeneity becomes more extreme with time and by 15 h only four nuclei are clearly labelled (two in the plane of focus of Figure 1l and Figure 4a and b). This heterogeneity of labelling within metameres does not appear to be an artifact of the whole mount preparation as it is also visible in sections (Figure 4c and d).

The epidermis

In the epidermis the strongly labelled *Ubx* metamere appears to extend from the A/P compartment boundary in T3 to the A/P compartment boundary in A1. The epidermis pattern differs from that in the ventral nervous system in that the labelling drops off more rapidly posteriorly, although there is labelling over roughly the same range. The labelling also extends anteriorly beyond the Keilin's organs in T2, i.e., into anterior T2 (Figures 2g and h).

Within *Ubx* metameres in the epidermis there appears to be a gradient of *Ubx* protein expression from posterior to anterior. This can be seen in Figure 2c but is more obvious in the section shown in Figure 4f.

The mesoderm

In the analysis of the distribution of *Ubx* transcripts (Akam, 1983) it was noted that the pattern in the mesoderm is rather different to that in the epidermis. This is also true of the *Ubx* protein products. In Figure 2a and b we can see the labelling of the mesoderm dorsally. There is no labelling detectable in T3, weak labelling in A1 and stronger labelling in A2 followed by a slow decline more posteriorly. A similar pattern is seen ventrally (Figure 5) although it is apparent that here there is a good signal in A1. The organization of the mesoderm is complicated. The mesodermal blocks appear to be centred on the *Ubx* metamere boundaries dorsally and thus seem to be segmentally arranged. There is however a displacement of the blocks of mesoderm nuclei as one moves from dorsal to ventral (Figure 5c and d) as has been noted for the larval muscle pattern (Crossley, 1978).

Ubx products are also expressed in the visceral mesoderm. There is one block of labelling visible in embryo sections and later in muscles along the midgut (Figure 4e and f and Figure 6). In Stage 13 embryo whole mounts there is also labelling in muscles along a region of the hind gut (Figure 6).

Heterogeneous expression of Ubx proteins in imaginal discs

In a previous publication (White and Wilcox, 1984) we analysed the distribution of *Ubx* products in imaginal discs. We found no labelling of the eye-antennal disc, the first leg disc or the genital disc. There was weak labelling over discrete areas of the second leg and wing discs. In the third thoracic discs, the third leg and haltere discs, the nuclei showed strong labelling and most if not all cells of the disc epithelium appeared to be labelled. Here we examine the patterns of labelling of the third thoracic discs in more detail. Although in whole mounts of third leg discs all nuclei appear to be labelled, the labelling does seem to be more intense over the posterior region of the disc (Figure 8a). In sections the heterogeneity of the labelling is obvious (Figure 8). The area of brightly labelled nuclei shows a clear correlation with the posterior lineage compartment (Steiner, 1976). There are, however, labelled nuclei in both compartments. There is also heterogeneity in the labelling in the proximo-distal axis. Comparison with the published fate map for the first leg disc (Schubiger, 1968) suggests that, at least in the posterior compartment, the presumptive tibia and first tarsal segment are the most intensely labelled. The labelling is low in the coxa. Examination of whole mounts of discs evaginated *in vitro* (data not shown) supports the evidence from sections that the distal tip shows little if any labelling.

The labelling is also heterogeneous in the haltere disc (Figure

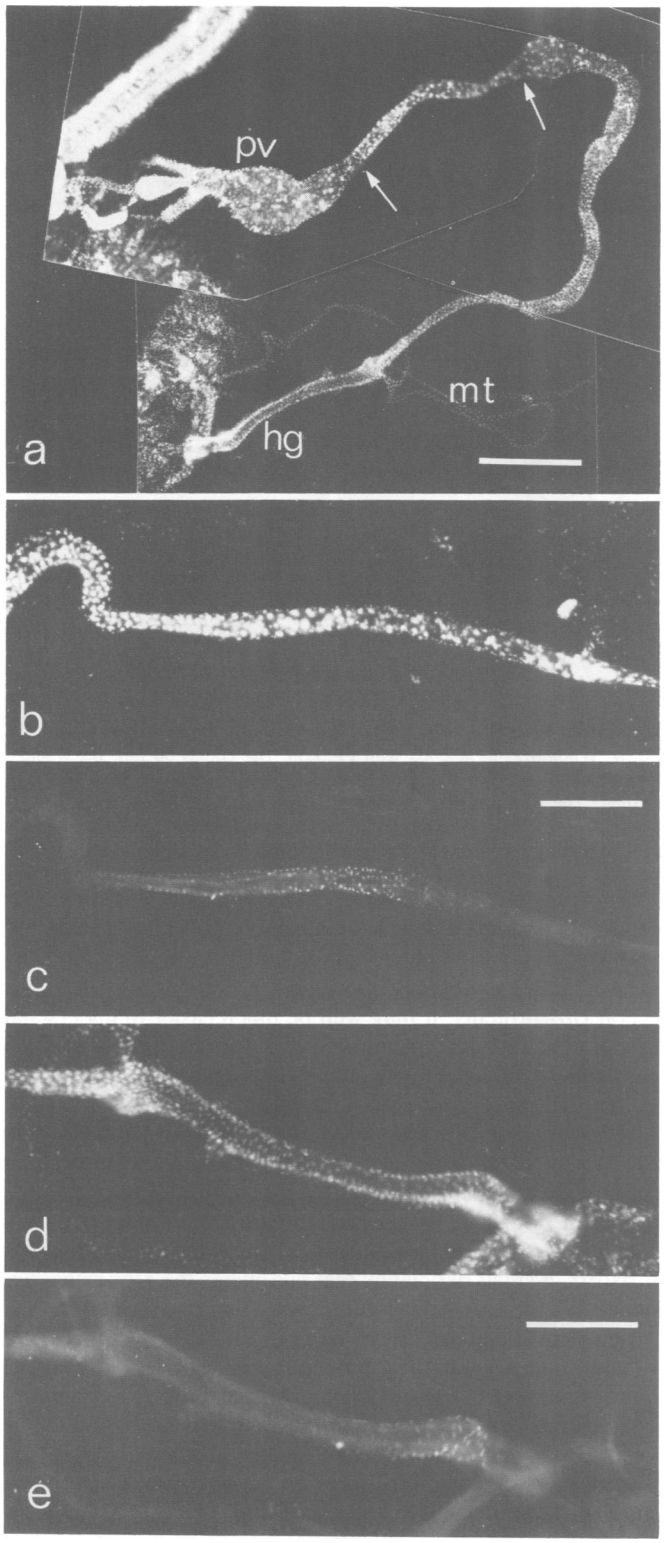

Fig. 6. *Ubx* protein expression in the visceral mesoderm. **a:** Hoechst labelling of late embryonic gut. pv: proventriculus; mt: malpighian tubules; hg: hind gut. The arrows indicate the extent of the labelled area in preparations labelled with FP.3.38 (see **c** below). Bar equals 100 μm. **b** and **c**: *Ubx* proteins in the midgut. **b:** Hoechst labelling. The predominant nuclei are those of the endodermal gut cells. **c:** FP.3.38 labelling. The *Ubx* proteins are expressed over a discrete region of the visceral mesoderm but not in the endoderm nuclei. Bar equals 50 μm. **d** and **e**: *Ubx* proteins in the hind gut. **d:** Hoechst labelling. **e:** FP.3.38 labelling. Peripherally located nuclei are labelled over a short region of the hind gut. These are probably the nuclei of muscles surrounding the hind gut. Bar equals 50 μm.

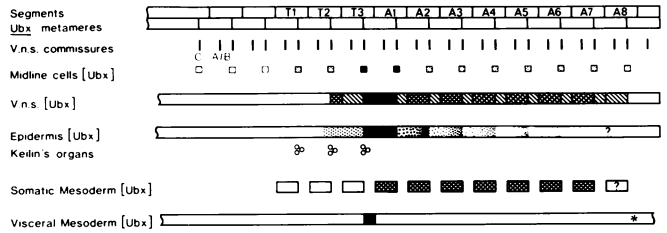

Fig. 7. Schematic representation of the pattern of *Ubx* protein expression in stage 11 – 13 embryos. The level of *Ubx* expression ([Ubx]) is indicated by shading. The *Ubx* metameres appear to correspond to parasegments (Martinez-Arias and Lawrence, 1985). The commissure pattern in the ventral nervous system (vns) can also be interpreted as a set of 14 units with each unit containing an anterior C and a posterior A/B commissure. There are fourteen clusters of midline cells which appear to lie at the anterior boundary of each parasegment (it should be borne in mind that we do not know precisely where these cells originate). In the epidermis the heterogeneity of the *Ubx* expression within metameres is indicated; there is also heterogeneity in the major block of labelling but this has been shaded black to indicate its coincidence with the major block of labelling in the ventral nervous system. *Ubx* expression in the epidermis extends anterior to the Keilin's organs in T2 but its anterior limit has not been defined. The posterior limit of *Ubx* expression in the epidermis is also unclear. In the somatic mesoderm it is not clear whether A8 expresses *Ubx* proteins. The position of the labelled block in the visceral mesoderm is arbitrary. *: Labelling in the hind gut see Figure 6.

9). The most prominent labelling is found over the presumptive distal area of the disc. The disc is more strongly labelled posteriorly than anteriorly and the sharp discontinuity indicated in Figure 9e may correlate with the A/P compartment boundary. However, the A/P compartment boundary in the haltere disc has not been precisely located.

Discussion

The results presented in this paper concern four major features of the distribution of *Ubx* protein products; (i) the tissue distribution; (ii) the extent of the distribution in the longitudinal body axis in different tissues; (iii) the identification of a repeat unit in the *Ubx* distribution – the *Ubx* metamere – and (iv) the identification of heterogeneity of *Ubx* product expression both between and within *Ubx* metameres.

The tissue distribution of *Ubx* protein products agrees well with that of *Ubx* transcripts detected by *in situ* hybridization with a *Ubx* 5′ exon probe (Akam, 1983). Transcripts were seen in the ventral nervous system, the mesoderm and the epidermis. The extents and patterns of labelling were similar to those reported here for the *Ubx* proteins. The presence of spatially restricted *Ubx* protein expression in these different tissues clearly suggests that the *Ubx* proteins play a role in these diverse developmental pathways. Gynandromorphs and clonal analysis have established a role for *Ubx* function in the epidermis (Lewis, 1964) and BX-C functions appear to be required in the mesoderm (Lawrence and Johnston, 1984). Although BX-C mutations have effects in the nervous system (Palka *et al.*, 1979; Green, 1981; Jimenez and Campos-Ortega, 1981; Teugels and Ghysen, 1983,1985; Thomas and Wyman, 1984), a cell autonomous function for the BX-C here has not yet been established.

How does the pattern of expression of *Ubx* proteins relate to the requirement of *Ubx* function as deduced from the analysis of mutants? Clonal analysis and gynandromorphs have revealed that *Ubx* function is required from the A/P compartment boundary in T2 to the A/P compartment boundary in A1 for the development of adult cuticular structures (Lewis, 1963; Morata and Kerridge, 1981; Miñana and Garcia-Bellido, 1982) and this region has been dubbed the *Ubx* anatomical domain (Sanchez-

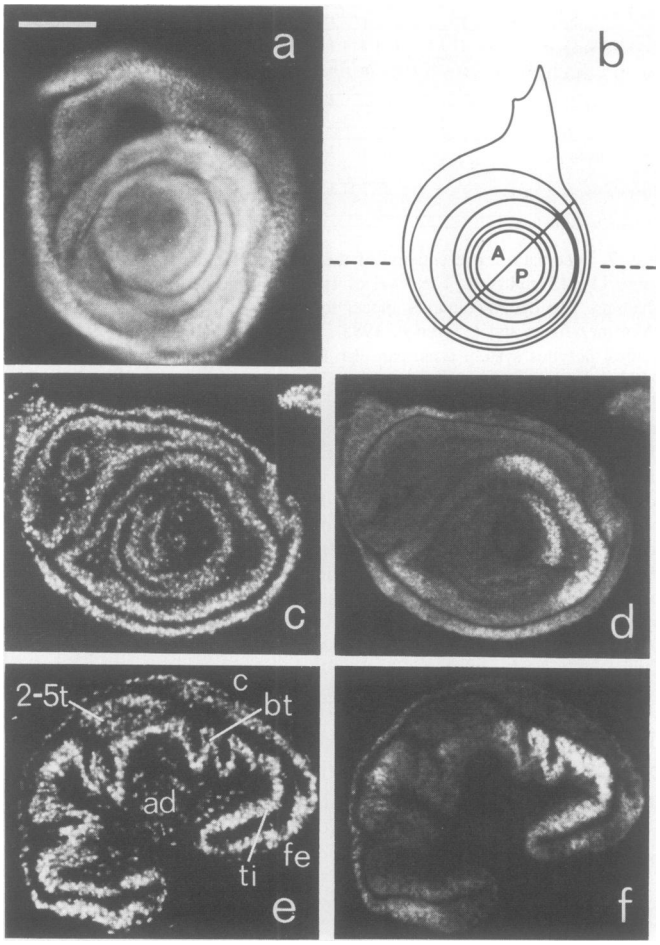

Fig. 8. Heterogeneity in *Ubx* protein distribution in the third leg imaginal disc. **a:** whole mount labelled with FP.3.38. Most if not all nuclei express *Ubx* protein but the labelling is stronger over the right-hand side (posterior) part of the disc. Bar equals 50 μm. **b:** diagram of first leg disc showing the position of the A/P compartment boundary (from Steiner, 1976). The dashed line shows approximate line of section of **e** and **f**. **c** and **d:** longitudinal section. **c:** Hoechst labelling. **d:** FP.3.38 labelling showing heterogeneity in *Ubx* protein expression over the disc. The prominent block of labelling is restricted to the posterior compartment. **e** and **f**: transverse section. **e:** Hoechst labelling. ad: adepithelial cells; 2−5t: second to fifth tarsal segments; bt: basitarsus; ti: tibia; fe: femur; c: coxa. **f**: FP.3.38 labelling revealing heterogeneity of *Ubx* expression over the disc epithelium. The adepithelial cells are unlabelled.

Herrero *et al.*, 1985). Observations on larval phenotypes of mutants affecting *Ubx* function (Hayes *et al.*, 1984; Struhl, 1984) also support a predominant role for *Ubx* function in this domain. However, a more posterior effect of *Ubx* has been noted in a comparison between embryos deficient for the entire BX-C (Df P9) and those deficient for *Ubx* function (Df bxd^{100}) (Lewis, 1981). The *Ubx* protein distribution in the epidermis is consistent with the functional requirements although it is clear that one would not have predicted 'the *Ubx* anatomical domain' from the protein pattern. The labelling extends many segments posteriorly from A1 and also, anteriorly, the labelling extends beyond the A/P compartment boundary in T2. It is interesting nevertheless that the area that can be designated as requiring *Ubx* function from the observation of cuticular phenotype should be bounded by A/P compartment boundaries rather than segmental boundaries. This clearly correlates with the boundaries of the *Ubx* metamere. As is emphasized in Figure 7, different tissues show different patterns of labelling and it should be noted that in the midline cells the labelling extends to T1.

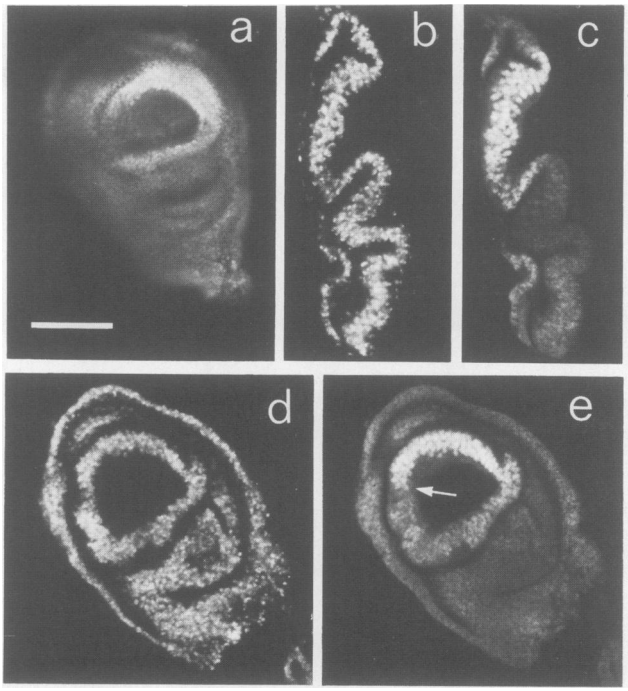

Fig. 9. Heterogeneity in *Ubx* protein distribution in the haltere imaginal disc. **a**: Whole mount labelled with FP.3.38. Bar equals 50 μm. **b,c,d** and **e**: longitudinal sections. **b** and **d**: Hoechst labelling. **c** and **e:** FP.3.38 labelling. The *Ubx* proteins are most strongly expressed in the presumptive distal part of the disc. The discontinuity arrowed in **e** may correspond to the A/P compartment boundary with posterior being on the upper right.

The monoclonal antibody FP.3.38 recognizes at least three *Ubx* products in protein blots from imaginal discs (White and Wilcox, 1984) and embryos (unpublished results). Thus the immunofluorescence analysis probably represents a composite picture for several *Ubx* proteins. It may well be much easier to understand the different labelling patterns in the various tissues and the heterogeneity of labelling within tissues when we can analyse the distribution of individual products.

Although most *Ubx* transcripts appear to show homology to the 5′ exon within which the antigenic determinant recognized by FP.3.38 is encoded it is possible that not all *Ubx* protein products are recognized. It is unlikely that FP.3.38 recognizes any protein other than *Ubx* products as in embryos homozygous for Df bxd^{100}, a small deficiency that eliminates the *Ubx* transcriptional unit, there is no labelling (White and Wilcox, 1984).

The repeat unit in the *Ubx* protein pattern − the *Ubx* metamere − is out of phase with the segmental repeat unit both in the nervous system and in the epidermis. In the epidermis it appears to be delimited by A/P compartment boundaries. Thus the *Ubx* metameres correspond to parasegments (Martinez-Arias and Lawrence, 1985). The identification of 14 clusters of midline cells that lie at the anterior boundaries of *Ubx* metameres supports the idea that the ventral nervous system is composed of 14 parasegments (Martinez-Arias and Lawrence, 1985).

This study revealed a surprising amount of heterogeneity in the levels of *Ubx* protein expression in nuclei within individual *Ubx* metameres in the embryonic nervous system and epidermis. The labelling of nuclei within compartments in the third leg and haltere imaginal discs is also markedly heterogeneous. These patterns of heterogeneity are very reproducible and suggest that the function of *Ubx* proteins may extend further down the pathway of developmental decisions than merely to specify which segmental developmental pathway a cell should follow.

Materials and methods

Flies

Drosophila melanogaster (Barton wild-type strain), raised at 25°C, were used throughout.

Immunofluorescence

Labelling of embryo whole mounts with the FP.3.38 monoclonal antibody, goat anti-horseradish peroxidase and Hoechst dye has been described previously (White and Wilcox, 1984). The monoclonal antibody DOV 4 has also been reported previously (White *et al.*, 1984). For double labelling with FP.3.38 and DOV 4 the specimens were first labelled with FP.3.38 and photographed and then were labelled with DOV 4. A dilution of 1:1000 of ascites fluid was used for DOV 4. The second antibody used in each labelling was Texas Red conjugated sheep anti-mouse immunoglobulin (Amersham).

For sections of embryos and imaginal discs a polyacrylamide embedding procedure was used (Hausen and Dreyer, 1981). Embryos were dechorionated and fixed for 5 min at room temperature in a modification of the Zalokar fixation (Zalokar and Erk, 1976). Equal parts of heptane and 3.5% paraformaldehyde phosphate-buffered saline (PBS) were mixed and the upper phase used as fixative. The vitelline membrane was removed manually. The embryos were embedded in polyacrylamide and sectioned. The sections were allowed to attach to poly-L-lysine-coated slides (1 mg/ml; Sigma) and then fixed for 15 min at 4°C in 3.5% paraformaldehyde/PBS. They were then permeabilized and labelled in the same way as the embryo whole mounts. Imaginal discs were treated similarly except that the initial fixation was for 15 min in 3.5% paraformaldehyde/PBS at 4°C.

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Note added in proof

For a detailed analysis of the distribution of *Ubx* transcripts in *Drosophila* embryos see Akam,M.E. and Martinez-Arias,A. (1985) *EMBO J.*, **4**, 1689-1700.