Ultrabithorax gene expression in Drosophila imaginal discs and larval nervous system

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Summary

Using a monoclonal antibody and image-processing procedures, the patterns of expression of the Ultrabithorax (Ubx) gene product have been characterized in Drosophila larvae. As reported previously, the metathoracic imaginal discs stain most intensely with anti-Ubx, with some mesothoracic and no prothoracic expression detectable. In the metathoracic discs, the greatest modulation in anti-Ubx staining is along the proximodistal axis. Ubx is generally expressed at higher levels in the posterior regions of metathoracic discs, although relatively high anterior expression is found in some areas. Expression in the mature wing disc is confined to the squamous peripodial membrane cells; in younger wings, Ubx expression fills the posterior half of the peripodial side of the disc. The mesothoracic leg stains with a pattern that is qualitatively similar (but not identical) to that of the metathoracic leg; Ubx is expressed in some anterior regions of the mesothoracic leg, in parasegment 4. Double staining with anti-Ubx and anti-engrailed reveals that discontinuities in Ubx expression that have been suggested to correspond to compartment borders do not coincide with the compartment boundaries in some cases. In the larval ventral ganglion, Ubx expression is greatest in parasegments 5 and 6, as in the embryonic nervous system.

Key words: Drosophila, imaginal discs, Ultrabithorax, gene expression, nervous system.

Introduction

Ultrabithorax (Ubx), located in the left (proximal) end of the bithorax complex, is the most extensively studied of the Drosophila homeotic loci. Correct regulation of the Ubx locus is necessary for the proper specification of thoracic and abdominal segments; in particular, loss of Ubx function leads to homeotic transformations in the meso- and metathorax and the first abdominal segment (T2, T3 and A1) (Lewis, 1978; Morata & Kerridge, 1981; Hayes, Sato & Denell, 1984; Struhl, 1984; Casanova, Sanchez-Herrero & Morata, 1985). Molecular studies indicate that the Ubx gene is extremely large, with a primary transcript of over 70 kilobases (kb) (Bender et al. 1983; Beachy, Helfand & Hogness, 1985; Hogness et al. 1985). Differential splicing of the primary transcript can yield mRNA molecules of between 3-2 and 4-7 kb (Akam & Martinez-Arias, 1985; Beachy et al. 1985; Hogness et al. 1985). The Ubx protein product(s) has a relative molecular mass of approximately 44 x 10^3 (White & Wilcox, 1984; Hogness et al. 1985).

The regulation of Ubx expression has been examined at the molecular level using a variety of techniques. In situ hybridization of nucleic acid probes to mRNA is a sensitive method for detecting Ubx transcription and has been used extensively to examine the segmental distribution of Ubx transcripts (Akam, 1983; Akam & Martinez-Arias, 1985). Unfortunately, this method requires sectioning of the tissue to be examined and detailed reconstructions of two- or three-dimensional patterns from serial sections is time consuming. An alternative to in situ hybridization is to localize the Ubx protein with specific antibodies. This has been done using rabbit antisera and a monoclonal antibody, both raised against lacZ/Ubx fusion proteins (White & Wilcox, 1984, 1985a; Beachy et al. 1985). From all of these studies, a number of conclusions concerning Ubx expression can be drawn.

Following segmentation of the embryo, Ubx is expressed primarily from the posterior compartment of the second thoracic segment (T2p) through the anterior of the eighth abdominal segment (A8a). In
the embryonic ectoderm, the expression of the gene appears to be organized into metameric blocks, which correspond to the parasegments (Martinez-Arias & Lawrence, 1985). Each parasegment includes the posterior compartment of one segment and the adjacent anterior compartment of the next segment. Expression is relatively weak in parasegment 5 (T2p/T3a), high in the more posterior parasegment 6 (T3p/A1a) and then decreases as one moves posteriorly through the abdominal region. In both the embryonic epidermis and nervous system, Ubx expression is not necessarily uniform within a parasegment. In all cases, anti-Ubx staining is found primarily in cell nuclei, consistent with the idea that Ubx, like other homeobox-containing genes, codes for a DNA-binding protein (Laughon & Scott, 1984; Primorica et al., 1985). Staining of disc whole mounts. Thus, in its gross aspects, staining of the larval discs seems to follow the parasegmental pattern of the embryonic ectoderm. A finer analysis, however, reveals that the patterns of Ubx expression are very heterogeneous within each imaginal disc. This was best illustrated by White & Wilcox (1985a), who found significant proximodistal and other variations in the levels of Ubx expression in the T3 discs. The highly heterogeneous expression of Ubx in imaginal discs suggests that the protein may be doing more than simply specifying segmental, or parasegmental, identity in these epithelia.

Before the fine regulation of Ubx in discs can be studied at the molecular or genetic level, it is necessary to know the wild-type patterns of expression in detail. Examinations of Ubx expression in imaginal discs have been rather cursory to date, largely due to technical problems. The approach generally taken is to examine anti-Ubx staining of disc whole mounts. Unfortunately the background fluorescence levels often are quite high in these preparations. Thus, faint specific immunofluorescence is difficult to characterize or may go undetected, and it is at least partly for this reason that the published accounts of anti-Ubx staining in discs contain discrepancies and ambiguities. The background fluorescence of whole mounts results primarily from nonspecific sticking of antibodies to the detergent-treated discs. Also, the convoluted nature of the disc epithelia leads to large differences in the background staining in different regions, in addition to variations between different preparations. A partial solution to this problem is to cut sections of discs and the best previous data on disc patterns come from the sections of White & Wilcox (1985a). A complete reconstruction of the disc-staining patterns from serial sections would be very time consuming, however, and has not been undertaken.

The background fluorescence problem also can be overcome using image-processing techniques (Brower, 1986). During the normal incubation with secondary antibody, the tissue is incubated also with an antibody, labelled with a different fluorochrome, that binds only nonspecifically. Using videomicroscopy and an image processor, the fluorescent image from the nonspecific antibody and the immunofluorescence from the specific antibody (which also contains a nonspecific component) can be subtracted from one another, leaving only the image resulting from specific antibody binding. In the work reported here, the background subtraction method has allowed the mapping of aspects of Ubx expression that previously had gone undetected, particularly in the mesothoracic discs. The proposed parasegmental nature of Ubx expression in discs has been examined also, using antibodies against the engrailed (en) gene product (DiNardo, Kuner, Theis & O’Farrell, 1985).

**Materials and methods**

**Antibody staining**

Wild-type Drosophila melanogaster (Barton) were raised at 24–25°C. Larval heads were cut off, turned inside out and extraneous tissues, such as fat body, were removed. Some tracheae and other connectives were cut to ensure that the discs and ventral ganglion of each head were exposed to the solutions. The larval heads were transferred to the various staining solutions with forceps. The discs and ganglia were dissected from the heads before mounting.

Tissue was fixed for 10 min in 4% formaldehyde, 1% NP-40, 0.1 M Pipes, 2 mM MgSO4, 1 mM EGTA, pH 6-9. Following fixation, the tissue was blocked for 1 h in 150 mM-NaCl, 50 mM-Tris-HCl, 0.5% NP-40, 10% fetal calf serum, pH 7-4. All subsequent antibody dilutions and washes, except for the final wash, were in this solution, except that the serum concentration was lowered to 5%. After blocking, the tissues were incubated for 2 h, with regular stirring, in monoclonal antibody FP.3.38 (White & Wilcox, 1984), which was diluted approximately 1:1000 from ascites fluid. (This antibody probably recognizes all Ubx-derived protein products; White & Wilcox, 1984.) The heads were washed for a total of 1 h (with three changes of solution)
and incubated for 2 h in fluorescein-conjugated goat anti-mouse antibody (Antibodies Incorporated) and rhodamine-conjugated goat anti-guinea pig antibody (Cappel Labs). For Ubx/en double labelling, the heads were first incubated simultaneously in mouse anti-Ubx and rabbit anti-en antibodies (generously provided by Steve DiNardo; see DiNardo et al. 1985, for a description of this antiserum); a rhodamine-conjugated goat anti-mouse (Cappel) and fluorescein-conjugated goat anti-rabbit (Antibodies Inc.) were used as secondary antibodies. The heads were finally washed as above, rinsed in the same solution without NP-40, washed as above, rinsed in the same solution without NP-40, and mounted in a solution of 70 % glycerol, 30 % 0.1 M-Tris, pH 9, to which was added 1–2 % n-propyl gallate to retard bleaching. The slides were examined using Zeiss plan-neofluor lenses and epi-illumination.

**Video processing**

Images were detected with a DAGE-MTI Model 66 ISIT camera and these images were fed into a Hughes Aircraft Co. Model 794 Image Processor. For standard immunofluorescence, the only processing was simple contrast enhancement by gamma compensation. For background subtraction, the image of the background rhodamine fluorescence was stored in memory using the INTG1 function in the manual mode and the image of the specific fluorescein fluorescence was subtracted from the memory image using the C-SUB function in the manual mode. This mode of operation allows the relative intensities of the images being compared to be varied continuously and generally a number of different ‘exposures’ were recorded for each specimen. In addition, the contrast between faint and no fluorescence was enhanced with the gamma compensation function; this setting remained constant for all subtractions. All images were recorded using a Panasonic NV-9240XD video recorder.

**Results**

Descriptions of anti-Ubx staining of imaginal discs have been published already (White & Wilcox, 1984, 1985a; Beachy et al. 1985); however, there are a number of discrepancies among the reports. (These accounts deal with other aspects of Ubx expression, such as embryonic patterns and, in some cases, the characterizations of imaginal discs are rather cursory. The most detailed representation is provided by White & Wilcox, 1985a.) The results presented here will focus on aspects of Ubx expression that have not previously been described, although some duplication with the earlier reports, especially with respect to general features, is inevitable. Also, features of the patterns that are particularly prominent or reliable will be emphasized, as these will be most useful in subsequent mutant analyses. The intensity of anti-Ubx immunofluorescence varies considerably within a single disc, particularly for the metathoracic discs. It often is not possible to depict the staining in all regions with a single exposure and contrast levels must be chosen that best illustrate particular features. Therefore, intensity differences may not be reflected accurately from one figure to another.

Unless stated otherwise, all descriptions are for staining on the columnar epithelial side of the flattened epithelial sac that constitutes each disc. The anteroposterior and proximodistal axes of the discs are defined with respect to the positions of the adult structures that will derive from a given region. (See Bryant, 1978 and Adler, 1982 for fate maps of discs.) Typically, the most distal adult structures derive from the central region of the columnar epithelium of each disc and the cells near the disc margin contribute to proximal cuticular structures.

**Metathoracic (T3) discs**

These discs stain much more intensely than the more anterior discs. Virtually all of the cells of the metathoracic discs express some Ubx protein; the only clear exception to this is the most distal region of the leg, where no antibody binding was detected, even with the sensitive subtraction procedure (Figs 1–3; see also White & Wilcox, 1985a).

In the leg (Fig. 1), the greatest levels of immunofluorescence are observed on a set of folds between the central knob and the more peripheral regions. Although the only published leg disc fate maps are for the prothoracic (T1) leg, comparisons suggest that these strongly staining folds probably correspond to the presumptive tibia and basitarsus. (This interpretation is in agreement with that of White & Wilcox, 1985a). In the circumferential axis, the strongest staining on the folds is in the posterior medial region, with high levels also seen in the posterior lateral and anterior-most regions. Closer to the disc margin, staining is greatest on the anterior side of the disc.

In the haltere (Fig. 3), the greatest variation in Ubx staining also is along the proximodistal axis. The distal regions and, especially, the large fold around the central pouch (the presumptive capitellum), stain much more intensely than other parts of the disc. The anterior edge of the pouch stains less than the rest. Other variations in staining intensity are observed in proximal regions.

**Parasegmental expression in metathoracic discs?**

Both the leg and haltere discs display anteroposterior differences in staining and both patterns feature distinct boundaries in Ubx staining near the putative compartment boundaries in some regions (Figs 1, 3). It has been suggested that these sharp Ubx boundaries correspond to the compartment boundaries (White & Wilcox, 1985a,b), in accord with the parasegmental nature of Ubx expression observed in embryos.
This correspondence was examined in detail for two regions of the metathoracic leg and one region of the haltere, by double-staining discs with antibodies against the Ubx and en proteins. Genetic requirements for en function and molecular studies of en expression indicate that en protein is a reliable

Fig. 1. Metathoracic leg disc, stained with anti-Ubx; standard immunofluorescence. Anti-Ubx staining is greatest on the folds between the central end knob and the disc margin; these folds represent the presumptive tibia and basitarsus of the adult leg. Staining is greatest in the posterior medial quadrant of the folds, with a sharp discontinuity near the anteroposterior compartment boundary (large arrow, see also Fig. 4). (In all figures except Fig. 2, posterior is to the left.) The small arrow marks the less-distinct discontinuity in staining near the lateral anteroposterior compartment boundary. Near the disc margin, staining generally is most intense on the anterior side. This figure, and Figs 3 and 4, are exposed to best illustrate intradisc differences in staining intensities; although not obvious from these figures, almost all of the nuclei of the metathoracic discs stain with anti-Ubx; an exception is illustrated in Fig. 2.

Fig. 2. Metathoracic leg disc, side view, stained with anti-Ubx; background subtraction method, specific fluorescence is dark. The end knob, which will make the most distal portion of the adult leg, is the white tissue at the 'top' of the disc (arrow); even in this overexposed image, the end knob does not stain with anti-Ubx antibody. The black dots overlying the end knob are nuclei of the peripodial membrane.

Fig. 3. Haltere disc, stained with anti-Ubx (3A) and anti-en (3B); standard immunofluorescence. Anti-Ubx staining is greatest on the large fold surrounding the central pouch; on this fold and the rest of the epithelium, there is generally more immunofluorescence in the posterior half of the disc. The discontinuity indicated by the large arrow in A has been suggested to represent the anteroposterior compartment boundary, however, comparison with the anti-en staining (B) indicates that this discontinuity lies anterior to the compartment border. A less-distinct change in anti-Ubx staining intensity is often detectable at the compartment boundary (small arrows). Bars, 50 μm.
marker for posterior compartments in the thoracic segments (Morata & Lawrence, 1975; DiNardo et al. 1985; Brower, 1986). Indeed, although the compartments were first defined by cell lineage restrictions, the expression of, or requirement for, the en gene may now be the best characteristic by which to define posterior compartments in the epidermis.

On the haltere disc, the expression of Ubx declines abruptly as one moves from posterior to anterior along the large fold on the dorsal side of the presumptive capitellum, and White & Wilcox (1985a) suggest that this Ubx-defined boundary may be coincident with the anteroposterior compartment boundary. However, double staining of haltere discs demonstrates that the Ubx- and en-defined boundaries do not coincide (Fig. 3). Occasionally, it is possible to detect a difference in Ubx staining across the en-defined compartment boundary on the fold, but Ubx modulation at this compartment border is not as great as it is at the more anterior boundary.

On the metathoracic leg, expression of Ubx is particularly great on the epithelial folds that give rise to the tibia and basitarsus, and discontinuities in antibody staining are evident in both the medial and lateral halves of these folds (Fig. 1). The most distinct Ubx boundary lies in the medial half of the disc and double staining with anti-en antibodies indicates that this Ubx discontinuity lies within the posterior compartment, about four to five cell diameters from the compartment border (Fig. 4). As in the haltere, a weaker medial discontinuity in Ubx expression is sometimes observed at the en-defined compartment border. On the lateral half of the leg disc, the Ubx-defined boundary is not so sharp, although there appears to be at least a rough correspondence between the compartment and Ubx boundaries (not shown).

**Mesothoracic (T2) discs**

The staining intensity of mesothoracic leg discs is very much reduced relative to the metathoracic leg and the specific immunofluorescence is best visualized using the background subtraction method (Fig. 5). In its gross aspects, the expression of Ubx in the mesothoracic leg is similar to the pattern of expression in the metathoracic counterpart. The ring of epithelial folds between the central and peripheral regions stains much more intensely in the posterior than the anterior. The region of strongest anterior staining corresponds to the homologous region of greatest anterior Ubx expression in the metathoracic leg (compare Figs 1 and 5). As in the metathorax, staining also is observed in the peripodial membrane layer (not shown). One significant difference between the two sets of legs is that in the mesothorax, the peripheral region of the disc also stains more intensely in the posterior half than in the anterior; in fact, the posterior margin stains about as intensely as the more central posterior folds, although the overall low levels of immunofluorescence make precise comparisons difficult.

Antibody binding to the wing imaginal discs also is most easily visualized following background subtraction (Fig. 6). Anti-Ubx staining is confined to the nuclei of the squamous peripodial membrane and the tracheal cells associated with the disc. Even with the sensitive methods used here, absolutely no Ubx expression was detected in the columnar epithelium which gives rise to the adult wing, in agreement with the findings of others (White & Wilcox, 1984, 1985a).

**Other discs and earlier developmental stages**

No anti-Ubx staining is detectable in the prothoracic (T1) leg or eye–antenna discs or in the dorsal prothoracic imaginal nest of cells at the base of the anterior spiracle (the humeral disc).

![Fig. 4. Medial region of metathoracic leg disc, stained with anti-Ubx (A) and anti-en (B); standard immunofluorescence. The arrows are in the same locations in both panels. The distinct discontinuity defined by Ubx expression (large arrow) lies posterior to the compartment border defined by en expression (small arrow). However, a minor difference in anti-Ubx staining is sometimes observed nearer the compartment border. Bar, 50 μm.](image-url)
**Ubx** protein can be detected in nuclei of meta- and mesothoracic disc cells during the entire third larval instar. The levels of expression are rather low early in the third instar, relative to that seen in mature discs. Although the small size of young discs make it difficult to map the early patterns of expression in

![Figure 5](image-url)  
**Fig. 5.** Mesothoracic leg disc, stained with anti-**Ubx**; standard immunofluorescence (A) and with background subtraction method (5B, specific staining is dark). The specific staining of mesothoracic leg discs is faint compared to the metathoracic legs, making it difficult to discern the pattern of **Ubx** expression from the background staining by standard immunofluorescence (A). Following subtraction to eliminate nonspecific fluorescence, it is clear that **Ubx** is expressed primarily in the posterior half of the disc (B). Some anterior staining is consistently observed, however, and is strongest in a region that is homologous to the region of greatest anterior staining in the metathoracic leg (arrow; compare with Fig. 1).

![Figure 6](image-url)  
**Fig. 6.** Columnar epithelium (A) and peripodial membrane (B) surfaces of a wing disc stained with anti-**Ubx**; background subtraction method, specific staining is dark. There is no staining of the columnar epithelium; staining on this face (A) is detected only on nonepidermal structures, such as the tracheae. (The diffuse dark staining observed on the left and top parts of the disc derives from out-of-focus nuclei of the peripodial layer.) On the opposite side of the disc, **Ubx** protein is found in the nuclei of the squamous peripodial membrane cells (B). On the posterior (left) side, the squamous epithelium becomes columnar more gradually than on the anterior side and this is reflected in the pattern of anti-**Ubx** staining. Only in the posterior dorsal region does the staining reach the disc margin (arrows).

![Figure 7](image-url)  
**Fig. 7.** Columnar epithelium (A) and peripodial membrane (B) surfaces of a wing disc from an early-to-mid third instar larva, stained with anti-**Ubx**; background subtraction method as in Fig. 6. (The terms 'columnar epithelium' and 'peripodial membrane' are used to denote the two surfaces of the flat sac of disc epithelium, even though the columnar and squamous characters of the respective epithelia are not fully developed at this stage.) As for the older wing disc, only the tracheal nuclei stain on the columnar epithelial face (A). In this particular disc, the foreign particle on the upper part of the disc can be used to focus on the nonstaining epithelium. (B) On the opposite face, **Ubx** is expressed in posterior cells only. In contrast to the mature disc, the **Ubx**-expressing nuclei extend to the posterior margin, but do not cross to the columnar epithelial face of the disc. Bars, 50 μm.
detail, some features are distinguishable. In meta-
thoracic discs from early third instar larvae, anti-\textit{Ubx}
staining often appears greater on one half of the discs,
presumably the posterior side. The peripodial layer of
the metathoracic discs also stains at early times. One
somewhat unexpected finding is that, early in the
third instar, the staining of the haltere disc is notice-
ably more intense than the staining of the meta-
thoracic leg.

In wing discs from early- to mid-third instar larvae,
the entire posterior half of the peripodial side of the
disc stains with anti-\textit{Ubx} (Fig. 7). It is particularly
noteworthy that the posterior edge of the \textit{Ubx}-
expressing domain appears to coincide precisely with
the margin of the disc. Later, the posterior border of
\textit{Ubx}-expressing cells does not extend to the margin,
except in the dorsal region close to the disc stalk.
(Compare Figs 6B and 7B.)

\textbf{Ventral ganglion}

The larval ventral ganglion is compressed relative to
the embryonic nervous system and the segmental
organization is not so clearly defined morphologically
(e.g. compare with the embryonic nerve chords
illustrated by White & Wilcox, 1984, 1985a; and
\textit{Beachy et al.} 1985). Although segmental boundaries
in staining tend to be somewhat less distinct in larvae,
blocks of \textit{Ubx} expression, approximately one seg-
ment wide, are evident (Fig. 8). The segmental distri-
bution of \textit{Ubx} protein in the larval ganglion is similar
to that reported for the embryonic nervous system,
with staining being most intense in a band that
appears to correspond to T3p/A1p (parasegment 6),
with weaker staining in T2p/T3a (parasegment 5) and
more posterior regions (Fig. 9). As in the embryonic
nerve chord, labelling is observed in midline cells of
T1 (parasegment 4). Anti-\textit{Ubx} staining is greatest in
the ventral half of the larval ganglion; on the dorsal

\textbf{Fig. 8.} Ventral ganglion stained with anti-\textit{Ubx},
standard fluorescence. (As for the discs, anterior is to
the right in Figs 8–10.) The pattern of staining is similar to
the pattern seen in embryonic ganglia (White & Wilcox,
1984, 1985a; \textit{Beachy et al.} 1985). The strongest band of
immunofluorescence corresponds to parasegment 6, or
T3p/A1a (large arrow), with the more anterior band
being in parasegment 5. Weaker staining is observed in
more posterior parasegments. The only staining anterior
to parasegment 5 in the nervous system is to a group of
midline cells in parasegment 4 (small arrow). Most of the
cell bodies of the ganglion are in the ventral and lateral
regions and the curvature of this intensely-staining region
leads to the extreme brightness at the lateral edges of the
ganglion.

\textbf{Fig. 9.} Ventral ganglion stained with anti-\textit{Ubx} (white)
and anti-\textit{en} (black); the opposite contrasts are produced
by subtracting the anti-\textit{en} image from the anti-\textit{Ubx} image,
using the image processor. The three thoracic \textit{en}-
expressing bands are indicated. The midline cells typically
express both proteins, however comparisons of the two
individual images reveals that many of the \textit{en}-expressing
cells in T2 fail to stain with anti-\textit{Ubx} (not shown).

\textbf{Fig. 10.} Ventral ganglion stained with anti-\textit{Ubx};
standard immunofluorescence. Most of the cell bodies of
the ganglion are located ventrally and the previous figures
are focused in that plane. This figure shows the staining
pattern in the dorsal region of the ganglion. Although
there are many fewer nuclei, there is still a relatively
strong region about one segment wide, flanked on each
side by a weaker staining region, with decreasing
fluorescence intensities toward the posterior end of the
ganglion. Bars, 50 \mu m.
The mesothoracic leg discs have been reported to T2p, at the anterior edge of t/fo-expressing parasegment 5, do not stain detectably with the anti-Ubx antibody. Close examinations of double-stained ganglia reveal that, even in the posterior region of each segment, many more nuclei stain with anti-Ubx than stain with anti-en. Nuclei are observed that stain with both antibodies, including the midline nuclei. Interestingly, many of the en-expressing nuclei in T2p, at the anterior edge of Ubx-expressing parasegment 5, do not stain detectably with the anti-Ubx antibody.

Discussion

By using videomicroscopy and image-processing procedures, the patterns of Ubx gene expression in imaginal discs have been characterized in detail. In particular, the background fluorescence subtraction method (Brower, 1986) has permitted the mapping of Ubx expression in the mesothoracic leg, where the levels of expression are low. The patterns of Ubx expression also have been compared with the distribution of the en protein, as the domains defined by these two regulatory genes have been proposed to coincide in some cases. In many respects, the results confirm or add more details to the descriptions of others (Akam, 1983; White & Wilcox, 1984, 1985a; Beachy et al. 1985). Several findings have been unexpected or are otherwise noteworthy, however, and these will be discussed below.

Mesothoracic leg

The mesothoracic leg discs have been reported to exhibit ‘little or no anti-Ubx staining’ (Beachy et al. 1985) or to show ‘weak labelling over discrete areas’ (White & Wilcox, 1985a). Presumably because of the low levels of expression (e.g. Ingham, 1985), the immunofluorescence staining pattern for this disc has not previously been illustrated. Image-processing methods reveal that the pattern of the mesothoracic leg is largely similar to the pattern of the more posterior metathoracic leg, although the amount of staining is very much reduced. In particular, there is faint anti-Ubx immunofluorescence detectable in some anterior cells of the mesothoracic leg (T2a), clearly in regions corresponding to parasegment 4. This result was rather surprising, as epidermal expression of Ubx in embryos is often described as extending anteriorly only through the posterior compartment of the mesothorax (T2p, parasegment 5). Another case of more anterior expression is found in the report of White & Wilcox (1985a), who described staining in the embryonic epidermis anterior to the mesothoracic Keilin’s organs, presumably in parasegment 4.

The function of Ubx in the mesothoracic leg is unknown. Elimination of Ubx function by somatic recombination results in a homeotic transformation of mesothorax to prothorax, but only if mutant clones are generated at the blastoderm stage and only posterior leg regions are affected (Morata & Ker-ridge, 1981). This transformation has been said to define the postprothorax (ppx) function of the bithorax complex and probably reflects regulatory interactions between homeotic genes very early in development (Struhl, 1982; Casanova et al. 1985). Elimination of Ubx function later in development, even in the late embryo, produces no discernible phenotype in the mesothoracic leg. Of course, the legs of the different Drosophila segments are much more similar to one another than the derivatives of the dorsal discs (wing and haltere) and minor abnormalities may be difficult to detect. Also, it is possible that Ubx expression in the mesothoracic leg disc serves no function that is detectable in the cuticular structures; even if this is so, Ubx may still act to direct some epidermis-associated patterning process, such as the projection of sensory neurones or the attachment of muscles.

Parasegments in imaginal discs?

The blocks of Ubx expression observed in embryos, the Ubx metameres, appear to correspond to the posterior compartment of one segment and the adjacent anterior compartment of the next-most-posterior segment (Akam & Martinez-Arias, 1985; White & Wilcox, 1984, 1985a; Beachy et al. 1985). That is, Ubx expression is organized roughly into metameres corresponding to parasegments (Martinez-Arias & Lawrence, 1985), rather than segments. Overall, the correspondence of the parasegment and Ubx metamere borders in embryos is good, but not always exact; for example, some Ubx protein expression has been observed in parasegment 4 in embryonic epidermis (White & Wilcox, 1985a).

If Ubx expression is regulated in parasegmental units in imaginal discs, then expression within each disc should reflect the anteroposterior compartmentalization of the discs (Martinez-Arias & Lawrence, 1985). Ubx protein can be detected in most of the metathoracic haltere and, while anti-Ubx staining is generally greatest in the posterior part of the haltere, the most distinct boundaries defined by Ubx expression do not correspond to the compartment boundary. In the mesothoracic wing disc, Ubx is found only in the cells of the peripodial membrane; most of the posterior compartment fails to express Ubx at all. In the metathoracic leg, the most distinct anteroposterior discontinuity in Ubx expression appears at first glance to correspond with a compartment border. However, double staining with anti-en
demonstrates that the two borders do not coincide. Moreover, in the proximal, or peripheral, regions of the metathoracic leg discs, anti-\(Ubx\) staining is greatest in the anterior and, in both metathoracic discs, the greatest variations in \(Ubx\) expression are along the proximodistal axis. Some variations in \(Ubx\) expression are observed to coincide with anti-\(Ubx\)-defined compartment boundaries, although the modulation of \(Ubx\) expression at these boundaries typically is very minor or even undetectable. (The discs illustrated in Figs 3 and 4 are among the clearest examples of this compartmental modulation.) Thus, while some regulatory relationship may still exist between compartments and \(Ubx\) late in development, it is clear that some major regulatory influences on \(Ubx\) expression in imaginal discs are not organized compartmentally.

The primary importance of imaginal discs as developmental units is indicated by numerous regeneration experiments. From the classical work of Hadorn and subsequent studies (reviewed by Gehring, 1972; Bryant, 1978), it is clear that disc type is the primary unit of determined state by the end of the third larval instar. The findings with leg discs reported here suggest that discs, rather than parasegments, are also more important units for regulation of \(Ubx\) expression patterns in larvae. For example, if \(Ubx\) regulation was organized strictly in parasegmental units, expression in the anterior mesothoracic leg (T2a) should be accompanied by expression in the posterior prothoracic leg (T1p), as both are in parasegment 4. However, no prothoracic expression is observed. As the spatially complex patterns observed in discs may have evolved by modification of the embryonic parasegmental organization, the anteroposterior differences observed in discs could be evolutionary (and ontogenetic) vestiges of the more primitive parasegmental patterns.

Overall, then, the expression of \(Ubx\) in discs appears to correlate more with disc type or, on a finer scale, with disc morphological features, than with parasegment type. In this vein, it is interesting to note that the expression of \(Ubx\) in larval muscles appears to be organized in segmental, not parasegmental, units and bithorax complex mutants also seem to define segmental domains in the muscles (Hooper, 1986).

Localization of \textit{Antennapedia} (\textit{Antp}) (Wirz, Fessler & Gehring, 1986) and \textit{Sex combs reduced} (\textit{Scr}) (Glicksman & Brower, unpublished observations) proteins in discs indicate that fine regulation of heterogeneous expression within disc epithelia is a general feature of homeotic genes. The patterns of expression suggest that these genes are likely to be important in detailed patterning events within discs. Mutations with phenotypes that are localized to specific regions of discs have been mapped in both the \(Ubx\) and \textit{Antp} loci, and clustering of similar mutations has indicated particular genetic domains that may be important for the localized expression of the genes (Peifer & Bender, 1986; Abbott & Kaufman, 1986). The phenotypes of these mutants generally involve segmental transformations, however, and it is not clear how the role of homeotic genes in segmental specification may be related to a potential role in patterning within segments. Clearly, more work is required to address this question.

I thank Sharon Jaffe for technical assistance, and Steve DiNardo for generously providing the anti-\textit{en} antibodies. Marcie Glicksman and John Little provided helpful criticisms of the manuscript. Supported by grants from the NIH (R01 GM34112 and K04 HD00659) and the NSF (DCB-8608164).

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(Accepted 6 May 1987)