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Segments, Lineage Boundaries and the Domains of Expression of Homeotic Genes

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Segments, lineage boundaries and the domains of expression of homeotic genes

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[Plate 1]

It is now possible to monitor directly the pattern of activity of homeotic and segmentation genes in the *Drosophila* embryo. Precisely bounded domains of expression are established in the blastoderm, at the time when cells became committed to specific segment identities. Some patterns of expression appear in their final form; others evolve rapidly during formation of the blastoderm. Transcripts of the homeotic gene *Ultrabithorax* accumulate at high levels in a single parasegment of the blastoderm, and in a block of seven parasegments of the extended germ band. The boundaries of these *Ubx* domains appear to lie precisely at presumptive A–P compartment boundaries.

During formation of the germ band, the abundance of *Ultrabithorax* transcripts shows a transient segment 'pair-rule' modulation. I suggest that this reflects an interaction between the *Ultrabithorax* gene and a segment pair-rule function, which may serve to establish the precise correlation between lineage boundaries and the domains of *Ultrabithorax* expression.

Introduction

The *Drosophila* embryo becomes visibly segmented 5 h after fertilization, when a repeating pattern of grooves and ridges appears in the extended germ band (Poulson 1950; Campos-Ortega & Hartenstein 1985). By this time, lineage restrictions isolate the cell populations which will give rise to each segment of the fly, and to anterior and posterior compartments within each segment (Garcia-Bellido *et al.* 1973; Garcia-Bellido 1975; Lawrence 1981). Cells acquire segment identities well before this. If cells are removed from an embryo that has just completed formation of the blastoderm, and transplanted to a new position in a host embryo, they retain the committment to their original fate (Illmensee 1978; Simcox & Sang 1984).

Ten years ago, Garcia-Bellido (1975) suggested that the acquisition of segmental identities and the establishment of lineage restrictions between compartments were intimately related. Building on the work of Lewis (1963), who had suggested that segment identities are established by particular patterns of homeotic gene expression, Garcia-Bellido argued that the domains of homeotic gene expression would be bounded by, and possibly result in, the lineage restrictions identified at segment and compartment boundaries. Since then, a large number of other mutations affecting the process of segmentation have been identified (Nusslein-Volhard & Wieschaus 1980; Nusslein-Volhard *et al.* 1982). Mutations at these loci result not in the homeotic transformation of one segment into another, but in disruption of the metameric repeat pattern itself: the loss of segments from particular body regions ('gap' mutations); the deletion of alternate metameric units, resulting in pair-wise segment fusions ('pair-rule' mutations) and the deletion of regions within each metameric repeat, resulting in abnormal pattern and polarity within each segment.

It is now possible to observe directly the patterns of expression of homeotic genes and of genes controlling segmentation in *Drosophila*. Here I review the first results of these experiments, and suggest a relationship between lineage boundaries, segmentation functions and homeotic gene expression.

Precise spatial regulation of homeotic and segmentation genes is established in the early embryo

Lineage boundaries reflect abrupt differences between adjacent cells. If the domains of action of homeotic genes are lineage compartments, then we would expect to see equally abrupt boundaries in the patterns of homeotic gene expression. Such boundaries are clearly seen when *in situ* hybridization is used to monitor the distribution of homeotic gene transcripts in the early embryo. For example, transcripts of the *Ultrabithorax* (*Ubx*) gene of the bithorax complex (BX-C) are expressed in a large region of the posterior part of the embryo. As early as the end of the cellular blatoderm stage, just as gastrulation begins, the anterior margin of this zone is defined by the juxtaposition of cells that contain maximal levels of *Ubx* transcripts and those that contain no detectable transcripts (Akam & Martinez-Arias 1985; see figure 1, plate 1). Similarly sharp boundaries have been observed for homeotic genes of the Antennapedia complex (ANT-C) (Levine *et al.* 1983; McGinnis *et al.* 1984), and also for the transcripts of genes involved in establishing or maintaining the segmental repeat pattern itself (Hafen *et al.* 1984; Fjose *et al.* 1985; Kornberg *et al.* 1985; Ish-horowicz *et al.* 1985).

Although relatively few genes have yet been examined, it is clear that the very earliest patterns of transcription include two very different types (figure 2). One is exemplified by the segment pair-rule gene *fushi-tarazu* (*ftz*; Hafen *et al.* 1984). In the syncytial blastoderm this gene

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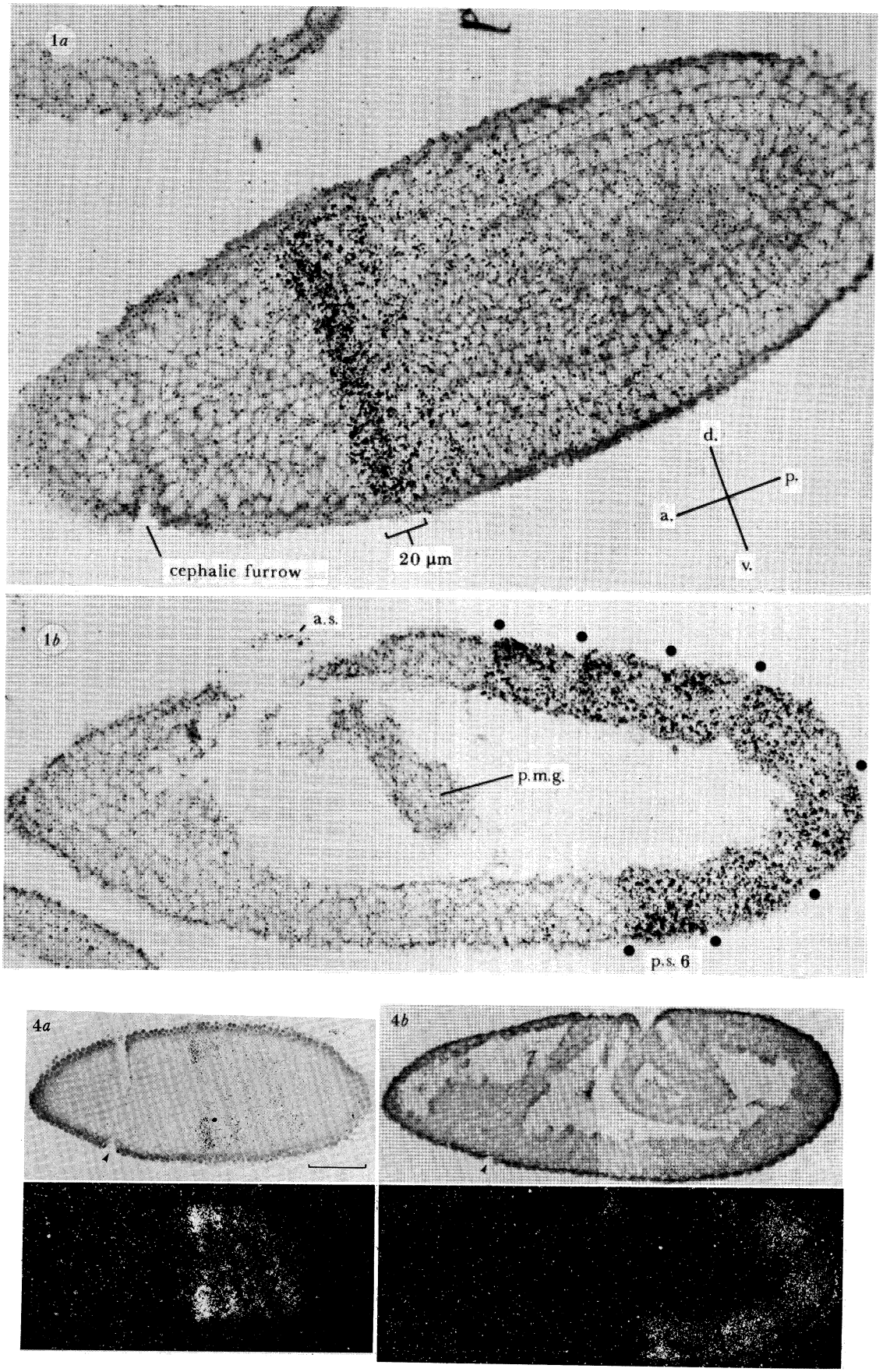
Description of plate 1

Figure 1. Bounded domains of *Ultrabithorax* expression in the early *Drosophila* embryo. Both panels show radioautographs of embryo sections which have been hybridized with a probe for transcripts homologous to the 5′ end of the *Ubx* gene. (*a*) Montage of sections from a very early gastrula (about 3.5 h after fertilization at 25 °C). *Ubx* transcripts are most abundant in a single metameric primordium of the embryo, visible as a narrow stripe of cells which are labelled with silver grains. This is probably the primordium for parasegment 6. (*b*) Near sagittal section of an embryo shortly after germ band extension (5–6 h after fertilization at 25 °C), hybridized under the same conditions as above. The head is at the left; the thoracic and abdominal regions of the germ band extend around the posterior pole of the embryo (right) and onto the dorsal surface. *Ubx* transcripts are now abundant in seven parasegments of the germ band, parasegments 6–12. Sharp boundaries demarcate this zone. In parasegment 6 the ectoderm (outer cell layer) is uniformly and strongly labelled. In parasegments 7–12 differential labelling of the ectoderm is observed within the anterior and posterior regions of each parasegment. Small groups of ectodermal cells in parasegments 5 and 13 also express *Ubx* at this stage, but they are not clearly visible in this section (reprinted from Akam & Martinez-Arias 1985). a., Anterior; p., posterior; d., dorsal; v., ventral; a.s., amnio serosa; p.m.g., posterior midgut; p.s.6, parasegmemt 6. Dots indicate the boundaries of parasegments 6–12.

Figure 4. Modulation of the abundance of *Ubx* transcripts in alternate segments. The two embryos show stages in the expression of *Ubx* intermediate between those illustrated in figure 1. Each radioautograph is illustrated in bright- and dark-field illumination.

In (*a*) germ band extension is just beginning. The section grazes the edge of the embryo. In (*b*) germ band extension is nearly complete. The arrowhead marks the cephalic furrow in each case. Four labelled primordia (probably parasegments 6, 8, 10, 12) are separated by three less strongly labelled zones (parasegments 7, 9, 11).

These sections have been hybridized with a *Ubx* 5′ probe which includes exons of the 4.7 kilobase early *Ubx* RNA (probe A122; see Akam & Martinez-Arias 1985). Much of the hybridization observed here is probably to nascent transcripts of the *Ubx* unit. The pair-rule pattern is less clear when the probe used is homologous to exons of the major stable embryonic RNAs. (Anterior is to the left; scale bar, 50 μm).



FIGURES 1 AND 4. For description see opposite.

(*Facing p.* 180)

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is transcribed throughout the whole region of the embryo that will give rise to the segmented germ band, but the distribution of transcripts rapidly evolves a more complex spatial pattern. As cells form, alternate zones each three to four cells wide accumulate high and low levels of *ftz* transcripts. From experiments that map the adult morphology onto the blastoderm stage embryo, we know that each of these zones is the size of a single segment primordium (Lohs-Schardin *et al.* 1981). A second class of pattern is exemplified by early transcripts of the *bithoraxoid* (*bxd*) region of the bithorax complex. Transcripts of this gene first appear at about the same time as those of the *ftz* gene, but already they are restricted to a region within the presumptive germ band, in this case the primordia for the abdominal segments. Their distribution remains much the same throughout the process of cellular blastoderm formation and gastrulation (Akam *et al.* 1985; unpublished results).

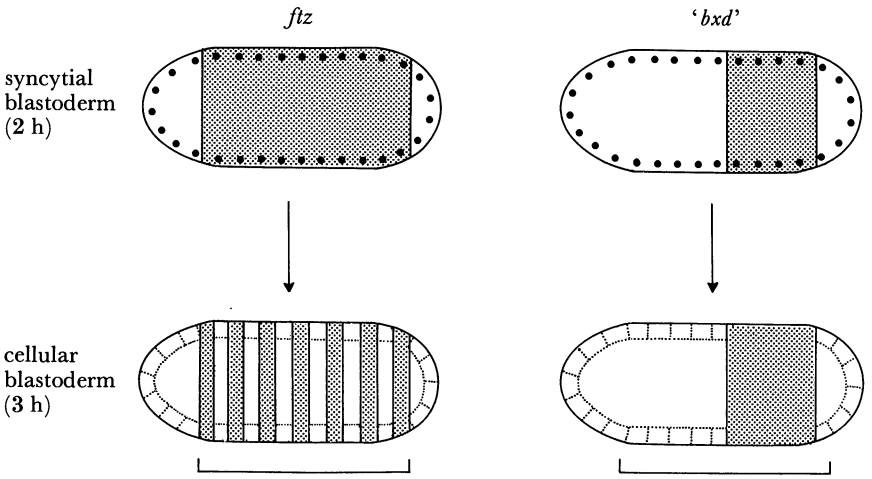

FIGURE 2. Contrasting transcription patterns in the blastoderm. The diagram illustrates patterns of expression for the segmentation gene *ftz* and the homeotic gene *bxd*. Both are first transcribed before cells form in the blastoderm: *ftz* is initially transcribed throughout the region of the embryo which will give rise to the segmented germ band (but not in primordia for the head or endoderm), whereas *bxd* is expressed in only a restricted region of the germ band. The distribution of *bxd* transcripts changes little during cellularization of the blastoderm (Akam *et al.* 1985; unpublished results), but the *ftz* pattern evolves a series of seven stripes (Hafen *et al.* 1984).

It seems likely that the patterning mechanisms underlying these two distributions are very different. In the case of the *bxd* transcript, we would guess that the pattern is defined by maternal positional information, for it is established at or very soon after the first detectable transcription of the embryo's genome (Zalokar 1976). For *ftz*, it seems more likely that the metameric domains are actually generated by the interaction of genes active during formation of the blastoderm, and do not reflect an underlying prepattern. As we shall see below, expression of the homeotic gene *Ultrabithorax* combines features of both of these patterns.

Transcription patterns that reflect subdivisions within each metameric repeat have not been detected in the early stages of blastoderm formation. However, at or shortly before gastrulation the segmentation gene *engrailed* (*en*) is transcribed in a single column of cells within each metameric unit (Fjose *et al.* 1985; Kornberg *et al.* 1985). The sequential expression, first of genes that are transcribed in metameric domains, and then of genes that are differentially expressed within each metamere may prove to be a general feature of *Drosophila* development.

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We would like to know precisely when lineage restrictions are established with respect to the appearance of these bounded zones of expression. Unfortunately this cannot be tested, as lineage restrictions can only be defined with respect to cells generated by mitosis after the blastoderm stage (Lawrence & Morata 1977). In the ectoderm the first of these divisions occurs during germ band elongation (Poulson 1950; Campos-Ortega & Hartenstein 1985), well after transcription patterns are established. At this time lineage restriction is fully effective at both segment and A–P compartment boundaries.

How do the observed boundaries of homeotic and segmentation gene expression relate to lineage boundaries?

If the transcription zones for patterning genes in the blastoderm were all to lie precisely in or out of phase with one another, defining uniquely a set of metameric boundaries, we might reasonably expect these to coincide with the earliest lineage restrictions. This appears not to be the case. We find that the anterior boundary of *Ubx* transcription in the blastoderm does not coincide with the edge of a *ftz* stripe (Akam & Martinez-Arias 1985). Thus the transcription of *ftz* and *Ubx* cannot both respect the same metameric units. The pattern deletions associated with other segment pair-rule mutations lie in several different registers with respect to the segment boundaries (Nusslein-Volhard *et al.* 1982). If these deletions are any guide to transcription patterns, then multiple phases in the blastoderm will be defined by the activity of these genes. Not all could coincide with future segment or compartment boundaries.

The transcription of *ftz*, and probably others among the segmentation genes, is transient. The activity of these genes may relate principally to the process of pattern generation, and not to the transmission of determined states. The Garcia-Bellido hypothesis concerns specifically those selector genes whose activity is maintained throughout development. How do the expression domains of these genes relate to segments and compartments?

Studies with probes for both transcripts and proteins show that the expression of *Ubx* defines a repeating metameric unit (Akam 1983; White & Wilcox 1984, 1985; Akam & Martinez-Arias 1985; Beachy *et al.* 1985). This is clear when segmentation first becomes visible, in the extended germ band (figure 1). Seven metameres within the germ band express *Ubx* in most or all cells. The most anterior of these units contains a particularly high level of *Ubx* transcripts; within each of the more posterior units a repeating motif of differential *Ubx* expression is observed. The limits of these '*Ubx* metameres' are precisely in register with the superficial grooves which are the first external sign of segmentation in the embryo. Until recently, these grooves were thought to be the presumptive segment boundaries, but recent evidence suggests that they define 'parasegment' boundaries, within each segment (Martinez-Arias & Lawrence 1985). Although there is no direct evidence to locate lineage restrictions in the embryo at this stage, the pattern of expression of the *engrailed* gene can be used to define the limits of posterior compartments. (The *engrailed* gene product is thought to be required in posterior compartments to maintain the lineage restriction between A and P cells (see Lawrence & Morata 1975).) By this criterion the superficial grooves lie precisely at the A–P compartment boundary within each segment (Ingham *et al.* 1985). If so, the domains of *Ubx* expression in the early embryo are indeed bounded by lineage restrictions.

Later in development, the pattern of *Ubx* expression can be related directly to overt

segmentation. A repeating metameric motif is again clearly visible in the larval epidermis and in the nervous system (White & Wilcox 1984, 1985; Akam & Martinez-Arias 1985; Beachy *et al.* 1985). It is clearly out of register with segments, but its boundary lies close to or at the presumed location of A–P compartment boundaries in these tissues (figure 3). One repeat unit, parasegment 6, is uniquely defined in both tissues.

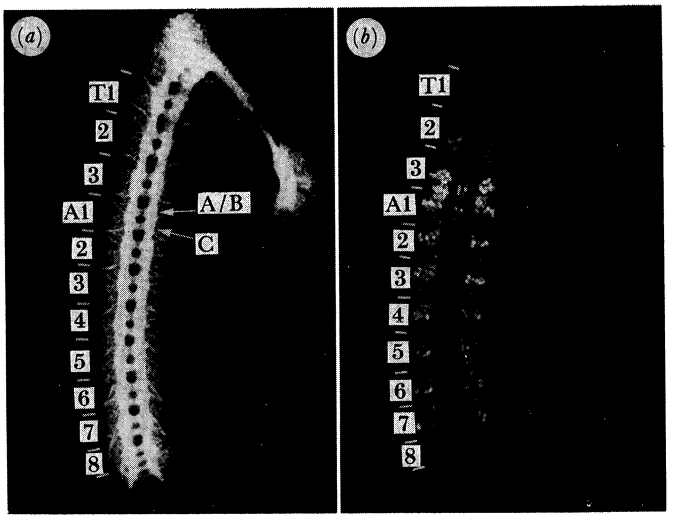

FIGURE 3. Distribution of *Ubx* proteins in the embryonic nervous system. A whole-mount preparation of the nervous system of a 13–14 h *Drosophila* embryo has been stained with antibodies to tubulin (*a*) and *Ubx* proteins (*b*). The ladder of lateral connectives reveals the segmental repeat. *Ubx* proteins define a repeating motif which is out of frame with segment boundaries, but coincident with the presumed parasegmental units of the nervous system. Parasegment 6 is prominently labelled (reprinted from White & Wilcox 1984).

It is not yet clear how the expression domains of other homeotic selector genes are related to that of *Ubx*, and to visible segmentation. High levels of *Antennapedia* transcripts characterize two metameres in the extended germ band, and a single metamere in the later nervous system (Hafen *et al.* 1983; Levine *et al.* 1983; A. Martinez-Arias, personal communication), but the available data do not distinguish whether these metameres are segmental units, parasegmental units or neither. My prejudice is to think that in the early embryo the boundaries of selector gene expression will define a unique metameric repeat, the parasegment. However, the possibility remains that selector genes other than *Ubx* will show segmental rather than parasegmental distributions. Either possibility would be consistent with a close relationship between the known early lineage restrictions and homeotic gene function.

I imply above that in the early embryo, and in later development, the domains of homeotic gene expression are always precise metameric units. We already know that this picture is incomplete. In the case of *Ubx*, even in early development there are groups of cells outside the principal *Ubx* domain which contain *Ubx* transcripts. These, ectodermal cells in parasegments 5 and 13, probably do not define complete developmental compartments. Later, the pattern of *Ubx* expression becomes very complex, and only some aspects of the apparent complexity relate to compartmental domains (Akam 1983; Akam & Martinez-Arias 1985; White & Wilcox 1985). This is particularly clear in the imaginal discs. *Ubx* is expressed throughout the discs of

the third thoracic segment (T3), as predicted by genetic analysis (Lewis 1978), but *Ubx* proteins are also detectable in regions of the T2 discs which, at least in the wing, are not compartment-bounded. One discontinuity in the abundance of *Ubx* transcripts lies at the A–P compartment boundary in the T3 discs, but other marked variations in abundance are not obviously related to lineage boundaries.

These observations suggest that the parasegmental regulation of *Ubx* is only one among many factors that govern the final distribution of *Ubx* transcripts and proteins. What seems clear is that, early in development, metameric regulation of *Ubx* is established in units that come to be bounded by lineage restrictions. This early pattern underlies the more complex distribution of *Ubx* products which is observed in later development.

A relationship between segmentation functions and *Ubx* expression

I have argued that, in the early embryo, *Ubx* and probably other homeotic genes are expressed in domains that are bounded precisely at segment or parasegment boundaries. If so, there must surely be some interaction between the mechanism that establishes lineage boundaries and the control of genes in the Antennapedia and bithorax complexes. The segment selector genes do not themselves establish the segmental repeat pattern, and it is likely that they play at most subsidiary role in maintaining the compartmental lineage restrictions. For example, complete lack of BX-C function allows development of the normal repeating pattern of segmental units, even though the segment identities are changed (Lewis 1978), and clones of cells lacking BX-C functions do not transgress compartment boundaries (Morata & Garcia-Bellido 1976). In contrast, the phenotypes of segmentation genes suggest that these functions establish or maintain the segment pattern itself; a primary role for maintaining lineage restrictions *in vivo* has been shown directly for *engrailed* (Lawrence & Morata 1976; Kornberg 1981; Lawrence & Struhl 1982). It therefore seems most likely that the product of some segmentation gene will interact with the homeotic selector genes to define the precise boundaries of their expression.

We were surprised to observe in the pattern of expression of *Ubx* clear signs of such interactions, first with a pair rule function and subsequently with *engrailed* or some related function (Akam & Martinez-Arias 1985). These interactions are visible as periodic spatial modulations of the abundance of *Ubx* transcripts (figure 4). The pair-rule modulation is transient, most clearly visible during gastrulation. At this time increasing levels of *Ubx* transcripts are observed throughout abdominal regions of the germ band. Hybridization reveals four stripes of cells which accumulate *Ubx* transcripts, separated by three relatively unlabelled regions. The size and spacing of the labelled regions suggest that they define parasegments 6, 8, 10 and 12. By the time that germ band elongation is complete, these stripes can no longer be detected; *Ubx* transcripts are then abundant in all seven metameres within the same span (figure 1). *Ubx* transcripts remain uniformly distributed throughout this region of the mesoderm, but in the ectoderm a subsequent modulation becomes apparent within each metameric repeat; an abrupt change in the abundance of *Ubx* expression occurs at each parasegment boundary, with more *Ubx* transcripts in the anterior of each presumptive segment than in the posterior.

Genetic analysis shows little sign of a pair-rule motif in the expression of BX-C functions. Since the effect is transient, we might question whether it has any developmental significance.

I would like to suggest that it is perhaps a consequence of the mechanism that relates domains of *Ubx* function to parasegment boundaries. In what follows I relate this suggestion to the sequence of stages which we see in the pattern of expression of *Ubx*.

The spatial regulation of *Ubx* expression

Ubx transcripts are first detectable a short while after the onset of *ftz* and *bxd* transcription, during the early stages of cellularization in the blastoderm (Akam & Martinez-Arias 1985). At this time their spatial pattern is best described by a quantitative ('normal') distribution, centred near the middle of the presumptive abdomen (figure 5). This wide distribution may not be precisely bounded, and largely overlaps that of the *bxd* transcripts, and probably also

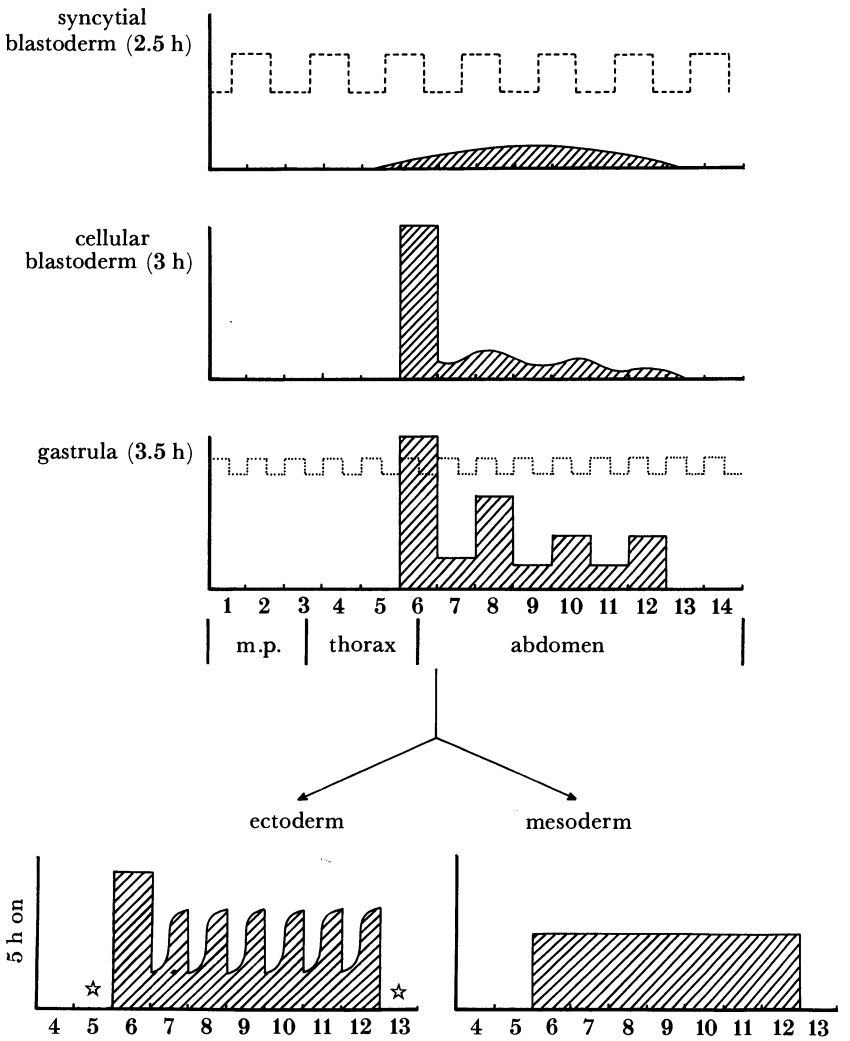

FIGURE 5. Stages in *Ubx* expression. Hatched areas show schematically the relative abundance of *Ubx* transcripts at four stages in the developing embryo. The horizontal scale represents the segmented primordia of the embryo posterior to the head, including mouthparts (m.p.) thorax and abdomen. Parasegments are numbered. The repeating patterns of *ftz* (dashed line) and *engrailed* (dotted line) transcripts are indicated at the time when each first becomes prominent.

Stars indicate that small groups of cells in the ectoderm of parasegments 5 and 13 also express *Ubx* in the extended germ band.

those of other homeotic genes at the same stage. We surmise that its location is determined with respect to the whole egg axis, directly or indirectly by maternal positional information.

It is at this time that the precise banded distribution of *ftz* transcripts is evolving (Hafen *et al.* 1984). Theoretical arguments suggest that the size and precise boundaries of these *ftz* zones can be established much more rapidly and accurately by a mechanism dependent on local interactions (for example, a reaction diffusion mechanism) than by a mechanism that depends on each cell sensing its position along the egg axis (Meinhardt 1982).

The *Ubx* distribution rapidly comes to display metameric domains. First, the primordium for parasegment 6 accumulates high levels of *Ubx* transcripts, while the level of transcripts in the more posterior regions remains low. Then, following the transient appearance of their pair rule stripes, *Ubx* transcripts become abundant in the seven consecutive parasegments of the principal *Ubx* domain. Both parasegment 6 and this larger *Ubx* domain reappear as motifs in the subsequent expression of *Ubx*. These two regions also correlate with the genetically defined domains within which certain (*bithoraxoid*) functions of the *Ubx* unit are required (Lewis 1978; 1981). Presumably therefore they have some developmental significance.

The precise boundaries of *Ubx* expression become apparent while the pair rule modulation of *Ubx* is visible. I suggest that both result from an interaction between some patterned component of the segmentation mechanism and the activity of the *Ubx* gene. I anticipate that this interaction serves to modify the probability that a given cell will or will not come to express *Ubx* stably. The effect of such an interaction will be to 'chop' the continuous distribution of *Ubx* activity into a discontinuous one, and to ensure that the bounds of *Ubx* expression coincide with the segmentation pattern. The modulation of *Ubx* transcript abundance that we observe could be an incidental consequence of this interaction or, if *Ubx* products are themselves autoregulatory, could reflect directly the mechanism that alters the probability of achieving stable expression.

This suggestion assumes that the component of the segmentation mechanism that interacts with *Ubx* will itself be expressed in (and perhaps define) parasegmental stripes. Thus it would not be the *ftz* product. Candidates for the gene encoding such a product are other known pair-rule genes.

Later in development, the abundance of the *ftz* gene product falls, and probably the activity of the postulated 'pair-rule chopper' falls also. The modulation of *Ubx* transcript abundance subsides, but precise boundaries of *Ubx* activity in phase with parasegments have now been established. Then, as the activity of *engrailed* and other intrasegment patterning functions rises, a second round of interactions occurs in the ectoderm, leading to the modulation of *Ubx* expression within each segment that persists throughout development (figures 4 and 5).

We do not know what makes *Ubx* expression in parsegment 6 differ from that in the wider *Ubx* domain, or what establishes the limited expression of *Ubx* in parasegment 5. It is possible that products of the segment selector genes themselves also interact in the blastoderm to alter heritable states of *Ubx* expression (Struhl 1982).

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References

Akam, M. E. 1983 The location of *Ultrabithorax* transcripts in *Drosophila*. *EMBO. J.* **2**, 2075–2084.

Akam, M. E. & Martinez-Arias, A. 1985 The distribution of *Ultrabithorax* transcripts in *Drosophila* embryos. *EMBO J.* **4** 1689–1700.

Akam, M. E., Martinez-Arias, A., Weinzierl, R. & Wilde, C D. 1985 The function and expression of *Ultrabithorax* in the *Drosophila* embryo. *Cold Spring Harb. Symp. quant. Biol.* **50** (In the press).

Beachy, P. A., Helfand, S. L. & Hogness, D. S. 1985 Segmental distribution of bithorax complex proteins during *Drosophila* development. *Nature, Lond.* **313**, 545–551.

Bender, W., Akam, M. E., Karch, F., Beachy, P. A., Peifer, M., Lewis, E. B. & Hogness, D. S. 1983 Molecular genetics of the bithorax complex in *Drosophila melanogaster*. *Science, Wash.* **221**, 23–29.

Campos-Ortega, J. A. & Hartenstein, V. 1985 *The embryonic development of Drosophila melanogaster*. Berlin: Springer-Verlag. (In the press.)

Fjose, A., McGinnis, W. J. & Gehring, W. J. 1985 A homeobox containing gene isolated from the *engrailed* region of *Drosophila* and the spatial distribution of its transcripts. *Nature, Lond.* **313**, 284–289.

Garcia-Bellido, A. 1985 Genetic control of wing disc development in *Drosophila*. In *Cell patterning, Ciba foundation symposium*, vol. 29, pp. 161–182. Amsterdam: Elsevier.

Garcia-Bellido, A., Ripoll, P. & Morata, B. 1973 Developmental compartmentalization of the wing disc of *Drosophila*. *Nature, new Biol.* **245**, 251–253.

Hafen, E., Levine, M., Garber, R. & Gehring, W. 1983 An improved in situ hybridization method for the detection of cellular RNAs in *Drosophila* tissue sections and its application for localizing transcripts of the homeotic Antennapedia gene complex. *EMBO J.* **2**, 617–623.

Illmensee, K. 1978 *Drosophila* Chimaeras and the problem of determination. In *Genetic mosaics and cell differentiation* (ed. W. J. Gehring), pp. 51–70. Berlin, Heidelberg and New York: Springer-Verlag.

Ingham, P. W., Martinez-Arias, A., Lawrence, P. & Howard, K. 1985 Expression of *engrailed* in the parasegment of *Drosophila*. *Nature, Lond.* (In the press.)

Ish-horowicz, D., Howard, K. R., Pinchin, S. M. & Ingham, P. W. 1985 Molecular and genetic analysis of the hairy locus in *Drosophila*. *Cold Spring Harb. Symp. quant. Biol.* **50** (In the press.)

Kornberg, T., Siden, I., O'Farrell, P. & Simon, M. 1985 The *engrailed* locus of *Drosophila*: *in situ* localization of transcripts reveals compartment specific expression. *Cell* **40**, 45–53.

Lawrence, P. A. 1981 The cellular basis of segmentation in insects. *Cell* **26**, 3–10.

Lawrence, P. A. & Morata, G. 1976 Compartments in the wing of *Drosophila*: a study of the *engrailed* gene. *Devl Biol.* **50**, 321–337.

Lawrence, P. A. & Morata, G. 1977 The early development of mesothoracic compartments in *Drosophila*. *Devl Biol.* **56**, 40–51.

Lawrence, P. A. & Struhl, G. 1982 Further studies of the *engrailed* phenotype in *Drosophila*. *EMBO J.* **1**, 827–833.

Levine, M. S., Hafen, E., Garber, R. L. & Gehring, W. J. 1983 Spatial distribution of *Antennapedia* transcripts during *Drosophila* development. *EMBO J.* **2**, 2037–2046.

Lewis, E. B. 1963 Genes and developmental pathways. *Am. Zool.* **3**, 33–56.

Lewis, E. B. 1978 A gene complex controlling segmentation in *Drosophila*. *Nature, Lond.* **276**, 565–570.

Lewis, E. B. 1981 Developmental genetics of the bithorax complex in *Drosophila*. In *Developmental biology using purified genes*, ICN-UCLA Symp. Mol. Cell. Biol. (ed. D. D. Brown and C. F. Fox), vol. 23, pp. 189–208.

Lohs-Schardin, M., Cremer, C. & Nusslein-Volhard, C. 1979 A fate map of the larval epidermis of *Drosophila melanogaster*: localized defects following irradiation of the blastoderm with an Ultraviolet laser microbeam. *Devl Biol.* **73**, 239–255.

Martinez-Arias, A. & Lawrence, P. A. 1985 Parasegments and compartments in the *Drosophila* embryo. *Nature, Lond.* **313**, 639–642.

McGinnis, W., Levine, M. S., Hafen, E., Kuroiwa, A. & Gehring, W. J. 1984 A conserved DNA sequence in homeotic genes of the *Drosophila* Antennapedia and bithorax complexes. *Nature, Lond.* **308**, 428–433.

Meinhardt, H. 1982 *Models of biological pattern formation*. London: Academic Press.

Morata, G. & Garcia-Bellido, A. 1976 Developmental analysis of some mutants of the bithorax system of *Drosophila*. *W. Roux Arch. Devl Biol.* **179**, 125–143.

Nusslein-Volhard, C. & Wieschaus, E. 1980 Mutations affecting segment number and polarity in *Drosophila*. *Nature, Lond.* **287**, 795–801.

Nusslein-Volhard, C., Wieschaus, E. & Jurgens, G. 1982 Segmentierung bei *Drosophila* – Eine genetische Analyse. *Verb. Dtsch. Zool. Ges.* **1982**, 91–104.

Poulson, D. F. 1950 Histogenesis, organogenesis and differentiation in the embryo of *Drosophila melanogaster*. In *Biology of Drosophila* (ed. M. Demerec), pp. 168–270. New York: Wiley.

Simcox, A. & Sang, J. 1983 When does determination occur in *Drosophila* embryos. *Devl Biol.* **97**, 212–221.

Struhl, G. 1982 Genes controlling segmental specification in the *Drosophila* embryo. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7380–7384.

White, R. A. H. & Wilcox, M. 1984 Protein products of the bithorax complex of *Drosophila*. *Cell* **39**, 163–171.

White, R. A. H. & Wilcox, M. 1985 Distribution of *Ultrabithorax* proteins in *Drosophila*. *EMBO J.* **4**, 2035–2044.

Zalokar, M. 1976 Autoradiographic study of protein and RNA formation during early development of *Drosophila* eggs. *Devl Biol.* **49**, 425–437.

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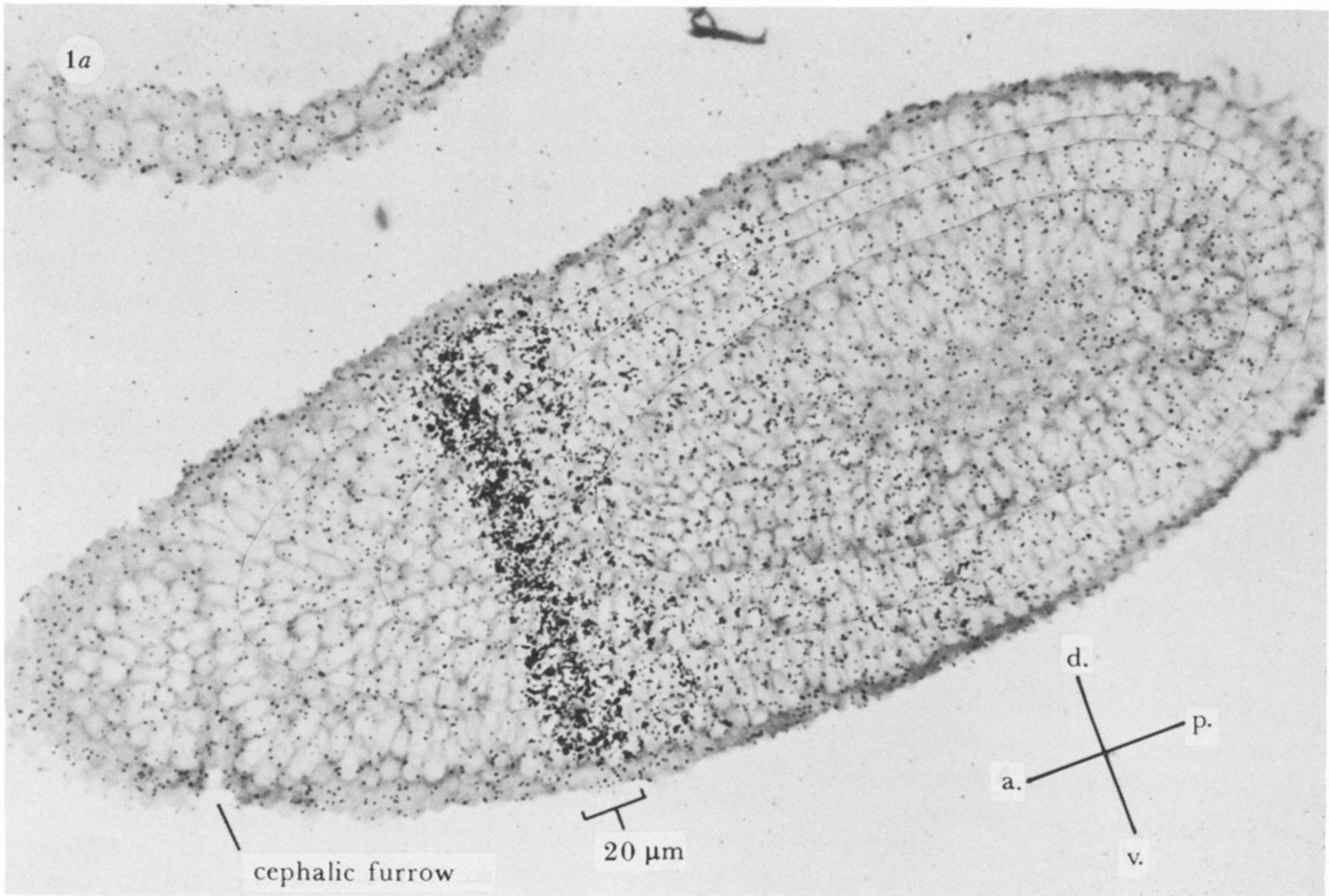

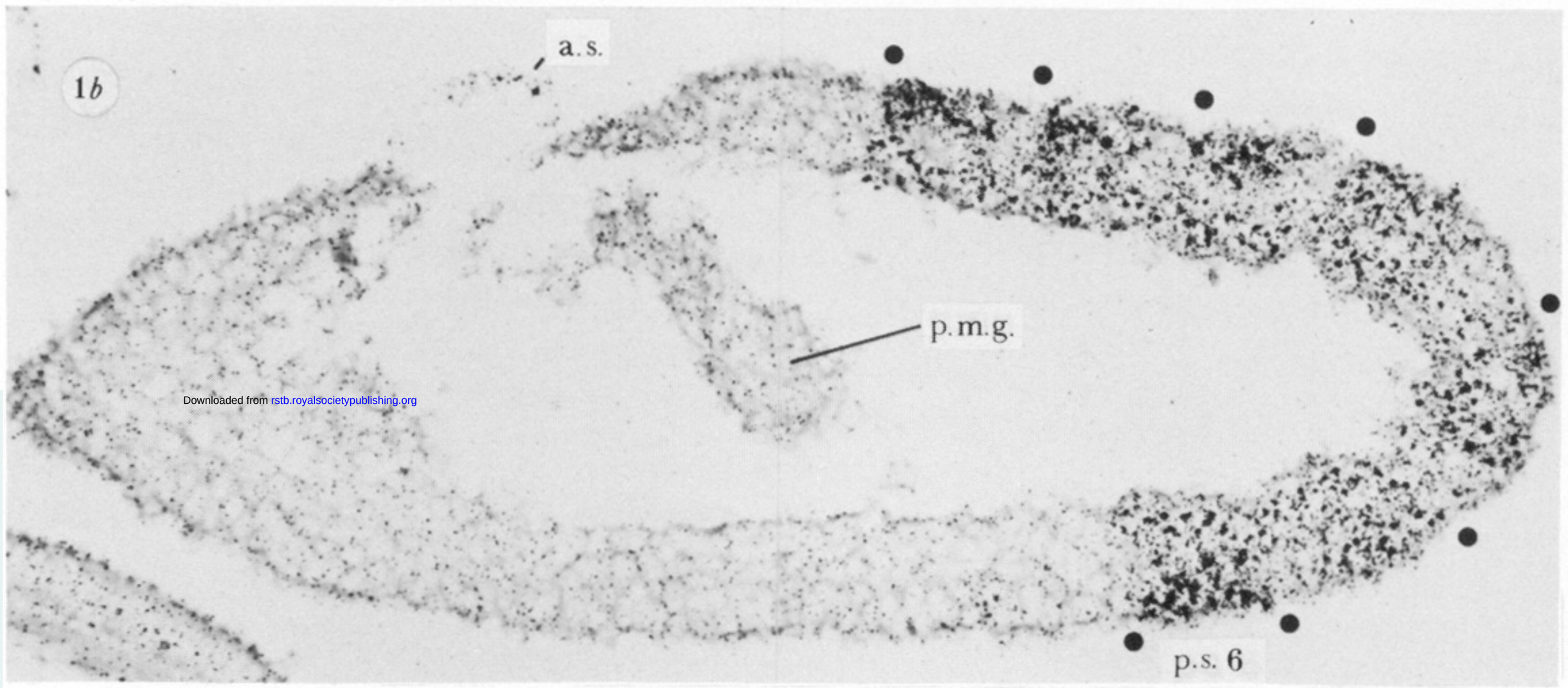

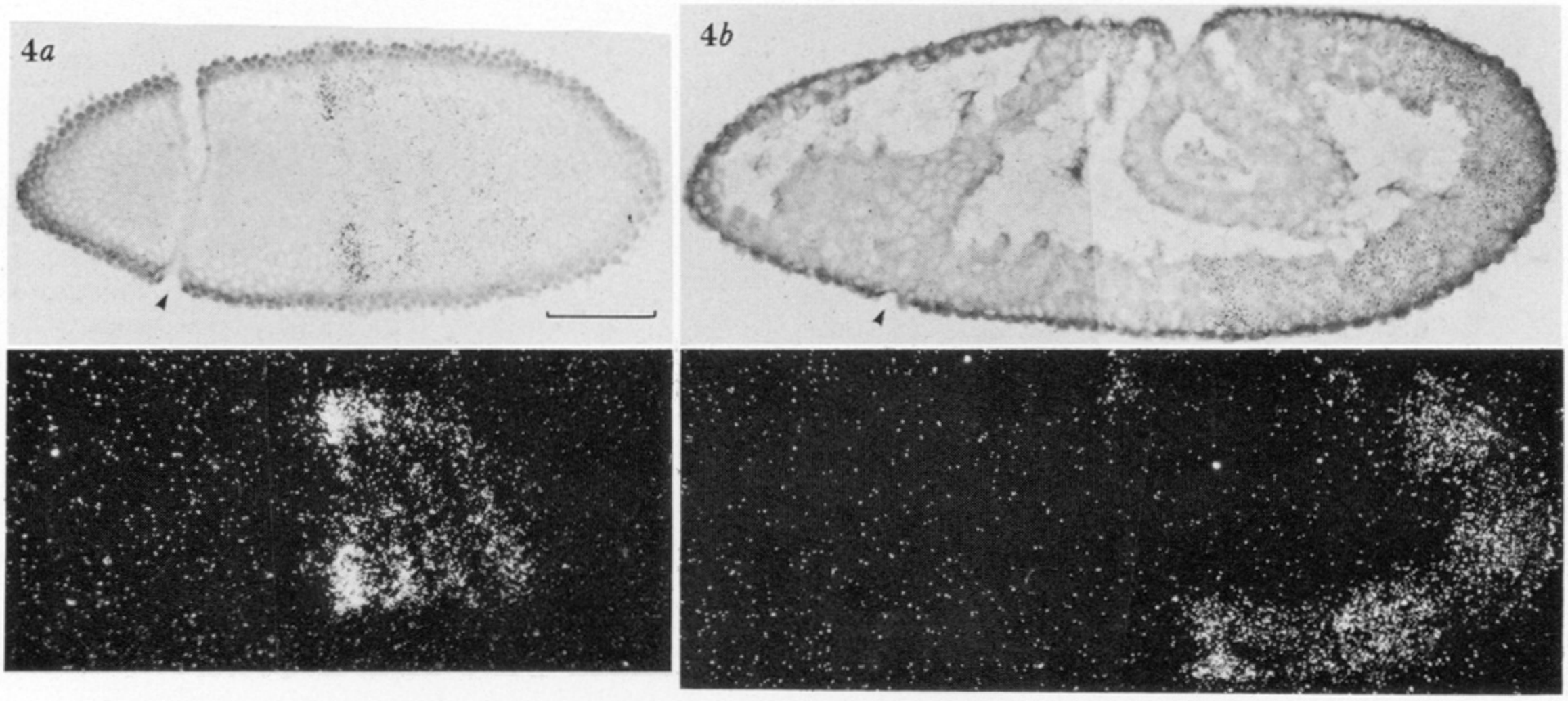

FIGURES 1 AND 4. For description see opposite.

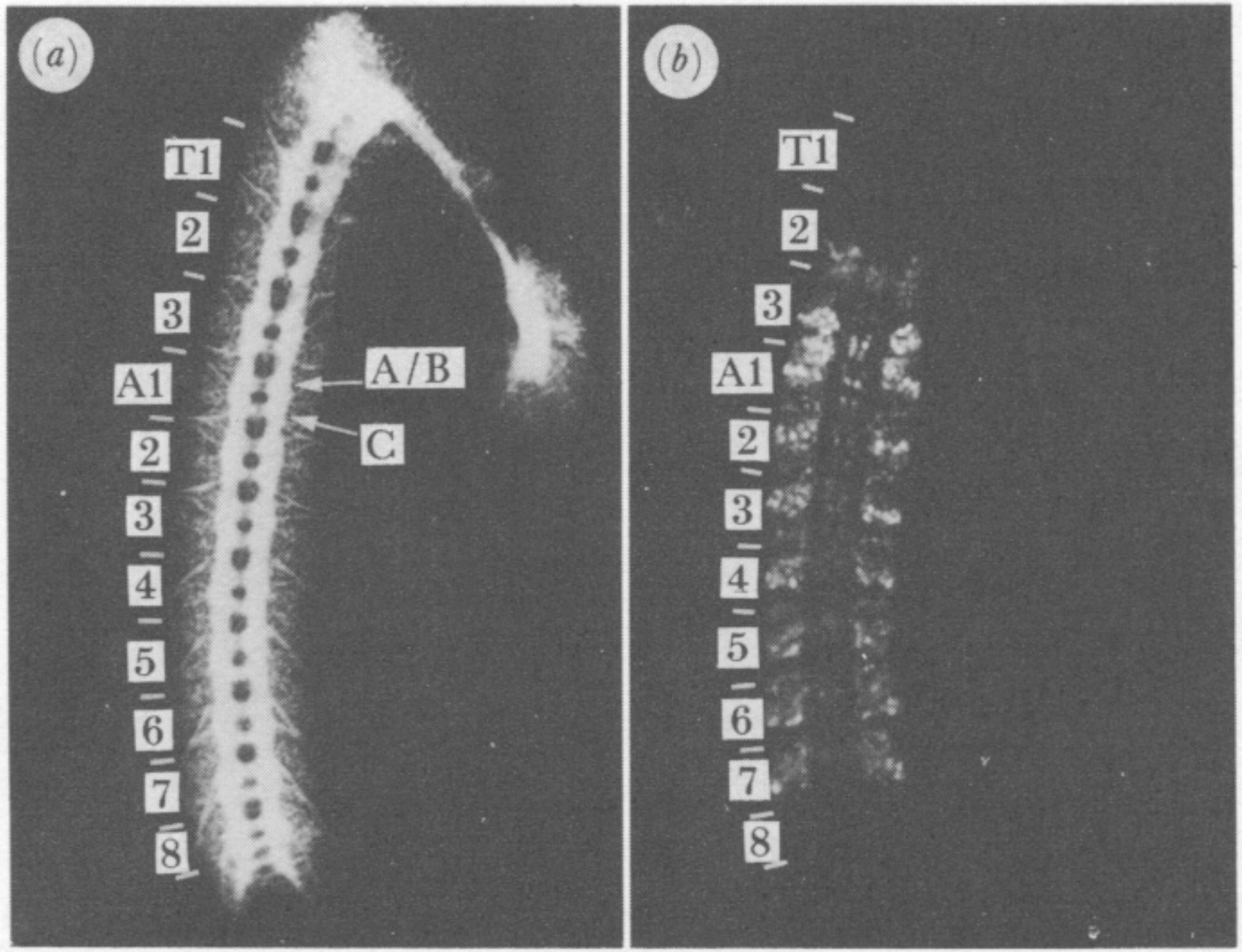

FIGURE 3. Distribution of *Ubx* proteins in the embryonic nervous system. A whole-mount preparation of the nervous system of a 13–14 h *Drosophila* embryo has been stained with antibodies to tubulin (*a*) and *Ubx* proteins (*b*). The ladder of lateral connectives reveals the segmental repeat. *Ubx* proteins define a repeating motif which is out of frame with segment boundaries, but coincident with the presumed parasegmental units of the nervous system. Parasegment 6 is prominently labelled (reprinted from White & Wilcox 1984).

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