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Genetics

Homoeosis in *Drosophila*: The Ultrabithorax larval syndrome

(determination/bithoraxoid/compartment)

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ABSTRACT **Recent results [Morata, G. & Kerridge, S. (1981) *Nature (London)* 290, 778–781] have shown that early Ultrabithorax$^-$ clones transform the posterior compartments of the adult meso- and metathoracic legs to prothorax. These transformations have not been seen in Ultrabithorax homozygous larvae, which are reported to show only transformations of the metathorax and the first abdominal segment to mesothorax [Lewis, E. B. (1978) *Nature (London)* 276, 565–570]. However, as the ventral surface of the larva does not exhibit sufficient markers to distinguish the posterior regions of these segments, cryptic larval transformations similar to those in the adult have been suggested (by Morata and Kerridge). We have further examined larvae of wild-type and various Ultrabithorax mutant genotypes, with particular attention to the dorsal surface. We find that Ultrabithorax homozygous larvae exhibit dorsal abnormalities consistent with transformations of the anterior metathorax and anterior first abdominal segment to mesothorax and of the posterior meso- and metathorax to prothorax as predicted by Morata and Kerridge; however, the posterior of the first abdominal segment remains untransformed. We suggest that in both larvae and adults the posterior first abdominal segment remains untransformed by Ultrabithorax mutations and that the unit of development with regard to the proximal bithorax complex consists of adjoining posterior and anterior compartments from neighboring segments rather than of segments themselves.**

Homoeotic mutations in *Drosophila* cause the replacement of body parts by others that normally develop elsewhere. Because of their highly specific and often dramatic effects, many homoeotic loci have been considered good candidates for genes that normally function to direct wild-type developmental pathways. The best-characterized homoeotic mutations define the tightly linked loci of the bithorax gene complex, which appear to control the development of the metathorax and all the abdominal segments (1, 2). The proximal loci of the complex control the more cephalic of these segments, and among them the Ultrabithorax (*Ubx*) locus is of special interest.

Individuals homozygous for *Ubx* mutations die as late embryos or larvae. In these homozygotes, thorax-specific markers appear in the first abdominal segment, and (in older larvae) mesothoracic spiracles develop in the metathorax and first abdominal segment (2). In doubly heterozygous combination with mutations at three other tightly linked loci, *Ubx* mutations have been shown to cause the analogous replacement of adult structures in the metathorax and first abdominal segment by those of the mesothorax (1). These and similar results have been interpreted as indicating that the mesothoracic level is the basal or ground state of development that is modified by the *Ubx*$^+$ gene product (and by other bithorax complex products) to give more posterior segmental identities (1, 3).

Recently, however, Morata and Kerridge (4, 5) examined homozygous *Ubx*$^-$ clones produced in a heterozygous background by mitotic recombination during development and observed that the adult clonal phenotype is contingent on the time of clone induction. Metathoracic clones induced later than 17 hr after egg laying differentiate mesothoracic structures in the adult, as expected; however, clones induced prior to 7 hr after egg laying differentiate prothoracic structures in the posterior compartments of both meso- and metathoracic legs. In addition, early clones in the postnotum sometimes differentiate bristles never seen in wild-type animals (see also ref. 1); Lawrence and Struhl (6) interpreted these bristles as prothoracic as well. Morata and Kerridge therefore proposed that the mesothorax is not the basal level of development with regard to the bithorax complex and that a locus (or loci) in the proximal bithorax complex is involved in mediating a developmental decision between the posterior compartments of the pro- and mesothorax.

The behavior of early clones in the posterior compartments is surprising in that the transformations appear to differ from those reported for homozygous embryos. However, all larval cuticular markers so far described—the mesothoracic spiracles and the ventral setal belts and sense organs—are in the anterior regions of segments. Morata and Kerridge therefore suggested (4) that *Ubx* lethal larvae might also contain an undetectable transformation of the posterior meso- and metathoraces to prothorax. We have further examined the phenotypes of early first instar larvae of various wild-type and *Ubx* mutant genotypes, paying particular attention to the dorsal surface. Here we describe dorsal characteristics that distinguish among the three thoracic and first abdominal segments, and we report on their status in the mutants.

MATERIALS AND METHODS

Stocks. The wild-type stocks examined were the Canton-S, Lausanne-S, Hikone A-S, Oregon-R, and Swedish-C strains. The Ultrabithorax genetic variants examined included point mutations, rearrangement breakpoints, and deficiencies. These mutants alleles were derived from the following stocks: *Ubx* point mutations—(*i*) Ubx^1 e^4/*In(3R)P*, *Dfd ca*, (*ii*) Ubx^{P8}/*In(3LR)TM1*, (*iii*) Ubx^{51}/*In(3LR)TM1*, (*iv*) Ubx^{54}/*In(3LR)TM1*, (*v*) Ubx^{104}/*In(3LR)TM1*, and (*vi*) Ubx^{03}/*In(3LR)TM1*; *Ubx* breakpoint mutations—(*i*) *In(3R)*-Ubx^{80}, Ubx^{80}/*red cv-c jvl* sbd^2 *ss* bx^{34e}, (*ii*) *In(3LR)*Ubx^{101}, Ubx^{101}/*Sb*, and (*iii*) *y*; *In(3LR)TM2*, Ubx^{130} e^s/*mwh bld* Sb^{63b}; *Ubx* deficiencies—(*i*) *Df(3R)*bxd^{100}/*Dp(3;3)P5,Sb* and (*ii*) *Df(3R)P9/Dp(3;3)P5,Sb*. All *Ubx* mutants were generously supplied by E. B. Lewis and L. Craymer. *Df(3R)P9* and *Dp(3;3)P5* are a deficiency and a duplication, respectively, of the entire bithorax gene complex (7). See Lindsley and Grell (8) for descriptions of the other mutations.

Inspection of Larval Phenotypes. Larvae were examined by phase-contrast or scanning electron microscopy. *Ubx* muta-

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Abbreviations: PRO, prothorax; MS, mesothorax; MT, metathorax; AB1, first abdominal segment.

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tions were examined in hemizygous condition over each of the deficiencies. As all combinations are larval viable, hatched larvae were mounted and mutant hemizygotes were subsequently identified by their distinctive transformation of the first abdominal ventral setal belt. For phase-contrast microscopy, eggs were collected and placed in distilled water to ensure that larvae had no opportunity to feed on hatching. Hatched larvae were mounted in lactic acid/95% ethanol (9:1) (2) and incubated at 45°C for at least 24 hr. For scanning electron microscopy, eggs were manually dechorionated and transferred with vitelline membrane intact to filter-sterilized 0.9% saline solution. Hatched larvae were fixed overnight in acidified 2,2-dimethoxypropane (9) and washed twice with absolute ethanol for several hours. The specimens were then critical point dried, coated with carbon and gold/palladium, and examined with an ETEC Autoscan U-1.

The dorsal and ventral surfaces of the thoracic and first abdominal segments were scored for the presence or absence of various sense organs as well as the morphology and pattern of denticles (ventral) and spinules (dorsal).

RESULTS

Wild-Type Larva. The different strains examined were indistinguishable with respect to all cuticular features but one, noted below.

The ventral surface. The ventral surface of the first instar larva has been described (2, 10, 11), and the reader is directed to those papers for specifics not included in the description that follows. The thoracic segments differ from abdominal ones by the presence of two pairs of distinctive sensory organs located symmetric to the midline in midsegment: medial trihairs known as Keilin organs (12) and, more laterally, sensory papillae [described elsewhere as "ventral pits" (2) or "black organs" (11)]. All thoracic and abdominal segments carry denticle belts near the anterior edge of the segment. The prothoracic and the first abdominal belts have distinctive characteristics. Although the meso- and metathoracic belts are similar (2, 11), we find that they can be readily distinguished. In the mesothoracic belt, the number of denticle rows varies, from three or four usually, across the lateral extent of the belt in a patchwork fashion, as if each larval cell secreted a small fringe of denticles. The denticles themselves are very small and fine, the anterior-most being slightly larger than the rest. In the metathoracic belt, denticles are arranged in one to three rows similar to the mesothorax; the anterior denticles are larger and more broadly based than those of the mesothorax, however, such that they have a triangular shape and appear darker under phase-contrast microscopy.

The dorsal surface. The basic features of the dorsal surface of the first instar larva have been described (11). The thoracic segments differ from abdominal ones by the presence of a single pair of sensory papillae similar to those on the ventral surface. At each lateral midline, all body segments except abdominal-8 have a distinctive long sensory hair that we refer to as a lateral sense organ. More dorsally, all segments have two pairs of small sensory hairs that are easily distinguished only in the prothorax, as they are surrounded by spinules in other segments.

All body segments carry spinules on their dorsal surface, and we find recognizable differences among them in each of the thoracic and first abdominal segments, both in their individual appearance at the anterior segmental border and in their pattern over the dorsal surface. We shall describe these aspects in turn.

The anterior spinules. The anterior limit of spinules lies at or near the segmental border (13), and in the thorax and first abdominal segment the anterior spinules are continuous laterally with the ventral setal belts. Although the morphological differences between the anterior spinules in three of these segments are ones of degree along a continuum, they are consistent enough to be useful in segmental identification. In the prothorax, the anterior-most spinules are rather erratically placed, elongate, somewhat tapered, and under phase-contrast microscopy have the pale appearance typical of more posteriorly placed spinules. In the mesothorax, two or three well defined rows of morphologically distinct spinules

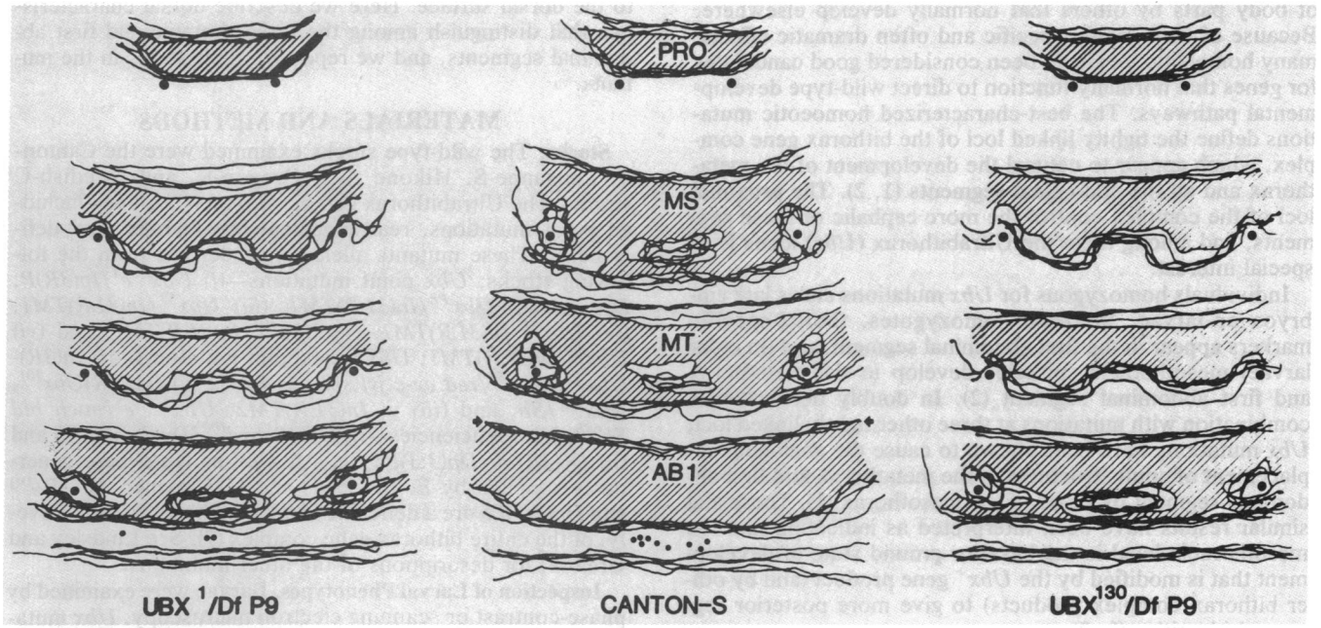

FIG. 1. Superimposed camera lucida tracings of the spinule patterns of five larvae of the indicated genotypes. Note the consistency of the patterns within genotype and the differences between wild-type and mutant patterns. The magnification of the camera lucida was adjusted to compensate for size differences among larvae. To standardize the patterns, the dorsal sensory papillae (large circles) were superimposed; in the wild-type AB1 the lateral edges of the anterior spinules (arrow) were used for standardization. Hooked spinules in the posterior first abdominal segment of the five larvae are represented as small circles; their numbers ranged from zero to three in different larvae. The cross-hatched area is covered by spinules in all five larvae of each genotype.

are present at the anterior edge: these spinules are relatively closely spaced, are short and thin, and appear noticeably darker than those immediately posterior, which resemble the spinules of the prothorax in this respect. In the metathorax, distinctive spinules are also present at the anterior edge; they are larger and more broadly based than those of the mesothorax and again appear darker than the norm. In the first abdominal segment the anterior-most spinules are larger yet and have a distinctive concave shape at the base.

The pattern of spinules. The following account is best understood by reference to Figs. 1 and 2, where Canton-S is illustrated.

The prothorax (*PRO*). The dorsal papillae are located in the anterior half of the segment. The outer pair of dorsal sensory hairs is located immediately anterior to the sensory papillae, the second pair being evenly spaced more medially. Spinules are restricted to the anterior medial portion of the segment, extending posteriorly from the anterior segmental border to the four sensory hairs. At their posterior limit, the spinules extend across the dorsal midline as far as the outer pair of sensory hairs; the lateral extent increases more anteriorly.

The mesothorax (*MS*) *and the metathorax* (*MT*). These segments exhibit a roughly similar pattern. They are distinguishable from the PRO in that the dorsal papillae are more posteriorly and laterally located in the segment and that the spinules cover the dorsal surface to a far greater extent, both posteriorly and laterally. The most posterior portion of both segments consists of naked cuticle. A "window" of naked cuticle is also present at the dorsal midline in the posterior region of the area otherwise covered by spinules, and small regions of naked cuticle are seen near the dorsal sensory papillae. In the MT the window is smaller and sometimes absent altogether, although overlap with the MS pattern is also seen. In the Oregon-R strain the spinules that define the posterior extent of the window are often absent; the spinules in more lateral regions are unaffected, however, and the pattern remains readily distinguishable from the pattern seen in *Ubx* mutants (see below). We have found that the most consistent difference between the spinule patterns of the MS and MT is in the lateral extent, spinules between the lateral sense organs and the dorsal papillae being recognizably denser in the MT.

The first abdominal segment (*AB1*). As noted before, the AB1 lacks the dorsal papillae of the thoracic segments. The spinule pattern is similar to that of the MT although it is somewhat more extended laterally and includes no naked cuticle. In addition, from one to five hooked spinules often appear near the posterior limit of the segment in the otherwise naked cuticle.

***Ubx* Larvae.** All *Ubx* hemizygotes examined exhibit the same features, although the severity of the transformations varies somewhat with allele. The following account describes a typical strong allele, as exemplified by Ubx^{130} or Ubx^{1} (Figs. 1 and 2).

The ventral surface. The PRO appears normal. It has been reported (2, 14) that the first abdominal denticle belt is replaced by a belt characteristic of the thorax, that Keilin organs appear in the AB1, and that ventral sensory papillae develop on abdominal segments 1–7. We would add to this that both the metathoracic and first abdominal belts are mesothoracic. Thus, the anterior regions of the ventral MT and AB1 apparently have been transformed to MS. It bears repeating here that the posterior ventral regions of all seg-

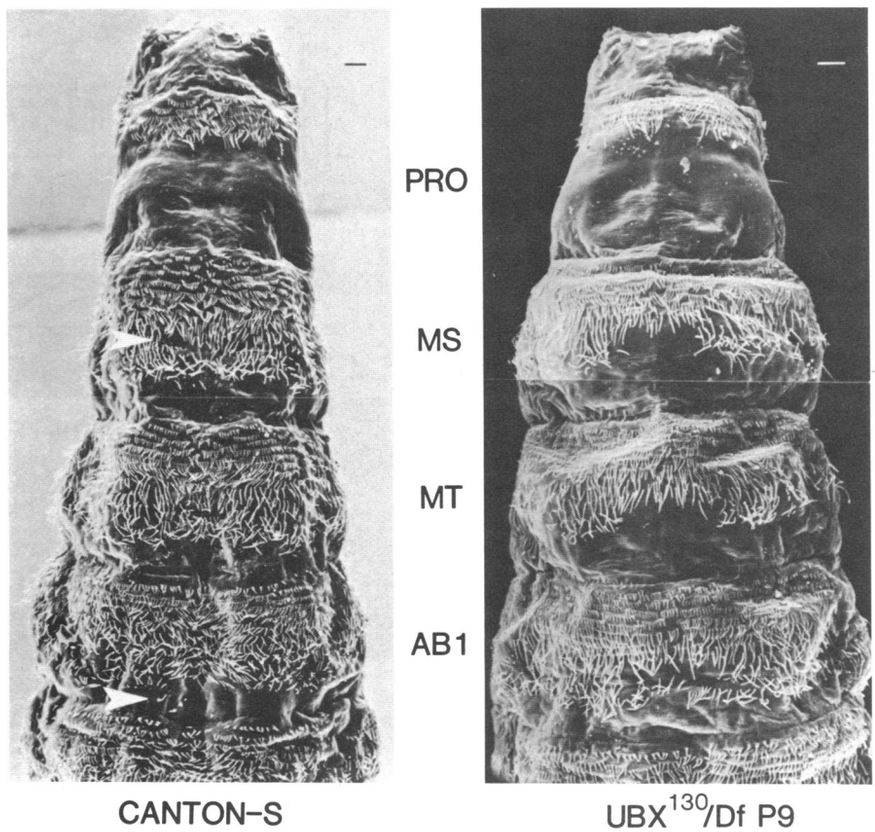

FIG. 2. The dorsal surface of the anterior segments of a Canton-S and an *In(3LR)TM2*, $Ubx^{130}/Df(3R)P9$ larva. In the wild-type larva, the mesothoracic window and posterior abdominal hooked spinules are indicated by arrows. In the mutant larva, the anterior regions of the MS, MT, and AB1 are similar in appearance. Note the posterior region of naked cuticle in the MS and MT as in the PRO. In the posterior of the AB1 spinules are present in positions typical of the wild type and, although this particular larva does not exhibit them, hooked spinules are seen in other larvae of this genotype. (Bars = 10.0 μm.)

ments consist of naked cuticle, and it is not possible to recognize transformations between them.

The dorsal surface. The PRO appears normal, but a number of changes are seen in the MS, MT, and AB1. In addition to their normal presence on thoracic segments, dorsal sensory papillae also develop on the AB1; however, they do not develop on more posterior abdominal segments as they do on the ventral surface. Major changes are also seen in the spinules of the MS, MT, and AB1.

The anterior spinules. The anterior-most spinules of the MS, MT, and AB1 are similar to those of the wild-type MS.

The pattern of spinules. The MS and MT: The spinule patterns of these two segments appear identical and are recognizably different from the wild-type pattern of either segment. The posterior extent of the spinules is reduced such that the spinules normally defining the posterior and posterio-lateral margins of the windows are absent, and the lateral extent of the spinules is also slightly reduced. We find that larvae deficient for the entire bithorax complex exhibit this pattern in abdominal segments 1–7 as well. We have noticed that a few *Ubx* alleles show a more extreme truncation of spinule pattern when homozygous than when hemizygous and will report elsewhere in detail on this finding.

The AB1: For descriptive purposes, the mutant AB1 is best thought of as composed of anterior and posterior regions, the transition point corresponding to the transition between spinules and naked cuticle in the MS and MT. In the posterior region, the posterior limit of spinules remains unaffected, and at their posterior limit spinules extend laterally to the lateral sense organs, as in wild-type. In addition, hooked spinules can be seen in the posterior naked cuticle as in wild-type. In the anterior region, however, the lateral extent of the spinules is somewhat reduced and naked cuticle is present at the midline where anterior and posterior regions join and in the regions surrounding the dorsal sensory papillae. The spinules of the anterior region thus exactly resemble those in the mutant MS and MT.

DISCUSSION

Two aspects of our results are of interest. First, in the thorax the mutant phenotypes are consistent with a transformation of posterior MS and MT to PRO as predicted by Morata and Kerridge (5): posterior spinules are replaced by naked cuticle, and the PRO furnishes the only serially homologous region of naked cuticle on the dorsal surface. The demarcation line between anterior and posterior regions apparently extends laterally through the window of naked cuticle and passes behind the dorsal sensory papillae. We have no evidence that these regions are equivalent to compartments. Our results suggest that the abnormal bristles seen in *Ubx* clones in the adult postnotum do indeed represent posterior PRO.

Second, the posterior region of the AB1 clearly remains completely untransformed. This finding is in contradistinction to the current view (refs. 2 and 15, Fig. 3*a*) that *Ubx* mutations transform the entire AB1. We have noted that the larval cuticular markers used by earlier workers cannot distinguish between the posterior regions of segments. Jimenez and Campos-Ortega (16) found a thorax-specific marker in the neuromeres of the larval nervous system and observed that in Ubx^1 mutants it is also present in the presumptive AB1. However, as the marker consists of a band of cells located at the anterior margin of the thoracic neuromeres it may not be indicative of the determined state of more posterior regions. Thus there is no evidence for a transformation of the posterior AB1 in *Ubx* mutant larvae.

Since *Ubx* mutations behave as recessive lethals they cannot be directly studied in the adult. However, the neighboring bithoraxoid (*bxd*) locus transforms the posterior region of the *Ubx* domain when in *trans* with *Ubx* mutations, and a discussion of *bxd* mutations is thus germane.

In adults, *bxd* mutations transform the posterior compartment of the MT to MS and it has been reported that they transform the entire AB1 to MT (2, 17). However, some recent evidence suggests that only the anterior AB1 is transformed. Kornberg (18) has reported the existence of a posterior compartment of the adult AB1, which he believes derives from the posterior dorsal histoblast nest. It occupies a very small region called the unpigmented band, which carries trichomes but no chaetae. Kerridge and Sang (17) have reported that in *bxd* third instar larvae the anterior dorsal histoblast nests of the AB1 are absent but the posterior nests remain intact. In addition, Minana and Garcia-Bellido (19) have recently reported that the posterior AB1 remains untransformed in adult *Ubx* gynandromorphs. We therefore examined adults of the genotype $bxd^1/Df(3R)bxd^{100}$ (data not shown). Because of the topography of the region and the fragile nature of the material it was not possible to score unambiguously every specimen, but in many flies the unpigmented band was clearly untransformed.

It remains necessary to consider the origin of markers specific for the posterior compartment in the fourth legs (17) and the very rare abdominal halteres (1) generated in *bxd* mutants, and we suggest that they may be derived from anterior cells. It is well established for mature leg discs that certain anterior disc fragments can regenerate the posterior compartment (20). In a more analogous situation to the present case, studies of temperature-sensitive cell lethality have shown that virtually complete legs can be formed from a small number of anterior cells and that respecification of cells from anterior to posterior compartments occurs before

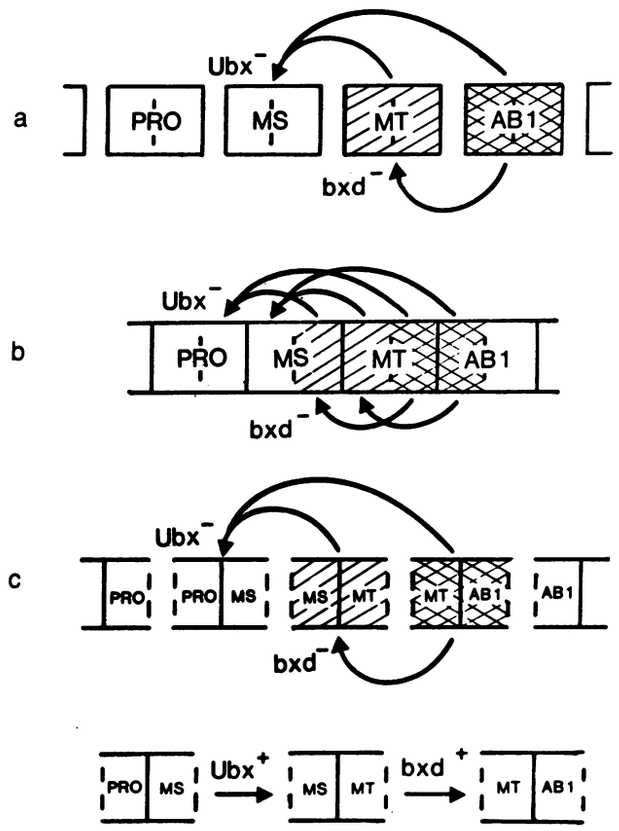

FIG. 3. (*a*) Classic view of Ubx^- and bxd^- transformation based on segmental units (1). (*b*) Ubx^- and bxd^- transformations currently postulated. (*c*) (*Upper*) The transformations pictured in *b* were redrawn in units of posterior–anterior compartments. (*Lower*) Proposed wild-type functions of Ubx^+ and bxd^+ transcripts.

the initiation of cell division in the blastema (21). In haltere discs, by contrast, regeneration of posterior compartment markers from anterior disc fragments has been reported to not occur (22). We suggest that the extremely low frequency of abdominal halteres (0.01%, ref. 17) may reflect an analogous difficulty in forming a blastema from purely anterior cells. Therefore, we feel that the best synthesis of the evidence is that in both larvae and adults the posterior AB1 remains untransformed by *Ubx* and *bxd* mutants. Thus, *Ubx* mutations alter determinative decisions in the posterior MS through the anterior AB1, a domain equivalent in size to the classic view but shifted anteriorly by one-half segment from it (Fig. 3*b*).

Recent molecular biological studies (15, 23) have shown that two large transcripts correspond to the regions defined by *Ubx* and *bxd* mutations and that a very complex pattern of processing of these transcripts occurs during development. When one considers the extreme transformations caused by breakpoints in the coding regions, however, a very simple if surprising picture emerges (Fig. 3*c*). *Ubx* mutations transform both the [posterior MS and anterior MT] and [posterior MT and anterior AB1] to [posterior PRO and anterior MS], while *bxd* mutations transform the [posterior MT and anterior AB1] to [posterior MS and anterior MT]. We therefore propose that the bithorax complex (and perhaps other similar homoeotic loci) does not recognize segments as developmental units but rather treats adjoining pairs of posterior and anterior compartments from neighboring segments as such units. In support of this idea, we emphasize that anterior–posterior compartmental boundaries and segmental boundaries are indistinguishable with respect to time of establishment and clonal inviolability during normal development (24, 25). Further, cuticular transplantation experiments (26) and mutations in the process of segmentation (27) suggest that the positions of segmental boundaries are assigned in a manner no different from that of other cuticular features. We propose that *with respect to the bithorax complex* the basal level of development is a unit comprised of [posterior PRO–anterior MS] (Fig. 3*c*). The bithorax complex genes would then alter this developmental level in a manner similar to that originally envisioned by Lewis (1). The Ubx^+ transcript would raise the basal level to [posterior MS–anterior MT] in two more posterior units; the bxd^+ transcript would further raise the second of these units to [posterior MT–anterior AB1].

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