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Larval Adipose Tissue of Homoeotic Bithorax Mutants of *Drosophila*

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In the homoeotic bithorax mutant combination bx^3 *pbx*/Ubx^{105} of *Drosophila melanogaster*, the metathoracic segment is transformed to a mesothoracic segment and the adult flies have an extra pair of wings in place of the paired halteres [Lewis, E. B. (1963). *Amer. Zool.* **3**, 33–56]. The morphology of the larval fat body, the number of cells in the fat body, and the distribution pattern of kynurenine autofluorescent materials (KAF+) in this tissue were compared in the homoeotic mutant and a wild-type strain. The mutant has an additional mass of adipose cells anterior to the posterior margin of the ventral commissure of the fat body. However, the total number of adipose cells in the two strains as well as the limits of the KAF+ cell population do not differ. Therefore, the bithorax transformation in the larval fat body involves rearrangement of the same cell population as that in the normal strain. This study suggests (1) that the bithorax mutant genes affect the pattern of cell segregation and/or migration of preblastoderm nuclei during embryogenesis and (2) that the larval fat body of *Drosophila* has a segmental origin.

INTRODUCTION

Five functionally related genes of the bithorax complex locus effect transformation of structures in the mesothorax, the metathorax, and the first abdominal segment of *Drosophila melanogaster* (Lewis, 1963, 1964). Among these genes, *bithorax* (*bx*) alters the anterior metathoracic segment such that it resembles the anterior part of the mesothorax and *postbithorax* (*pbx*) transforms the posterior part of the metathorax to the morphology of the posterior mesothorax. The regional differentiations of the segments affected by these two mutants are independent of each other since the double mutant strain (bx^3 *pbx*) shows complete transformation of the metathoracic region to a mesothoracic segment. In these flies, the halteres have been changed into a second pair of wings.

The cuticular structures of the adult fly arise from metamorphosis of the imaginal discs during the pupal period. In the larva, the various imaginal discs are morphologically distinguishable and their positions are anatomically distinct. Wing discs are larger than haltere discs, and the increase in cell number of the transformed haltere discs in bithorax mutants is evident in the larval stage. That bithorax gene action affects development considerably earlier than this time was first suggested by Gloor's (1947) demonstration that bithorax phenocopies result from ether treatment of embryos at 0–6 hr. Capdevila and Garcia-Bellido (1974) reexamined the kinetics of ether-induced phenocopies in wild-type individuals and various combinations of mutants in the bithorax complex and found that the phenocritical period for bithorax phenocopies includes the preblastoderm stage before the migrating syncytial nuclei have reached the cortex of the egg. Action of the bithorax complex at this early time in development suggests that larval structures should also show morphologic effects, provided their embryonic origin lies within the domain of the affected segments. Lewis (1964) noted that larvae of the *Ultrabithorax* (*Ubx*) strain as well as several double mutant combinations develop spiracles on the metathoracic and first abdominal segments in addition to the normal anterior pair of spiracles. These organs as well as the trans-

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formed adult structures are modifications in the morphology of the exoskeleton, so the question remains whether internal larval structures are also influenced by bithorax mutant transformations. In the adult, the transformed metathorax does not contain flight muscles (El Shatoury, 1956), indicating that modifications of internal structures may not coincide with those of cuticular origin.

The differentiation of the internal larval tissues of *Drosophila* is completed by 18 hr of embryogenesis (Poulson, 1950), and cells of these tissues, such as the salivary glands, malpighian tubules, and fat body, do not continue to divide during the larval period, but rather grow by increase in cell size. It is relatively easy to determine the number of cells in these tissues due to their large size, so the effects of a bithorax transformation on a larval tissue can be inferred by counting cell numbers in various regions of the transformed tissue and comparing this distribution pattern with that of the untransformed pattern. For such a comparison, we selected the larval fat body since it displays characteristic morphological and functional differentiation (Rizki, 1961; Rizki and Rizki, 1970) in the body segments that are affected by the bithorax genes.

MATERIALS AND METHODS

Ore-R wild-type and sbd^2 bx^3 *pbx/T(2:3)* Ubx^{105} larvae were grown at 24°C on standard corn meal-molasses medium seeded with live yeast. Fat bodies were dissected from mid-third-instar female larvae in *Drosophila* Ringer solution, fixed in Carnoy, and reacted with Feulgen reagent. Only intact and complete fat body dissections were used. The images of the whole-mount dissections were projected through an inverted microscope, and the tissue outlines along with the distribution of nuclei were traced for counting.

For examination of autofluorescence in fat body cells, the procedures used previously were followed (Rizki, 1964). White and early tan puparial stages were used for this aspect of the study to assure that the kynurenine autofluorescence pattern was fully developed and intense. Freshly dissected, whole-mount preparations were projected through a photographic enlarger onto a lantern slide plate, which was later developed to obtain an outline of the fat body tissue. The dissections were then examined and photographed with the fluorescence microscope. Since the field of the fluorescence microscope is limited to a small area excited by the 365-nm wavelength, several overlapping photographs of each specimen were required to record the pattern of kynurenine autofluorescence. A montage of these photographs was then compared with the photograph of the entire tissue outline to establish the limits of the autofluorescent cell distribution. In these preparations, total cell counts were not made.

RESULTS AND DISCUSSION

A photograph of a bx^3 *pbx/*Ubx^{105} adult is included with a photograph of an *Ore-R* wild-type male to illustrate the marked difference between the adults of the two strains used in this study (Fig. 1). The external morphology of the larvae of these strains is similar, but the pattern of regional differentiation of the larval fat bodies differs. As described previously (Rizki, 1969), the fat body of the *Ore-R* larva consists of a single layer of cells forming a continuous sheet of tissue extending from the anterior region of the body to the caudal segment. Figure 2a is a freshly dissected, complete fat body of an *Ore-R* larva suspended in Ringer solution. The fat body is bilaterally symmetrical, and the salivary glands with their common duct have been retained during dissection so as not to disturb attachments with the fat body. The anteriormost adipose cell mass, which is roughly triangular in shape, adheres to the salivary gland and overlies the cerebral hemispheres in the body of the larva. A single strand of adipose cells which are in close apposition to the salivary glands connects this piece to

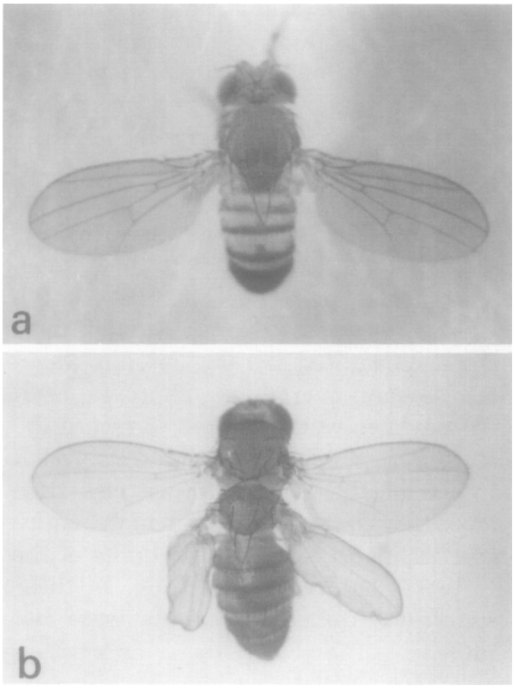

FIG. 1. (a) *Ore-R* adult male showing the mesothorax with a pair of wings and the metathorax with halteres; (b) bx^3 pbx/Ubx^{105} newly emerged male showing transformation of the metathorax to a well-formed mesothoracic segment with wings.

a V-shaped commissure which lies in the midventral region of the larva. The commissure joins the two main lateral masses of adipose cells at their anterioventral margin. The gonads are located approximately midway between the anterior and posterior limits of the main lateral adipose tissue masses, and a long strand of adipose cells, one end of which is connected with the postgonadal mass of the fat body on each side, runs dorsally parallel to the heart and dorsal aorta.

A freshly dissected fat body from a bx^3 pbx/Ubx^{105} larva is shown in Fig. 2b. The anteriormost triangular mass has retained its relationship with the salivary gland in a position comparable to that of the *Ore-R* fat body. However, the anterior margin of the large lateral fat masses has shifted forward with relation to the posterior edge of the ventral commissure so that the anteriormost triangular mass is almost duplicated in morphology. This modification is particularly apparent in the right half of the specimen in Fig. 2b. Additional strands of adipose cells connecting the commissure and more posterior portions of the lateral masses are present, but the morphology of the fat body posterior to these rearrangements is similar to that of the *Ore-R* fat body.

Since the transformed haltere disc of bx^3 pbx/Ubx^{105} contains more cells than its normal counterpart, it is reasonable to question whether the morphological alterations in the anterior portion of bx^3 pbx/Ubx^{105} fat body are achieved by extra cell division. In other words, are the additional cells anterior to the commissure a result of extra cell division of precursor cells for this specific region of the fat body? To answer this question, the total number of cells in fat bodies of *Ore-R* and bx^3 pbx/Ubx^{105} was counted. This information is summarized in Table 1. There is no difference between the two sets of data, suggesting that the difference in morphology between the two groups of fat bodies is due to a rearrangement of the cells in the affected regions rather than additional cell division. An explanation that appears less likely, since it is more complicated, is the possibility of a concomitant proliferation of cells within one region and inhibition of cell division in the adjoining regions of the fat body.

Shortly before pupariation, the cells in the anterior portion of the larval fat body accumulate kynurenine in cytoplasmic inclusions which can be seen in freshly dissected, unfixed fat body by examination with the fluorescence microscope (Rizki and Rizki, 1970). Under standard feeding conditions, there is a sharp line of demarcation between the anterior cell population that shows kynurenine autofluorescence (KAF+) and the posterior cells that lack this material (KAF−). The approximate position of this boundary (Figs. 2a and 4a) occurs within the region of the fat body that is affected in the bithorax transformation. Therefore, bx^3 pbx/Ubx^{105} fat bod-

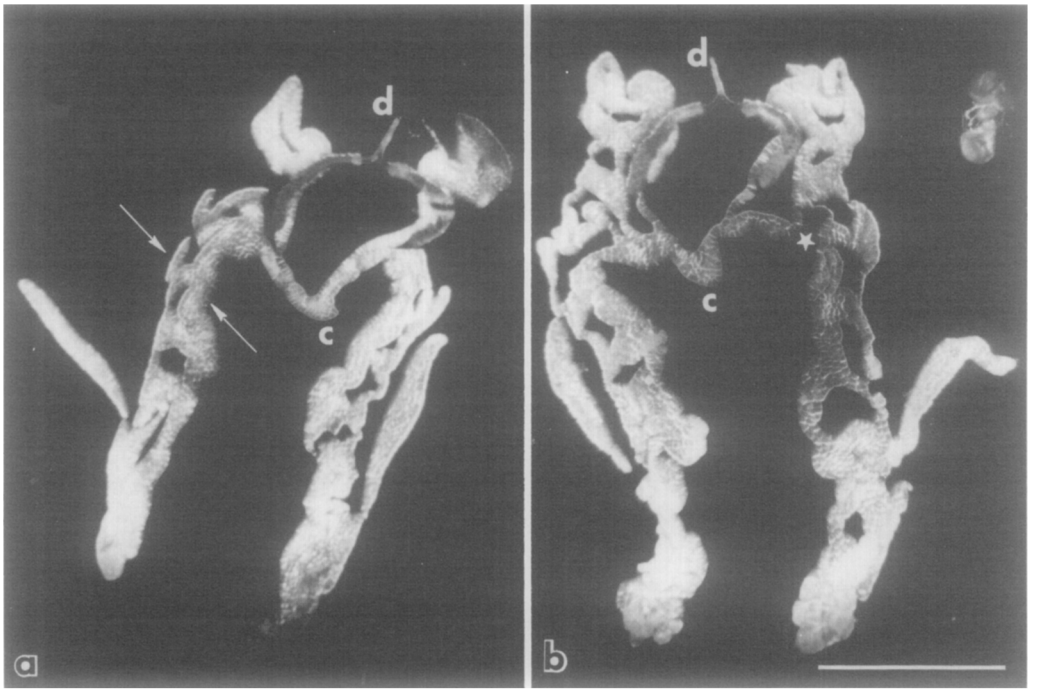

FIG. 2. Photographs of freshly dissected larval fat bodies from the *Ore-R* (a) and bx^3 pbx/Ubx^{105} (b) strains suspended in *Drosophila* Ringer solution. For information on the disposition of the fat body masses within the body cavity, see the text. The bilaterally symmetrical fat body is held together in the anterior region by the common salivary duct (d) and the V-shaped ventral commissure (c) of adipose cells which joins the two lateral fat body masses. Compare the arrangement of the fat body masses in the two specimens by noting their disposition along the salivary glands and the position of the strands of adipose cells connected to the ventral commissure. The arrows indicate the approximate posterior extent of KAF+ cells in the *Ore-R* specimen. The continuity of the adipose cells in specimen b has been broken (star) during manipulation for photography. Note the sizes of the mesothoracic and metathoracic discs from this specimen (b) in the upper right corner. The bar represents 1 mm.

TABLE 1

NUMBER OF CELLS IN THE LARVAL FAT BODY[a]

Ore-R	bx^3 pbx/Ubx^{105}
2318	2254
2064	2264
2263	2121
2026	2134
2383	1903
	2254
2211 ± 71	2155 ± 57

[a] $t = 0.44$, df 9, $P > 0.70$.

ies were examined with the fluorescence microscope to determine the distribution of KAF+ cells when the morphology of the anterior fat body has been modified by a bithorax transformation.

A photographic montage showing the posterior limit of KAF+ cells in a bx^3 pbx/Ubx^{105} fat body is shown in Fig. 3. This region of the same specimen projected through a photographic enlarger onto a photographic plate (inset) is included for purposes of comparison with Figs. 2b and 4b. The boundary between KAF+ and KAF− cells in bx^3 pbx/Ubx^{105} fat bodies is sharp and occurs in the lateral region adjoining the ventral commissure. This KAF+ cellular distribution pattern was consistent for all bx^3 pbx/Ubx^{105} specimens (N = 11) that were examined. Since the total number of cells in the fat bodies of *Ore-R* and bx^3 pbx/Ubx^{105} is the same but the boundary of the KAF+ cell population has moved forward with relation to the

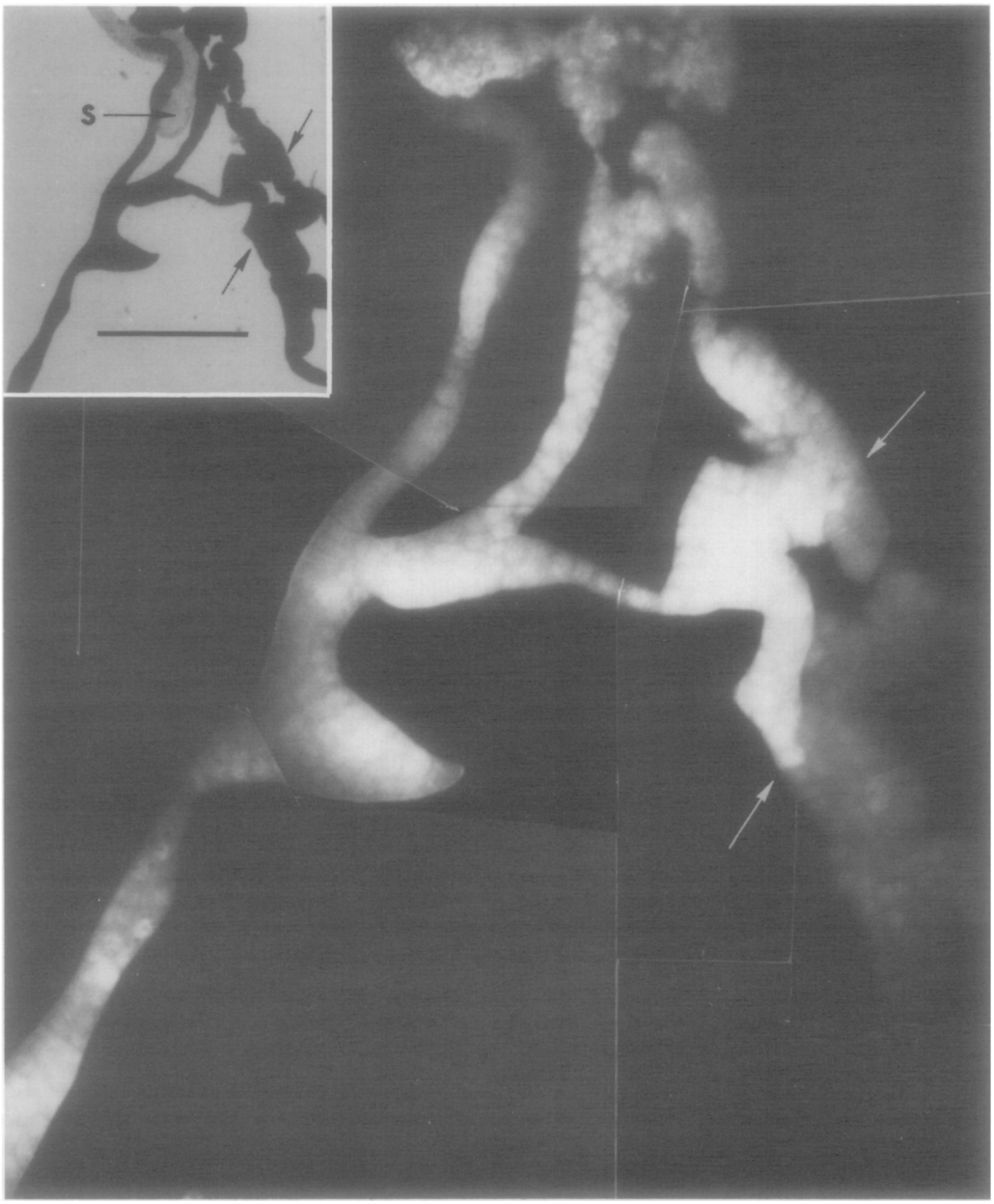

FIG. 3. A montage showing the distribution of KAF+ cells in bx^3 pbx/Ubx^{105} fat body. All cells in this montage are KAF+ (sky blue) except the cells extending from the position indicated by the arrows to the right margin of the frame. The variability in autofluorescence is an artifact due to two factors: (1) The coverglass is floating on the specimen in Ringer solution and all cells are not at the same optical level, resulting in unequal excitation from the uv light converging from the dark-field ultracondenser; (2) the field of excitation is small, so the center of each frame is more intense than the borders. The border between the KAF+ and KAF− cells was positioned in the center of the field for photography; the KAF− cells show autofluorescence due to pteridines which decay on exposure to uv light. The inset is a photograph of the same region projected through a photographic enlarger onto a photographic plate. All fat body tissue is uniformly dark and the salivary gland(s) is translucent. Note the absence of autofluorescence of the salivary gland. The bar represents 1 mm.

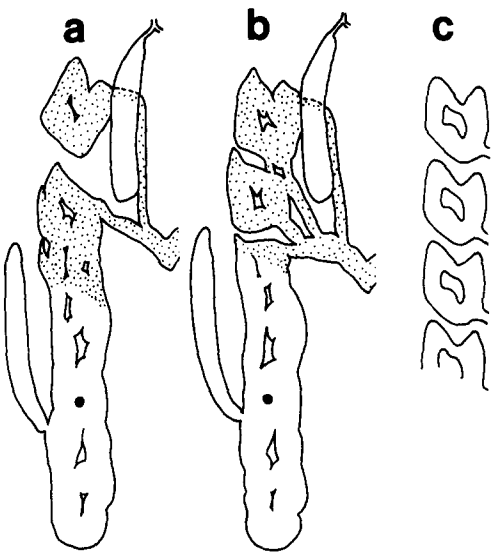

FIG. 4. Semidiagrammatic representation of the left half of the larval fat body of *Ore-R* (a) and bx^3 pbx/Ubx^{105} (b), showing the region of KAF+ cells (stippled). These diagrams are based on camera lucida drawings of fresh dissections and whole-mount preparations of dissections. For orientation, note the position of the salivary gland and common salivary duct; the ovary (solid circle) is located in the posterior half of the main lateral fat body mass. Diagram c represents the metameric origin of the larval fat body of *Drosophila* deduced from comparison of the normal and bithorax transformation.

posterior margin of the ventral commissure, it is apparent that the KAF+ region in bx^3 pbx/Ubx^{105} fat bodies approximates the total KAF+ region in *Ore-R* fat bodies. In the bithorax transformation, fat body cells have undergone anatomical rearrangement without functional modification of the rearranged cells.

When wild-type haltere disc cells are mixed with wild-type wing disc cells, the two cell types segregate, whereas the cells from haltere discs of bx^3 will integrate with wild-type anterior-wing disc cells and the transformed haltere disc cells of *pbx* will integrate with wild-type posterior-wing disc cells (Garcia-Bellido and Lewis, 1976). The cellular affinities displayed by the bithorax discs in mixing experiments are thus those of their transformed states, and Garcia-Bellido and Lewis concluded that the effects of the bithorax mutants are cell autonomous, reflecting their genotypic constitution rather than segmental origin. In the bx^3 pbx/Ubx^{105} larval fat body, altered patterns of cell adhesion are reflected in the altered organization of groups of fat body cells.

In addition to the developmental effect on cell surface properties associated with the transformation of the haltere disc, the bithorax mutations increase the number of cells in this disc. The effect on cell number has recently been confirmed by Morata and Garcia-Bellido (1976), who analyzed the frequency and sizes of clones induced after irradiation of wild-type and bithorax mesothoracic and metathoracic discs. Their study indicates that the initial as well as the final number of cells in the transformed haltere disc is the same as that of a normal wing disc. In evaluating the effects of the bithorax genes on adipose tissue, it should be recalled that the cells become nondividing following embryogenesis and increase their genomic content by polyteny, whereas the imaginal disc cells continue to multiply by mitosis during the larval period. Our observations on the larval fat body indicate that the total number of cells in this tissue is not affected by the bithorax transformation. Therefore, the tissue reorganization associated with the mutant genes was achieved by changes in the positional patterns of cells, involving either changes in the migration of the preblastodermal nuclei or changes in the distribution of cells at the early cellular blastoderm stage.

The pattern of regional differentiation of the larval fat body cells of *D. melanogaster* has been studied by utilizing mutant genes whose influence is limited to specific regions of this tissue (Rizki, 1969). Analysis of the distribution of fat body cells showing a KAF+ phenotype has been particularly useful, since this cell trait is autonomous as demonstrated by examination of gynandromorphs containing *v*+ (normal) and *v* (mutant lacking kynurenine synthesis) cells (Rizki and Rizki, 1968). On the standard *Drosophila* diet, the pattern of KAF+ cells in the *Ore-R* fat body is specific and shows

a discrete boundary between KAF+ and KAF− cells. These two cell types are side by side in the fat body and their junction occurs within the main lateral fat mass at a site not distinguishable by morphological landmarks or routine histological examinations. An explanation for this intercellular relationship within the fat body is proposed in Fig. 4. According to this model, the larval fat body is a metameric tissue in which cells of adjoining segments become morphologically integrated as a continuum during development. This scheme requires that the functional differentiation of fat body regions related to the segmental origin of the cells is retained. The fat body is thus an ordered mosaic of cell groups, and segmental reorganization in the bx^3 pbx/Ubx^{105} mutant combination results in new morphological relationships without affecting the KAF+ function of the cells.

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