DEVELOPMENTAL BIOLOGY 86, 363-372 (1981)

Compartments in the Abdomen of Drosophila and The Role of the *engrailed* Locus

THOMAS KORNBERG

Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

Received February 17, 1981; accepted in revised form April 27, 1981

A clonal analysis has shown that the dorsal surface of the first abdominal segment of *Drosophila melanogaster* is subdivided into anterior and posterior compartments. Cells of the posterior compartment grow up to but not beyond the anterior-posterior compartment border within the first abdominal segment and the intersegmental border that defines the boundary between the first and second abdominal segments. Growing within these boundaries, a narrow band of tissue clonally isolated from the adjoining tissue is formed. When these posterior cells are deficient for the *engrailed* locus, however, neither the compartment nor the segment border is maintained. The implications, that compartmentalization is essential for segmentation, and that all insect segments are subdivided by anterior and posterior compartments, are discussed.

INTRODUCTION

The cells that form the insect epidermis grow without defined lineage programs but with prescribed spatial limitations. Boundaries, some coincident with obvious morphological subdivisions, others featureless, transect the insect to limit the growth of the epidermal cells. These boundaries are lines demarking where cells of one group fail to mix with cells of a neighboring group, and thus represent geographical limits to the growth of an individual cell group.

In *Drosophila* and in the milkweed bug *Oncopeltus*, the individual segments have independent lineages from a very early stage: epidermal cells can grow up to and along a segment border, but can never cross it (Lawrence, 1973; Lawrence *et al.*, 1978). Garcia-Bellido *et al.* (1973, 1976) discovered that similar developmental restrictions can subdivide a single segment and termed the segmental subdivisions "compartments." For example, cell clones marked by somatic recombinations are found to be confined to grow entirely within either the anterior or posterior compartment of a mesothoracic (wing) segment, although the two compartments grow juxtaposted as a single imaginal disc throughout larval development; thus, the apparently homogenous sheet of imaginal disc cells which gives rise to the adult wing is in fact discontinuous. Subsequent work has shown that many of the other imaginal disc derivatives, the proboscis (Struhl, 1977), the antenna (Morata and Lawrence, 1979; Baker, 1978), the legs of all three thoracic segments (Steiner, 1976; Wieschaus and Gehring, 1976; Lawrence *et al.*, 1979), and the terminalia (Dubendorfer, 1977) are similarly subdivided into anterior and posterior compartments. Such restrictions limiting the fate of the epidermal cells are thought to reflect a developmental committment that may be genetically controlled.

The functional significance of these developmental discontinuities is not understood. Garcia-Bellido (1975) has proposed that compartmental subdivisions reflect the mechanism that reduces the developmental potential of groups of cells in a stepwise manner. This compartment hypothesis proposes that a small number of controlling genes (selector genes) effect these developmental switches by their expression in only one of a pair of compartments. Studies of mutations of the *engrailed* genes have supported this idea. Experiments with viable (Lawrence and Morata, 1976) and lethal alleles (Kornberg, 1981) indicate that the wild-type function of the *engrailed* gene is necessary for the normal development of the cells of the posterior wing compartment and for the maintenance of the boundary between the anterior and posterior compartments of the wing blade; in contrast, anterior cells develop normally in the absence of a wild-type *engrailed* function. The phenotype of the *engrailed* mutants is thus consistent with control of the posterior developmental pathway by the *engrailed* locus.

The importance of compartmental clonal restrictions as a general developmental mechanism must depend in part upon their ubiquity. A mechanism with broad significance should function in all of the adult segments in addition to the imaginal disc derivatives and should also function in the embryonic and larval tissues. To date, subsegmental compartments have been found only in the imaginal disc-derived structures of the adult epi-

0012-1606/81/120363-10$02.00/0

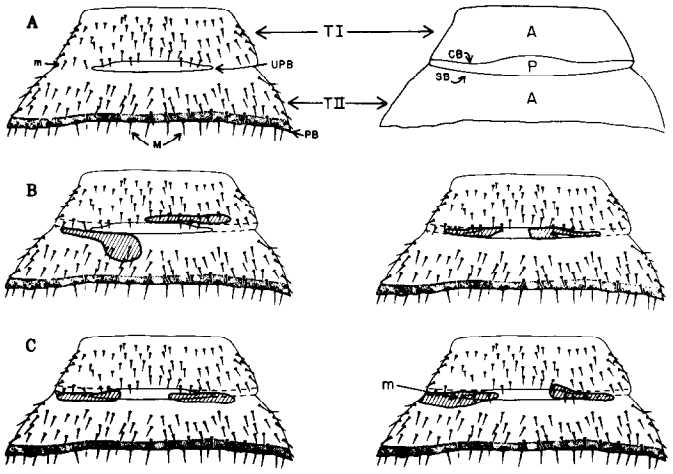

FIG. 1. Morphology and clonal subdivisions of the dorsal abdomen. (A) Camera lucida drawings of the entire tergite I and anterior portion of tergite II. The pertinent morphological details (left) and the location of the clonal restriction boundaries (right) are indicated. First tergit (TI); second tergite (TII); anterior compartment (A); posterior compartment (P); macrochaete (M); microchaete (m); unpigmented band (UPB); pigmented band (PB); compartment border (CB); segment border (SB). (B) The left figure depicts anterior clones in the first and second abdominal tergites that meet the compartment borders and segment, respectively. The right figure illustrates two clones in the posterior compartment of the first abdominal tergite that meet the segment and compartment borders. (C) These figures illustrate the occurence of tergite I bristles within the *engrailed* clones and the failure of these clones to respect the compartment or segment borders.

dermis but not in the epidermis of the adult abdominal segments or in internal organs. However, studies of lethal *engrailed* alleles indicate that the normal function of this gene is necessary for the normal development of the embryo and larva. Embryos homozygous for lethal *engrailed* alleles die late during embryonic developmental, defective in the morphogenesis of the head, thoracic, and abdominal epidermis (Kornberg, 1981). Given the specificity of the *engrailed* locus for the development of only the posterior cells in the adult compartments and the likelihood that the locus plays a similar role during different developmental periods, these results strongly suggest the existence of posterior compartments requiring *engrailed* function in the body segments where compartments have not yet been found—in the adult abdomen and in the segments of the embryo and larva. The experiments described here examine the cell lineage of the first segment of the adult abdomen and assess the requirement for the *engrailed* locus in this structure.

MATERIALS AND METHODS

Clonal analysis. Cells homozygous for the lethal en^{LA4} allele were produced by X irradiation with a dose of 1000 R. Clones produced by mitotic recombination were marked by the cell marker mutants *straw* (2-55.1; Lindsley and Grell, 1968) and *pawn* (Garcia-Bellido and Dapena, 1974). *straw* bristles and hairs are yellowish and pale; *pawn* bristles are short and pale at the tips; *pawn* abdominal hairs are very short. In combination, the hairs and bristles of the dorsal abdominal cuticle that are *straw* and *pawn* can be easily distinguished from the surrounding wild-type tissue (see Fig. 3). As diagrammed in Fig. 2, mitotic recombination between the locus of *straw* and the centromere results in clones of cells marked with *straw* and *pawn*; these clones grow more rapidly because they are homozygous for $M(2)c^{33a+}$ (Morata and Ripoll, 1975). However, the relative growth advantage of the $Minute^+$ cells in the abdominal epidermis is apparently not as great as the relative growth advantage of $Minute^+$ imaginal disc cells, because the absolute size of the tergite clones is smaller than the clones that have been observed in the imaginal disc-derived structures. Two crosses were made: Cross 1, for the production of control *straw pawn* clones to describe the normal development of the abdominal histoblasts,

$$♀\ stw\ pwn/SM5 \times ♂\ M(2)c^{33a}/In\ (2LR)bw^D,$$

Cross 2, for the production of en^{LA4} clones marked with *straw* and *pawn* to describe the development of *engrailed* abdominal histoblasts,

$$♀\ stw\ pwn\ en^{LA4}/SM5 \times ♂\ M(2)c^{33a}/In\ (2LR)bw^D.$$

Irradiation was at 72 ± 2 hr after egg laying and adults of the following genotypes were collected: $stw\ pwn/M(2)c^{33a}$ and $stw\ pwn\ en^{LA4}/M(2)c^{33a}$. After aging for several days, the flies were dissected in isopropanol and the anterior portion of the abdomen mounted in a mixture of Canada balsam and methyl salycilate (Lawrence *et al.*, 1979).

RESULTS

Developmental Compartments in the Abdominal Segments

The morphology of the adult *Drosophila* abdomen has been described in detail (Roseland and Schneiderman, 1979; Madhavan and Madhavan, 1980) and only the relevant features will be summarized here (see Fig. 1A). The dorsal surface, the tergite, of each of the seven anterior-most abdominal segments is constructed by nests of cells called histoblasts. The histoblast nests can be distinguished among the larval epidermal cells at the end of the embryonic period and they do not divide until the period of larval growth has concluded and metamorphosis has commenced. Dorsally in each segment there are four histoblast nests. Pairs of histoblast nests are located symmetrically to the fly midline, one

pair of anterior histoblast nests, each with approximately 15–17 cells, and one pair of posterior nests, each with approximately 5–7 cells. Approximately nine rapid cell divisions during the first 48 hr of pupal development produce in each segment the approximately 9000 cells that will secrete the adult cuticle of the tergite.

In the adult fly, the first abdominal tergite has in its anterior four-fifths a region populated with hairs and bristles (microchaetae). The posterior one-fifth of this tergite is covered by hairs but has no bristles. The portion of this posterior cuticle closest to the midline lacks pigmentation in an area that although sexually dimorphic, can be recognized in both sexes and is here called the unpigmented band. The second abdominal tergite is similar morphologically but differs in several respects. The anterior half has evenly distributed approximately 70 bristles that are slightly larger than tergite I bristles; the most posterior row of bristles occupies a pigmented band located in the midregion of the tergite and these bristles (macrochaetae) are larger still. The posterior half of the second tergite is lightly pigmented and in its more anterior portion is populated with hairs but no bristles. The most posterior region lacks pigmentation, hairs, or bristles and marks the intersegmental boundary. With some variation, the more posterior abdominal tergites are similarly constructed. Histological observations (Madhavan and Madhavan, 1980) and ablation experiments (Roseland and Berns, cited in Roseland and Schneiderman, 1979) have indicated that the posterior histoblast nests produce only the most posterior cells of the tergite, the intersegmental region (including both the intersegmental membrane and acrotergite), and that the anterior histoblast nests produce the remainder: the hairy and bristled regions and in the segments with macrochaetae, the more posterior hairy region.

In order to determine whether developmental segregations restrict the growth of the cells that construct the adult abdominal epidermis, mitotic recombination was used to induce marked cell clones during the larval period (see Fig. 2), and the position of the clonal descendants was recorded. The experiments described here have mapped the location of clones in the first abdominal tergite and in the anterior portion of the second tergite. (The absence of any hairs and bristles and its folded structure made scoring for clones in the posterior second tergite region impractical.) Two thousand abdomens from flies that had been irradiated as larvae were screened. On 287 of these abdomens, 301 *straw pawn Minute*$^+$ clones were found in the first and second tergites.

When comparing the relative position of the clones found on different abdomens, it is necessary to be able to relate the clones to landmarks within the tergites.

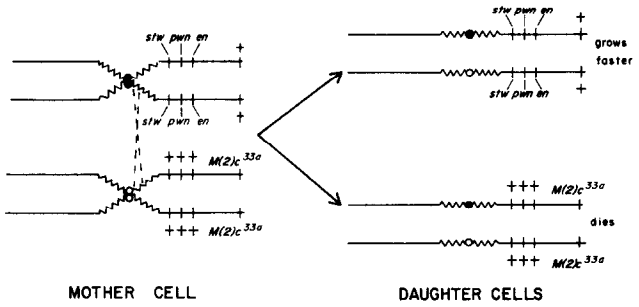

FIG. 2. Somatic recombination generating *straw pawn Minute*$^+$ clones. The second chromosome is diagrammed to show the results of crossing over between *straw* and the centromere. All *straw* clones are also homozygous for *pawn*, *engrailed*, and $M(2)c^{33a+}$.

Landmarks available include the bristles of the first and second tergites and the macrochaetae of the second tergite, the unpigmented band of the first tergite and the darkly pigmented band of the second tergite. While the bristles of both abdominal segments are not constant in number nor identically placed among different individuals, the unpigmented band of the first abdominal tergite can be used to indicate position with respect to the first and second abdominal tergites. Strikingly, all of the clones found respected the borders that define the unpigmented band (see Figs. 3A, B). Whereas clones within the bristled regions of the first and second tergites were quite irregular in shape, clones that met this band formed a straight border. Moreover, clones within the unpigmented band similarly defined these borders; some filled an area within the unpigmented band and defined both borders. Clones within the region were elongated in a lateral direction whereas most clones that did not meet these borders were more rounded in shape. In all, 88 *straw pawn Minute*$^+$ clones met the boundaries defined by the unpigmented band and none crossed them. The behavior of the cells in the unpigmented band suggests that its borders coincide with a developmental compartment and also define the region of a posterior compartment within the first abdominal segment. The posterior border of the posterior compartment defines the segmental border between abdominal segments I and II. Figure 1A illustrates the position of these developmental subdivisions and several representative clones that meet their borders are diagrammed in Fig. 1B.

Of the 301 control *straw pawn* clones detected, 117 were in the anterior compartment of tergite I, 28 in the posterior compartment of tergite I, and 156 in the anterior portion of tergite II. The small percentage of posterior clones is due in part to the difficulty in detecting clones anywhere but in its unpigmented medial region. Clones were observed to extend laterally beyond the unpigmented area but clones entirely outside this region were located in cuticle that frequently folded out

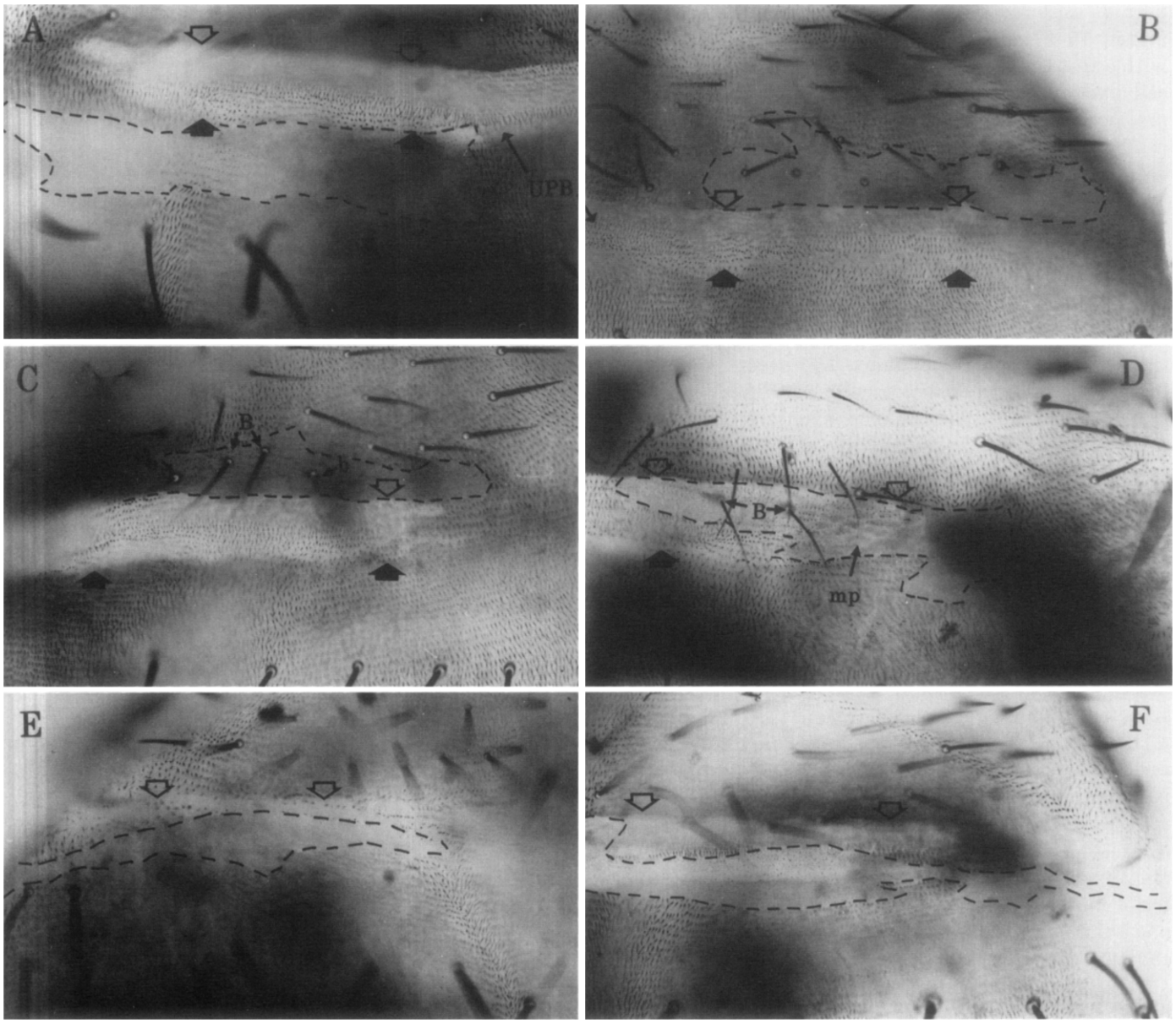

FIG. 3. Portions of the first and second tergites bearing clones marked with *straw* and *pawn*. The unpigmented band (UPB), wild-type bristles (B), *stw pwn* bristles (b), area of mottled pigmentation (mp), compartment border (▽), segment border (▲), and clonal boundary (---) are indicated. (A) Second tergite *stw pwn* clone defining segment border. (B,C) First tergite *stw pwn* (B) and *stw pwn* en^{LA4} (C) clones in the anterior compartment defining anterior-posterior compartment border. Note presence of both wild-type and mutant bristles in these clones. (D) First tergite *stw pwn* en^{LA4} clone in the posterior compartment. Note presence of wild-type bristles, mottled pigmentation, and abnormal shape of unpigmented band. (E,F) *stw pwn* en^{LA4} clones that cross the segment border.

of the primary plane of focus in these preparations and so could not be scored reliably. Since *straw pawn* bristles in these regions could be recognized, lateral clones in the bristled regions were scored. Comparing only those clones within the medial region of tergit I, the incidence of posterior clones (28) was approximately one-third that of anterior clones (87). This proportion suggests that the posterior compartment is one-third the size of the anterior compartment at the time of clone induction, a proportion that approximates the relative number of cells in the posterior (5–7 cells) and anterior (15–17 cells) histoblast nests. Given the demonstration that only the most posterior band of the tergites is produced by the posterior histoblast nests and that this posterior band is the position of the posterior compartment in tergite I, these correlations suggest an identity relationship between the posterior compartment and the posterior dorsal histoblast nest.

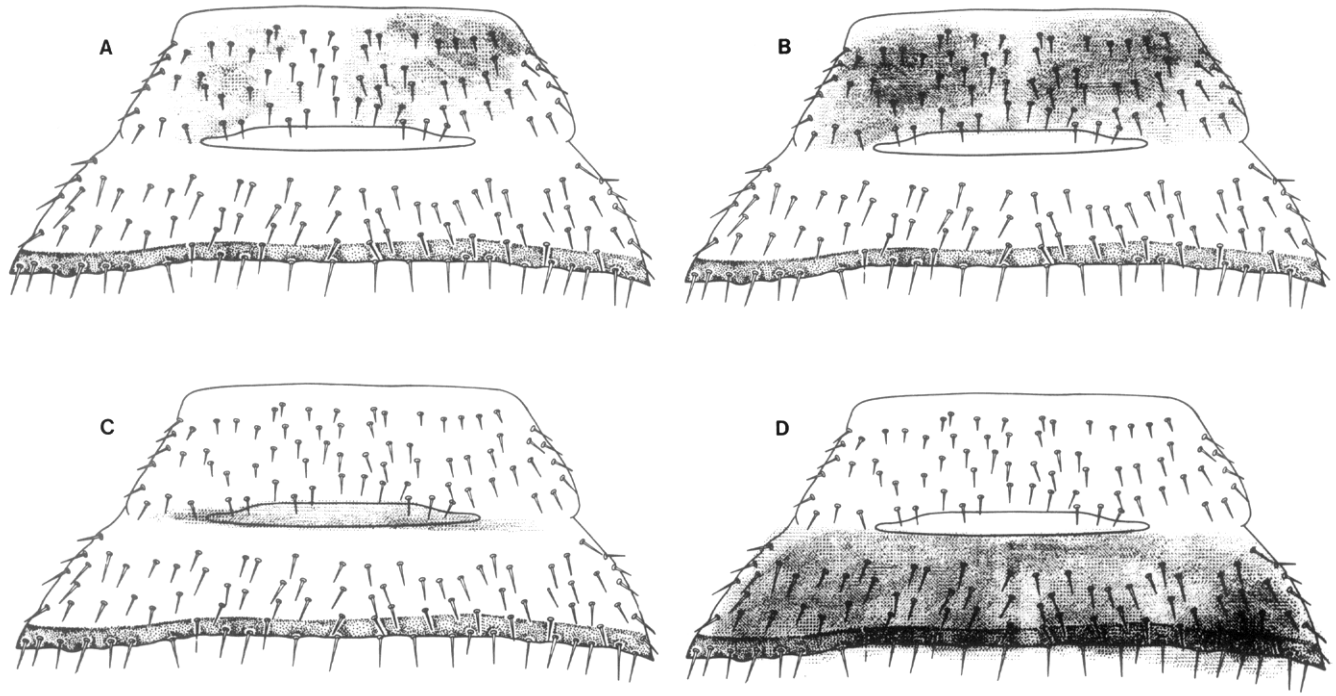

FIG. 4. Composite drawings of *straw pawn Minute*$^+$ clones. (A) 30 randomly selected tergite I clones. All clones that were found in the tergite I anterior compartment (B), the tergite I posterior compartment (C), and tergite II (D). Note that these clones respect the compartment and segment borders. This computer montage was generated with the UCSF Computer Graphics Facility.

Clones within the second abdominal tergite were observed in some cases to extend posteriorly beyond the pigmented band. This indicates that if an anterior-posterior compartment border exists within the second abdominal tergite, it does not coincide with the row of macrochaetae or the pigmented band. If the anterior histoblast nest represents the anterior compartment primordium, then these clonal data are consistent with the observation that the anterior nest in tergite II produces tissue posterior to the row of macrochaetae (Madhaven and Madhaven, 1980).

Figure 4 illustrates computer-generated images in which the position and shape of the *straw pawn Minute*$^+$ clones have been recorded with a graphics display system. Composite images depicting several clones from different abdomens were generated. In Fig. 4A, 30 randomly selected clones within the anterior compartment of tergite I are shown to illustrate how regions of overlapping clones are toned proportionally darker in these computer montages. In Figs. 4B–D are shown all clones within the anterior first tergite, posterior first tergite, and anterior second tergite, respectively. It can be clearly seen that the clones do not cross the compartment or segment borders.

The Requirement for the engrailed Gene

Although the homozygous viable en^1 allele perturbs the normal development of all three thoracic segments, the development of the abdominal segments in en^1/en^1 individuals is normal. Abdominal development is also normal in individuals heterozygous for en^1 or for any of the 58 en^{lethal} alleles that have been isolated to date.

The lethal alleles of *engrailed* cause development to arrest during embryogenesis (Kornberg, 1981) and they are therefore thought to be more severely deficient for *engrailed* function than en^1. In order to test whether abdominal development is affected when *engrailed* function is blocked in such lethal homozygotes, mitotic recombination was used to induce clones of a lethal allele of *engrailed*, en^{LA4}. (en^{LA4} was recovered after EMS mutagenesis, has no apparent chromosome breaks, and was cleansed of possible second site mutations by four separate recombinations.) These clones were marked with *straw*, *pawn*, and $M(2)c^{33a}$ using a protocol identical to that described above except that en^{LA4} was *cis* to *straw* and *pawn* and *trans* to the *Minute*. Two thousand two hundred sixty-five abdomens were examined and 427 clones were found in the scorable tergite regions of abdominal segments I and II. Clones within the anterior compartment of the first tergite or within the anterior region of the second tergite developed normally. Thirty-four tergite I and thirty-nine tergite II clones defined the anterior–posterior compartment border and intersegmental border, respectively. The position of these borders was indistinguishable from those defined by *engrailed*$^+$ clones.

In contrast, *engrailed* clones in the posterior compartment were abnormal in these respects: First, the unpigmented band was frequently enlarged and its borders less well defined when containing *engrailed* cells but no apparent pattern duplication or mirror image symmetry was associated with the *engrailed* clones.

Second, 10 of the 44 posterior clones were associated with cuticle with mottled pigmentation in the normally unpigmented band and contained bristles (1–5 bristles/clone) of a size characteristic of the first tergite anterior compartment. These bristles were *straw*$^+$ and *pawn*$^+$ and therfore not constituents of the *straw pawn engrailed* clone; the anterior areas immediately adjacent to these clones were depleted of bristles (see Figs. 1C and 3D). The abnormally placed bristles were associated only with posterior *engrailed* clones that grew along the anterior–posterior compartment border and no other bristles in the posterior compartment were observed in the more than 5000 abdomens that were examined in this study. The location of bristles within the posterior *engrailed* clones suggests that these *straw*$^+$ *pawn*$^+$ bristles were formed by cells of the anterior compartment that migrated into the *engrailed* posterior compartment. Movement of bristle-forming cells has been documented as a general mechanism of pattern formation in insects (Wigglesworth, 1940; Tokunaga, 1962; Garcia-Bellido and Merriam, 1971a,b; Lawrence *et al.*, 1979). Figure 3C illustrates this greater movement of bristle-forming cells relative to hair-forming cells in the anterior compartment of the first tergite: characteristically the marked hair-producing cells generate a coherent patch but the bristles within the patch may be of either mutant or wild-type genotype. In the case of the *engrailed* posterior clones (Fig. 3D), such movement of anterior compartment bristles into the posterior compartment is abnormal, and occurs as a nonautonomous consequence of having *engrailed* cells in the posterior compartment.

Third, some of the *engrailed* clones failed to respect the compartment or segment borders. Seven clones were found that crossed these borders a significant distance and Fig. 1C, 3E, and F illustrate several examples. Twenty-two clones were found that met but did not cross these borders. Three clones were found that were mostly within the anterior tergite II but included some tergite I territory.

These results suggest that the function of the *engrailed* locus is required in the cells of the posterior compartment of the first tergite, but not in the anterior compartments that it abuts. Failure of these posterior cells to express the *engrailed* function results in the inability to maintain either the compartmental or segmental borders, and this failure results in the abnormal morphogenesis of the posterior compartment, the abnormal movement of tergite I anterior cells into the posterior compartment of the first tergite, and the crossing of posterior compartment cells into the adjoining segment.

DISCUSSION

Compartments in the Adult Abdomen

Cell lineage analysis and studies of histological preparations have established that the cells that secrete the cuticle of the adult cephalic (Becker, 1957; Postlethwait and Schneiderman, 1971; Struhl, 1977; Baker, 1978; Morata and Lawrence, 1979), thoracic (Garcia-Bellido, 1968; Bryant, 1970; Garcia-Bellido and Merriam, 1971a; Steiner, 1976; Wieschaus and Gehring, 1976; Lawrence and Morata, 1977; Morata and Lawrence, 1979; Lawrence et al., 1979), and genital (Dubendorfer, 1977) structures grow logarithmically throughout the larval periods, segregated as individual imaginal discs. In contrast, the abdominal segments are formed from histoblast nests that neither separate physically from the larval epidermis nor divide during the larval period. The cells of these nests can be distinguished because they are smaller than the neighboring larval cells and begin a rapid period of cell division only upon pupariation (Garcia-Bellido and Merriam, 1971b; Guerra *et al.*, 1973; Madhavan and Schneiderman, 1977; Roseland and Schneiderman, 1979; Madhavan and Madhavan, 1980). The results presented here indicate that despite their very different programs of development, the histoblast and imaginal disc cells share a striking similarity: both are developmentally segregated into anterior or posterior compartments.

The failure of previous studies to detect compartmental subdivisions in the abdominal tergites (Garcia-Bellido and Merriam, 1971b; Lawrence *et al.*, 1978; Roseland and Schneiderman, 1979) can most likely be attributed to technical reasons. The cell marker mutants used in these studies, *yellow, forked,* and *multiple wing hair,* do not unambiguously mark the very small cell hairs of the tergite. As shown here, the clones of hair producing cells marked with *straw* and *pawn* clearly demonstrate the location of a restriction to growth within the first tergite. Because the posterior compartment produces no bristles, this compartment would not be revealed by the boundaries of clones delineated by marked bristles only.

Unfortunately, the methods used in this study to mark the epidermal cells of the first tergite cannot be applied to a clonal analysis of the more posterior abdominal segments, since the most posterior region of each of these segments (the region homologous to the posterior compartment of tergite I) produces no hairs or bristles that could serve to mark clonal borders. Several observations do however suggest that all of the other abdominal segments are likely to be subdivided

into compartments as well. First, Lawrence observed that each of the abdominal segments of *Oncopeltus* appeared to be subdivided into two unequal parts, a predominant anterior portion and a small posterior band, that from early embryogenesis have independent lineages (Lawrence, 1973). This is consistent with the demonstration of compartmental subdivisions of the *Drosophia* tergite I and suggests that all of the abdominal segments are subdivided into compartments in *Oncopeltus* and in *Drosophila.* Second, all of the abdominal tergites follow a similar developmental program—each develops from paired nests of cells—one anterior and one posterior. Given the indication that in tergite I, the anterior nest gives rise to the anterior compartment and the posterior nest to the posterior compartment, it would seem plausible that this correlation would also apply to the other abdominal segments. Third, some of the homeotic transformations of the bithorax complex effect intersegmental transformations between the other abdominal segments and the first abdominal segment (Lewis, 1978). Since the homeotic transformations are thought to occur between homologous groups of cells, the existence of compartments in the first abdominal segment implies that the other segments are similarly subdivided. This is not to suggest the restriction of the bithorax alleles to anterior or posterior compartments in the abdominal segments, but to point to the inherent homology that the transformations imply.

Demarcation of Segments

Several different methods have previously indicated that segment boundaries restrict the growth of clones. The induction of clonal patches by X irradiation in early *Oncopeltus* embryos (Lawrence, 1973) and in early *Drosophila* embryos (Lawrence *et al.*, 1978) indicated that cells are restricted to grow within their respective segment from a period at or shortly after blastoderm formation. Similarly, analyses of gynandromorph mosaic larvae of *Drosophila* have found an increase in sturt values at the segment borders. This indicates that the separate segments have independent lineages from about the time of the cellular blastoderm and that the larval epidermal cells from separate segments do not mix (Szabad *et al.*, 1979). The clonal analysis described here reaffirms the observation that segment borders restrict the growth of clones and this study positions the segmental border of the adult first and second abdominal segments with a precision that the morphological features alone did not permit. Madhavan and Madhavan (1980), in their elegant study of abdominal tergite development, found that the larval dorsal longitudinal muscles that persist for the first several days of adult life attach to the adult cuticle at the segment borders. Although the boundaries of some of the clones observed in this study coincided with the points of muscle attachment (not shown), this correlation, possibly due to the distortion of the abdominal structures in our preparations, was not consistent. The segment border defined by this study is therefore recognized solely by functional criteria—the inhibition of cell mixing at the segment border.

The mechanism that maintains the clonal separation of the different metameric segments is not understood. In the cuticle of the adult abdomen, the cells of neighboring segments join to produce a continuous sheet well before the mitotic divisions of these cells have ceased (Madhavan and Madhavan, 1980). As demonstrated here, clones of epidermal cells were observed to grow up to and along a segment border, but not to cross; the crossing of the segment borders was observed only in the absence of the *engrailed* function.

The engrailed Gene

Although a firm understanding of the role of the *engrailed* gene in the formation and the maintenance of compartments and of the role of compartments in development is not yet available, this study establishes a correlation between apparantly disparate aspects of the pleitropic *engrailed* phenotype. The *engrailed* phenotype, as expressed in en^1 homozygotes and in somatic clones of of *en* homozygous cells, has two principal features: the failure of posterior *engrailed* cells of the adult wing blade to respect the anterior-posterior compartment border, and, to a limited extent, the partial transformation of these posterior cells into patterns and structures characteristic of the wing blade anterior compartment (Lawrence and Morata, 1976; Kornberg, 1981). In embryos homozygous for *engrailed*-lethal mutations, a large number of abnormalities are evident, the most striking of which is the fusion of adjacent segments (Kornberg, 1981; Nusslein-Volhard and Wieschaus, 1980). In gastrulating *engrailed* embryos, the deep grooves that normally mark each of the segment boundaries are absent in an alternating pattern. en^{LA4} embryos, for instance, lack clear segmental grooves at the borders of the pro- and mesothoracic, metathoracic, and first, second, and third, fourth and fifth, and sixth and seventh abdominal segments. When later during embryonic development a cuticle is secreted, the belts of denticle hairs that normally mark each of the segments reflect the segmental fusion seen in the younger, gastrulating embryos. Figures 5A and B compare the denticle belts of wild-type and *engrailed* embryos and demonstrate the segment fusion of the *engrailed* mutant.

This study has analyzed the behavior of the *engrailed* cells in the posterior compartment of the tergite I where

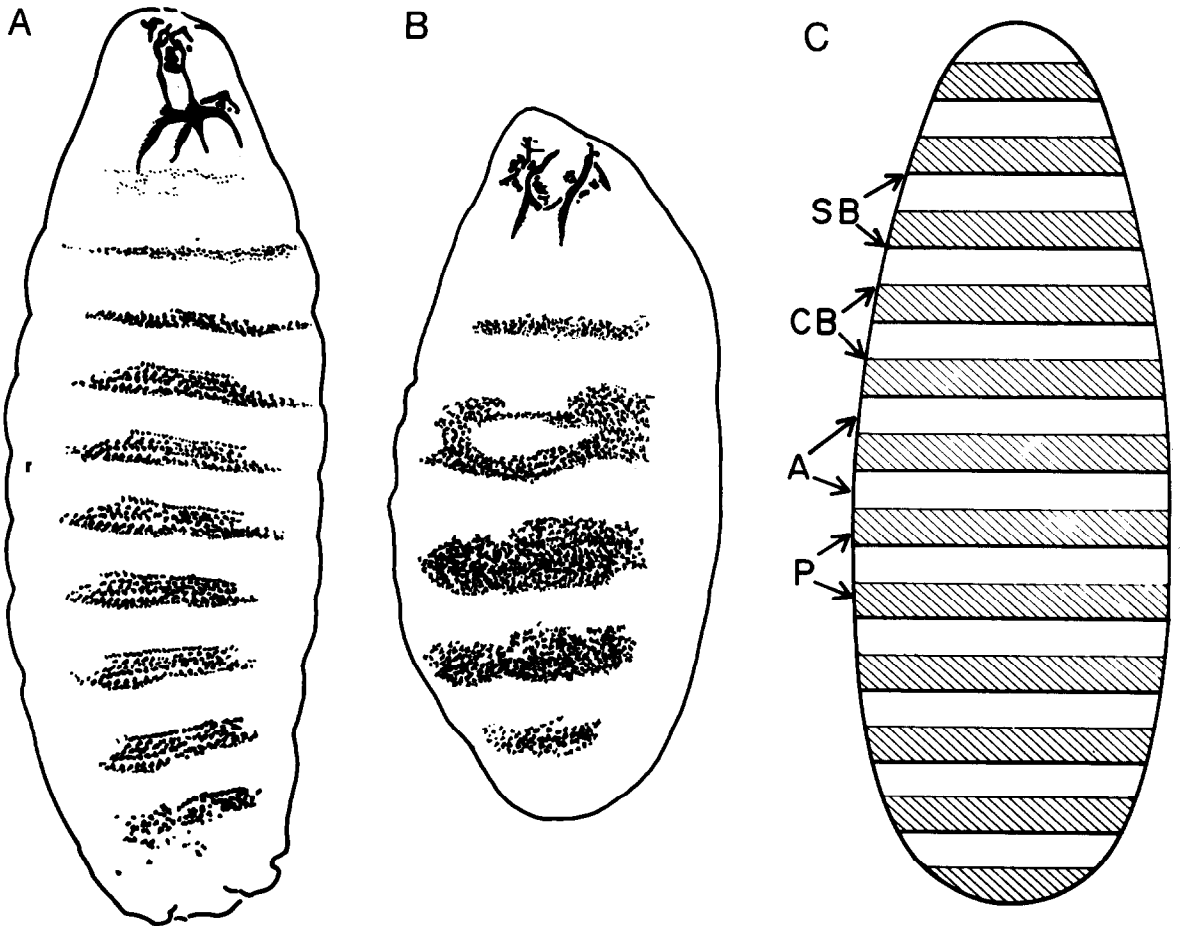

FIG. 5. Ventral external morphology of 18-hr embryos. (A) Wild-type embryo with denticle belts marking each of the metameric segments. (B) An *engrailed* embryo with fused denticle belts reflecting the fusion of adjacent segments. (C) Diagram of the segmented embryo with the postulated anterior (A, open areas) and posterior (P, crosshatched areas) compartments alternating in a "zebra-like" fashion; compartment border (CB); segment border (SB).

the effects of deficiency for *engrailed* on segment and compartment borders could both be examined. The finding that the anterior-posterior compartment border within the first tergite and the segment border between the first and second tergites are not maintained when confronted by *engrailed* posterior cells indicates a property shared with other aspects of the *engrailed* phenotype. As with the defective segmentation of *engrailed* embryos and the behavior of *engrailed* wing blade cells, there is a failure to maintain clonal restriction boundaries. During development, anterior and posterior cells normally confront each other at compartment and at segment borders. This apposition of two different cell types, a difference dependent upon the expression of the *engrailed* locus in posterior cells, appears to be essential for the maintenance of these borders; deficiency for the *engrailed* locus results in the dissolution of compartment and segment borders wherever they have been examined. A general application of this conclusion to all such clonal restriction boundaries leads to several predictions: If the retention of segment borders requires the confrontation of anterior and posterior cells, then anterior and posterior compartments should exist during all the stages when segmentation subdivides the developing organism, during embryonic and larval, as well as adult development. This would predict that previously unrecognized posterior compartments subdivide the embryonic and larval segments. The presence of posterior compartments in the embryonic and larval segments would account for the effects of the *engrailed* deficiency—the fusion of adjacent segments due to the failure to maintain the segment borders. The presence of anterior and posterior compartments in each of the embryonic and larval segments would result in an alternating "zebra-like" pattern of anterior and posterior compartments. A schematic representation of this model of compartments in the embryo is shown in Fig. 5.

Garcia-Bellido (1975) has proposed that compartments are the units in which determination decisions, under the control of selector genes, are expressed. The results reported here suggest an alternative, that compartments function to maintain segmentation. These results bear on the question of the relationship between compartments and segments as developmental units in two respects. (1) A segment border should be considered to be a compartment border as a boundary limiting the growth of cells and as a boundary limiting the expression of the *engrailed* gene. This is not meant to imply that the function of the *engrailed* locus is itself sufficient to define and maintain a segment border or to differentiate between segment borders and compartment borders. The *engrailed* locus is necessary for the border maintenance and its absence illustrates properties that are shared at compartment and segment borders. (2) A segment is a composite of two unequal parts, one anterior that does not express the *engrailed* function, the other one, posterior, that does. A segment is, therefore, not a unit composed of two equivalent parts. The significance of this inequality is manifested by the dissolution of segment borders in the absence of *engrailed*$^+$ posterior cells. If maintaining a segment border requires the apposition of two different cells types, of anterior and posterior cells, then a segment must necessarily be subdivided into two unequal parts. The anterior-posterior compartment subdivision is therefore a necessary consequence of the segmentation mechanism: one of the functions of these compartments is to maintain the segment borders. This concept is of course speculative but some of its predictions can be tested: anterior and posterior compartments should be found in those organ systems that are segmentally derived; organ systems lacking segmentation should not require this compartmentalization.

I thank Zehra Ali for her excellent assistance, Dr. Peter Lawrence for his hospitality and help in initiating this work, and Dr. David Agard for his computer programming and assistance. This research was supported by a Basil O'Connor Research Grant from the March of Dimes, Birth Defects Foundation and a Career Development Award from the American Cancer Society.

REFERENCES

BAKER, W. K. (1978). A clonal analysis reveals early developmental restrictions in the *Drosophila* head. *Develop. Biol.* **62,** 447-463.

BECKER, H. J. (1957). Uber Rontgenmosiak-flecken und Defektmutationen am Auge von *Drosophila* und die Entwicklungsphysiologie des Auges. *Z. Verebungs lehere* **88,** 333-373.

BRYANT, P. J. (1970). Cell lineage relationship in the imaginal wing disc of *Drosophila melanogaster. Develop. Biol.* **62,** 389-411.

DUBENDORFER, K. (1977). "Die Entwickling der männulichen und weiblichen Genital-Imaginalschiebe von *Drosophila melanogaster:* eine klonale analyse." Ph.D. Dissertation, University of Zurich.

GARCIA-BELLIDO, A. (1968). Cell lineage in the wing disc of *Drosophila melanogaster. Genetics* **60,** 181-188.

GARCIA-BELLIDO, A. (1975). Genetic control of wing disc development in *Drosophila. In* "Cell Patterning," Ciba Foundation Symposium, No. 29, pp. 161-182. North-Holland, Amsterdam.

GARCIA-BELLIDO, A., and DAPENA, J. (1974). Induction, detection and characterization of cell differentiation mutants of *Drosophila. Mol. Gen. Genet.* **128,** 117-130.

GARCIA-BELLIDO, A., and MERRIAM, J. (1971a). Parameters of the wing disc development of *Drosophila melanogaster. Develop. Biol.* **24,** 61-87.

GARCIA-BELLIDO, A., and MERRIAM, J. (1971b). Clonal parameters of tergite development in *Drosophila. Develop. Biol.* **26,** 264-276.

GARCIA-BELLIDO, A., RIPOLL, P., and MORATA, G. (1973). Developmental compartmentalization in the wing disc of *Drosophila. Nature New Biol.* **245,** 251-253.

GARCIA-BELLIDO, A., RIPOLL, P., and MORATA, G. (1976). Developmental segregations in the dorsal mesothoracic disc of *Drosophila. Develop. Biol.* **48,** 132-147.

GUERRA, M., POSTLETHWAIT, J. H., and SCHNEIDERMAN, H. A. (1973). The development of the imaginal abdomen of *Drosophila melanogaster. Develop. Biol.* **32,** 361-372.

KORNBERG, T. (1981). *engrailed:* A gene controlling compartment and segment formation in *Drosophila. Proc. Nat. Acad. Sci. USA* **78,** 1095-1099.

LAWRENCE, P. A. (1966). Development and determination of hairs and bristles in the milkweed bug, *Oncopeltus fasciatus* (Luggaeidae, Hemiptera). *J. Cell Sci.* **1,** 475-498.

LAWRENCE, P. A. (1969). Polarity and pattern in the postembryonic development of insects. *Advan. Insect Physiol.* **7,** 197-266.

LAWRENCE, P. A. (1973). A clonal analysis of segment development in *Oncopeltus* (Hemiptera). *J. Embryol. Exp. Morphol.* **30,** 681-699.

LAWRENCE, P. A., GREEN, S. M., and JOHNSTON, P. (1978). Compartmentalization and growth of the *Drosophila* abdomen. *J. Embryol. Exp. Morph.* **43,** 233-245.

LAWRENCE, P. A., and MORATA, G. (1976). Compartments in the wing of *Drosophila:* A study of the *engrailed* gene. *Develop. Biol.* **50,** 321-337.

LAWRENCE, P. A., and MORATA, G. (1977). The early development of the mesothoracic compartments in *Drosophila. Develop. Biol.* **56,** 40-51.

LAWRENCE, P. A., STRUHL, G., and MORATA, G. (1979). Bristle patterns and compartment boundaries in the tarsi of *Drosophila. J. Embryol. Exp. Morphol.* **51,** 195-208.

LEWIS, E. B. (1978). A gene complex controlling segmentation in *Drosophila. Nature (London)* **276,** 565-570.

LINDSLEY, D. L., and GRELL, E. L. (1968). Genetic variation of *Drosophila melanogaster. Carnegie Inst. Wash. Publ.* No. 627.

MADHAVAN, M. M., and MADHAVAN, K. (1980). Morphogenesis of the epidermis of the adult abdomen of *Drosophila. J. Embryol. Exp. Morphol.* **60,** 1-31.

MADHAVAN, M. M., and SCHNEIDERMAN, H. A. (1977). Histological analysis of the dynamics of growth of imaginal discs and histoblasts nests during the larval development of *Drosophila melanogaster. Wilhelm Roux Arch. Entwicklungsmech. Organismen* **186,** 269-305.

MORATA, G., and LAWRENCE, P. A. (1979). Development of the eye-antenna imaginal disc of *Drosophila. Develop. Biol.* **70,** 355-371.

MORATA, G., and RIPOLL, P. (1975). *Minutes:* Mutants autonomously affecting cell division rate in *Drosophila. Develop. Biol.* **42,** 211-221.

NUSSLEIN-VOLHARD, C., and WIESCHAUS, E. (1980). Mutations affecting segment number and polarity in *Drosophila. Nature (London)* **287,** 795-801.

POSTLETHWAIT, H. H., and SCHNEIDERMAN, H. A. (1971). Pattern formation and determination in the antenna of the homeotic mutant *Antennapedia* of *Drosophila melanogaster*. *Develop. Biol.* **24**, 477-519.

ROSELAND, C. R., and SCHNEIDERMAN, H. A. (1979). Regulation and Metamorphosis of the abdominal histoblasts of *Drosophila melanogaster*. *Wilhelm Roux Arch. Entwicklungsmech. Organismen* **186**, 235-265.

STEINER, E. (1976). Establishment of compartments in the developing leg imaginal discs of *Drosophila melanogaster*. *Wilhelm Roux Arch. Entwicklungsmech. Organismen* **180**, 9-30.

STRUHL, G. (1977). Developmental compartments in the proboscis of *Drosophila*. *Nature (London)* **270**, 723-725.

SZABAD, J., SCHUPBACH, T., and WIESCHAUS, E. (1979). Cell lineage and development in the larval epidermis of *Drosophila melanogaster*. *Develop. Biol.* **73**, 256-271.

TOKUNAGA, C. (1962). Cell lineage and differentiation on the male foreleg of *Drosophila melanogaster*. *Develop. Biol.* **4**, 489-516.

WIESCHAUS, E., and GEHRING, W. (1976). Clonal analysis of primordial disc cells in the early embryo of *Drosophila melanogaster*. *Develop. Biol.* **50**, 249-265.

WIGGLESWORTH, V. B. (1940). The origin of the sensory neurones in an insect, *Rhodnius prolixus*. *J. Exp. Biol.* **17**, 180-200.