THE ANATOMY OF A COMPARTMENT BORDER

The Intersegmental Boundary in Oncopeltus

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ABSTRACT

In the insect Oncopeltus (Hemiptera, Lygaeidae), after blastoderm formation, labeled cells in one segment never give rise to cells in another; clones always respect a sharply defined line, the segmental boundary. Similarly, demarcation lines defining “compartments” have been recently found within the imaginal disks of Drosophila and promise to be of first importance in developmental genetics. In Oncopeltus the segmental border is a straight line in a simple epithelial monolayer and is marked by a change in pigmentation that is visible in the electron microscope. Reconstructions from serial sections show that there is a change of cell shape at the boundary, but attachment desmosomes, septate junctions, and gap junctions link cells of different segments as well as cells of the same segment. The form of the epithelium at different stages of the molt cycle is described, and the possibility that there may be an abrupt change of cell adhesiveness at the segment boundary is discussed.

Insects, like vertebrates, are metamERICALLY segmented: each segment, while being homologous to the others, usually has characteristic structures and properties. This simple observation implies that the same basic ground plan can, as a result of evolution, be locally modified in a number of different ways—in insects some of the genes responsible for this are known. Evidence has come from an analysis of X-ray-induced clones of marked cells in Drosophila and has opened a new approach to developmental biology. In an important series of experiments on the dorsal mesothoracic disk, Garcia-Bellido et al. (9) have shown that the developing disk is subdivided progressively into “compartments” with rigidly defined boundaries. These compartments form early from a small number of primordial cells, a marked cell within a nascent compartment never subsequently giving rise to progeny cells in any other compartment. Within the compartment, the clones have variable borders, but at the edge they always define the same boundary lines. Work with genetic mosaics of homeotic mutants has suggested that compartments are critical units of development, not only in terms of the spatial disposition of cell lines, but also because particular wild-type alleles exclusively control their differentiation (8, 9).

Clonal analysis of the hemipteran Oncopeltus (13, 14) has shown that the segment boundary is a compartment border; clones generated after the late blastoderm stage may touch the border, but if they do they frequently run along it and never extend to the other side. In Oncopeltus the light microscope shows that there is an abrupt change of pigmentation coinciding with the border, as well as an alteration of cell shape (15). Identification of a particular cell border in the electron microscope would not be possible without a marker, which the pigment granules provide. We undertook three-dimensional reconstruction from serial thin sections and here report that although there is a discontinuity of cell shape at the border, the cell
junctions are typical of the rest of the segment, and we note no other structural feature that we can relate to this important developmental landmark. We discuss our results in relation to measurements of electrical coupling across the segmental boundary (23).

MATERIALS AND METHODS

Oncopeltus fasciatus Dall. were reared on milkweed seed and water at 29°C, 16 h in light, 8 h in dark. Whole mounts were prepared by fixing the clear cuticle with attached epidermis in Carnoy's fixative and staining in Hansen's trioxynhematein (11). For electron microscopy, larvae were dissected in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, and the ventral abdominal epidermis was left for 30 min in the fixative, postfixed in 1% osmium tetroxide in Veronal buffer (pH 6.8) for 10 min and stained en bloc in 1% uranyl acetate in the same buffer (21). Blocks were dehydrated in ethanol and propylene oxide and embedded in Araldite. Sections were stained in 5% uranyl acetate (10 min at 60°C) and lead citrate in water (5 min at room temperature) and examined in an AEI 68 electron microscope.

For serial reconstruction, oriented ribbons of sections were mounted on slot grids, every 10 sections photographed, and the cell profiles tracted onto Perspex sheets of the appropriate thickness. (The average section was estimated to be 60 nm in thickness.) Two selected cells were then further analyzed by the use of polystyrene cutouts to reconstruct solid scale models. For analysis of cell junctions large montages were prepared from plates photographed at ×5,000, and printed at ×12,700.

RESULTS

The epidermis is a continuous single-celled layer extending all over the external surface of the insect, as well as the foregut, hind gut, and tracheal system. It is bounded on one side by the secreted cuticle and on the other by a basement membrane. In the abdomen, segmentation is indicated by the reiterated patterns of pigmentation, muscle attachments, and slight indentations near the intersegmental borders.

Whole mounts show that there is a change in the apical profiles of cells at the border, passing from more isodiametric white cells at the posterior border of one segment to mediolaterally elongated orange cells at the anterior border of the adjacent segment (Fig. 1) (15).

Reconstruction of 20 cells from serial sections on plastic sheets has confirmed and extended these observations. In a fifth-stage larva 2 days after ecdysis, all the cells adjacent to the border are elongated mediolaterally, particularly at the apical surface. This elongation is much greater in the orange cells, and affects cells farther away from the margin than the white cells. There is an abrupt change of shape at the border, the reconstructions showing that the white cells are shorter and fatter than the orange cells. The volumes of two neighboring cells, one white and one orange, were estimated from solid models (Figs. 2, 3) to be 980 and 1,120 μm³, respectively. The cells are elongated mediolaterally near the cuticle to give a long apical perimeter and have a more isodiametric bulbous bowl, near the basement membrane, containing the nucleus. This shape implies that the cells, particularly the orange ones at the anterior margin of the segment, overlap more with their neighbors and have greater areas of lateral contact at the apical surface than at the basal.

Cell Junctions

We identified three types of specialized cell junction in the epidermis (Figs. 4-7). These are concentrated in a limited region of the apical part of the lateral cell membranes. Most apically (ca. 1 μm below the tips of the microvilli) there is invariably a single attachment desmosome (Fig. 8). Because it is seen in every section, this desmosome must be a complete ring binding neighboring cells together and extending all around every cell. Its appearance is similar to attachment desmosomes described in other insects (1, 12). Septate junctions (19, 22) are abundant and occupy 15%
of the lateral cell membrane in the sections studied from a 2-day old fifth-stage larva (Figs. 4, 5). The reconstruction of Perspex sheets shows that at the extreme apical tips of the cells there are frequently small clusters of interdigitations (e.g. Fig. 11) that are between the tapering tip of one cell and its immediate neighbors, the cell membranes being mostly linked by septate junctions. They do not occur in the basal parts of cells which lack all junctional specializations except for the very occasional gap junction (Figs. 4, 6). In the apical regions, interspersed between the septate junctions, the gap junctions are frequent and vary from about 75 to 2,000 nm in length.

The distribution of the three types of junction in a section from the 2-day old fifth-stage is shown in Fig. 8 which includes the segment border. Clearly, there is no marked correlation between the distribution of junctional structures and the border. All three types of junction link the orange, most anterior cell of one segment with its neighboring white cell from the adjacent segment; apart from the changes in pigmentation and cell shape, there would be no way of identifying the boundary from the section. With respect to the amount of distribution of junctions, there was no evidence for any differences between lateral cell membranes linking white or orange cells. Basal and apical gap junctions were found to link cells across the segment border. Representative areas illustrated in Figs. 9-11 make these points, as well as Table I.

Because of differences in cell shape the orange cells have about 30% greater length of apical lateral contact with their neighbors than the white cells (estimated from measurements of apical cell perimeters in whole mounts). Measurements of sections on three insects fixed during the period before the separation of the epidermis from the cuticle suggested that there is a more or less constant amount of junctional specialization in the lateral membranes in the apical parts of the cells (between the cuticle and about 8 μm from it) (Table I). It follows that there may be more junctional specialization per cell or per unit epidermal volume in the orange cells than in the white. The changes in form and volume of the cells are accompanied by increased or decreased lateral contact in the basal parts, but not by noticeable changes in the amount of septate junction (Table I).

For most of the fifth-stage molt cycle (ca. 162 h from ecdysis to ecdysis [17]) the epidermis remains firmly adherent to the cuticle, and little relative movement or change in cell shape occurs. However, once the epidermis has become free of the larval cuticle (about 80 h after ecdysis to the fifth-stage larva), the epithelial sheet becomes folded near the segment border and the cells
undergo changes in form (Figs. 12 and 13). Our impression is that the orange cells have been extended so that they mostly abut by that part of the lateral membrane which includes junctions. By contrast, the white cells are now apparently squashed together, and have long lateral membranes. Measurements on one individual have suggested that more gap junctions develop in the basal parts of the cells at the time of folding (Table 1).

We reconstructed 15 cells at this stage, over 420 sections (25 μm), and found that the position of the boundary as indicated by the pigmentation change shifted slightly in relation to the fold. Thus, in the first section (Fig. 12) the white and orange cells met precisely at the fold; in the 340th section (Fig. 13) the boundary had moved more or less one cell diameter posteriorly. All three types of intercellular junction linked the cells of two adjacent segments.
Figure 8 Drawing from a large montage to show the distribution of intercellular junctions in a 2-day old fifth-stage larva near the intersegmental boundary. This section was oriented in the anteroposterior axis. Septate junctions are shown as ladder-like structures, attachment desmosomes as toothed lines, gap junctions as single lines, unspecialized membrane apposition as double lines. The orange cells of segment 4 are shaded, the white cells of segment 3 unshaded. Note that the orange cells of segment 4 are narrower than the white cells of segment 3. The regions depicted in Figs. 9–11 are indicated. × 7,700.
TABLE I
The Length of Junction per Lateral Membrane in Larvae of Different Age

|                | Orange Cells |                         | White Cells |                         |
|----------------|--------------|--------------------------|-------------|--------------------------|
|                | Gap junction | Septate junction         | Gap junction | Septate junction         |
| Newly emerged fifth stage (n = 10 lateral membranes) | 0.7 ± 0.4 | 2.2 ± 0.9 | 1.0 ± 0.9 | 2.6 ± 1.6 |
| 48-h fifth stage (n = 10 lateral membranes)    | 1.0 ± 0.5 | 2.6 ± 0.7 | 0.9 ± 0.8 | 2.6 ± 1.3 |
| 70-h fifth before fold (n = 10 lateral membranes) | 0.6 ± 0.5 | 1.7 ± 1.0 | 0.8 ± 0.5 | 2.0 ± 0.6 |
| 72-h fifth after fold (n = 10 lateral membranes) | 1.7* ± 0.9 | 2.6 ± 1.5 | 2.1* ± 1.7 | 1.2 ± 1.6 |
| The lateral membrane separating the cell in one segment from its neighbour in another (n = 10) | 1.5 ± 0.8 |                         | 3.0 ± 1.4 |                         |

* There is a significant increase of gap junction in orange (P < 0.01) and white cells (P < 0.02) at the time of folding. The 10 cell membranes used are the 10 nearest to the intersegmental boundary.

The adult cuticle is secreted at the apical surfaces of the folded cells, and, at ecdysis, the fold becomes somewhat opened out.

DISCUSSION
During the last few years it has been noted that in insects, marked clones, while having variable shapes, may always respect certain defined boundary lines (7, 3). These observations can be interpreted as due to the separate origin of tissues which later come together, thus abutting cells of remote origin along a defined line. More detailed study has shown that this explanation may not apply to all cases: (a) in an analysis of the wing disk of Drosophila, Garcia-Bellido et al. (9) studied the shape of clones which have an enhanced rate of growth when compared to unmarked cells. They find that the enlarged clones are still confined to, and serve to demarcate rigidly defined areas on the wing (compartments). They show that the wing disk becomes progressively subdivided as development proceeds, new effective boundaries being established across previously unsegregated and unseparated groups of cells; (b) In Oncopeltus at midblastoderm stage, marked cells may give rise to progeny cells in several segments; but only one or two cell divisions later, at the time of cellularization of the blastoderm, similarly marked cells generate progeny confined only to a single segment (14). During much embryonic (5) and all larval growth, the epidermis remains as a single sheet with the epidermis from different segments forming a continuous monolayer of cells. The boundary between different segments is marked only by a change in cell shape and, in later instars, by an abrupt change in pigmentation (14). It is this color change that has allowed us to look at the detailed anatomy of the intersegmental boundary in the electron microscope.

We have been able to describe in detail the change in cell shape at the boundary and have noted the types of junction. Our data (Table I) are inadequate to rule out the hypothesis that differences in the amount of cell junctions may be important. This is mainly because sample sections in one plane cannot give a complete picture of the shape and distribution of junctions, but also because apparently identical structures may have diverse functions. Our results, however, do show that there are no gross differences in the cell junctions associated with the border; attachment and septate junctions, as well as gap junctions, are found as frequently between cells of different segments as between cells of the same segment. We see little that helps to explain why, during development, dividing cells in each segment remain on their own side of this remarkably straight line. The preferred orientation of cell divisions at the anterior edge of the segment (14) as well as the discontinuity of shape and the position of the kink when the epidermis is thrown into a fold (Figs. 12, 13) suggest that this continued lack of cell mingling may be due to differences in "adhesiveness" of the cells (20, 6). Perhaps the cells remain
FIGURE 9 Plate from the section shown in Fig. 8. Note that the white (w) and orange (o) pigment granules allow identification of the segment border (arrows). a = attachment desmosome; c = cuticle; g = gap junction; s = septate junction. × 13,400.
**Figure 10** Detail from white area shown in Fig. 8. × 21,000.

**Figure 11** Detail of the orange area shown in Fig. 8. × 23,000. a = attachment desmosome; g = gap junction; i = interdigitations; m = unspecialized membrane apposition; o = orange pigment granule; s = septate junction; w = white pigment granule.
strongly attached to neighboring cells of the same segment and only weakly associated with neighbors in the adjacent segment. Such properties could cause a discontinuity in shape where one mutually adhering sheet of deformable cells confronts another, especially where the entire structure is under pressure or tension. If this is so, it is of interest that the difference in cellular properties across the boundary finds no expression in the cell junctions visible in the electron microscope.

Further support for this view comes from the behavior of cells grafted between different sites in the abdomen; cells taken out and replaced in the same site soon re-establish themselves and contribute to a normal epidermal sheet, while those moved up or down the segment, or replaced in unusual orientations, take unevenly, giving crowded cells and changes of cell shape at the junction between host and graft (18, 2) (P. A. Lawrence, unpublished observations on Rhodnius, Oncopeltus, and Dysdercus). The segments of the abdomen are apparently homologous because grafts between equivalent places on adjacent segments take well (18) (Lawrence, unpublished observations), whereas grafts between, for example, anterior and posterior sites in different segments take unevenly.

This behavior, therefore, could be another expression of the segmental gradient that controls cell polarity, so that the discontinuity between adjacent homologous gradients could coincide with the segment boundary. For example, in Rhodnius a cut made at a critical time in the molt cycle has markedly different effects on cell polarity depending on whether or not the cut crosses the segment boundary (16).

In attempting to correlate the anteroposterior gradient with structural features, we have to remember that there could be only a limited period in each molt cycle when the gradient is set up, and therefore only a limited period when a discontinuity could be detected in the electron microscope.

Because gap junctions are thought to be the channel for electrical communication (10, 4), the presence of gap junctions between cells of different segments in Oncopeltus is in good accord with observations on electrical coupling between the epidermal cells of a similar insect Rhodnius. Warner and Lawrence (23) found that a cell was as well coupled to another in its own segment as to a similarly distanced cell in an adjacent segment, showing that this compartment border allows intersegmental exchange of small ions. They could not detect any preferred direction of small current flow through the border. The possibility that gap junctions may allow the passage of small ions carrying current and not molecules carrying information (23) should be borne in mind.

In conclusion, it is important to remember that our knowledge of the physiological and anatomical basis of compartments remains scanty and that these observations on the segmental boundary of Oncopeltus may not be typical of compartment boundaries in general, nor of those of Drosophila in particular.

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