Ciba Foundation Symposium 29 (new series)

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Chairman's introduction

S. BRENNER

MRC Laboratory of Molecular Biology, Cambridge

I think we may have a rather difficult few days ahead of us: there is a lot to assimilate, much of which may be unfamiliar to people working in special fields. We shall be discussing a variety of different experimental systems and organisms, and there will be a number of different ways, often intricate, of doing experiments. Interspersed between these descriptions will be speculations about the possible mechanisms involved. I should therefore like to ask speakers to make clear, as briefly as possible, exactly what sorts of experiments they have done and can do and to try to keep separate the experimental facts from the interpretations of them. If we can do this, we may be able to establish some communication between those who work with slime moulds and those who work with embryos and developing nerves. In addition may I remind those who work on the nervous system, that not many people are *au fait* with the behavioural and electrophysiological experiments which you do and you may have to provide us with some background.

You will see from the programme that we are not following a Linnaean order; we shall not have an insect day or an avian day, and this means that we may have to discuss the same thing several times. For example, the opening talk by Dr Lawrence on compartment borders in *Oncopeltus* is linked with Dr García-Bellido's talk on *Drosophila*, and thereafter we shall move down the scale to *Protozoa*.

One last point—it would be helpful if speakers were to mention the size of the objects they are working with; just say whether it is micrometres, millimetres or miles.

The structure and properties of a compartment border: the intersegmental boundary in *Oncopeltus*

PETER A. LAWRENCE

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Abstract García-Bellido *et al.* have described how groups of cells in developing *Drosophila* become subdivided into 'compartments'. Cells within any compartment have rigid prospective fates such that, while their progeny may give rise to variable regions within a compartment, they can never generate cells in any other compartment. Analysis of the position and shapes of clones allows definition of the compartment boundaries.

I report studies on a compartment boundary (likewise demonstrated by clonal analysis) in the hemipteran insects *Oncopeltus* and *Rhodnius*. The advantage of this border is that it can easily be identified in the light and electron microscopes. There is an abrupt change of cell shape at the border, which has been analysed by means of serial electron microscope sections. The types of cell junctions at the border and elsewhere are compared and shown to have no qualitative differences. The border is an effective barrier to the growth of peripheral sensory axons, although not apparently to dendrites. The intersegmental boundary allows passage of information relating to cuticle deposition, wounding response, tracheolar movement and intercellular coupling. Making wounds across the border leads to greater effects on polarity of epidermal cells than making similar wounds elsewhere on the tergites.

COMPARTMENTS

Methods for analysing growth of insects by means of genetically marked clones have been developing rapidly. The ability of X-rays to induce a high rate of somatic crossing over, as well as the existence of mutants which mark the cuticular secretion of each cell, has permitted a sophisticated analysis of cell lineage in *Drosophila*. Irradiation of staged eggs and larvae has shown that, progressively during development, groups of cells acquire particular properties which affect their behaviour and the behaviour of the clones of progeny that each cell generates. This is expressed partly in the shape of the clones: for example, it was noted (García-Bellido 1968; Bryant 1970; García-Bellido &

Merriam 1971) that clones produced by X-irradiation after the first larval stage, although variously shaped, never crossed from dorsal to ventral on the wing blade. If they reached the wing margin their edges ran exactly along the edge of the wing, without a single cell straying to the other side. Similarly, clones produced in the abdomen of the milkweed bug *Oncopeltus* by irradiation after the late blastoderm stage are restricted to individual segments, and if near to the edge of the segment, run along the boundary without crossing it (Lawrence 1971, 1973*a*).

Thanks to a detailed analysis by García-Bellido *et al.* (1973, 1974), the great importance of these observations is becoming apparent. They studied marked clones which had the capacity to grow so much more rapidly than the surrounding unmarked cells that if they had been unrestrained they would have covered several organs in the adult. In fact, these clones were restricted to certain defined regions (*compartments*), their edges frequently running along fixed boundary lines. By irradiating at different times García-Bellido *et al.* found that compartments were established progressively during development, clones produced later being confined to smaller compartments than those produced earlier. A compartment can thus be subdivided into a small number of subgroups of cells, which each acquire particular properties that define the structures they can eventually generate, and the space their progeny will occupy. The proper development of each new subcompartment may depend on the activity of a particular wild-type allele (García-Bellido *et al.* 1973, 1974; García-Bellido & Santamaria 1972; García-Bellido, this volume pp. 161–178), an observation that offers, for the first time, the prospect of analysing the role of the genome in pattern formation.

It is worth emphasizing that a compartment border differs from any other line drawn over the surface of an insect. Clones generated after the establishment of the compartment never cross this border in any individuals. No other line drawn on the body surface will have this property—it will be crossed by clones in at least some individuals.

I have gathered together here some information about a compartment boundary, mainly concerning the intersegmental border in *Oncopeltus*, where even large clones generated after the late blastoderm stage fail to cross from one segment to another, but frequently define the compartment boundary by running along it (Fig. 1). An advantage of studying *Oncopeltus* is that the clone is not seen in the cuticle, but is marked by altered pigmentation in the epidermal cells themselves (Lawrence 1973*a*, *b*). From the proportion of the total segment occupied by the average clone, I calculated that when the border is first established there are *about* 10 presumptive epidermal cells in each segment quadrant (dorsal or ventral, left or right). Thereafter an epidermal cell in any particular

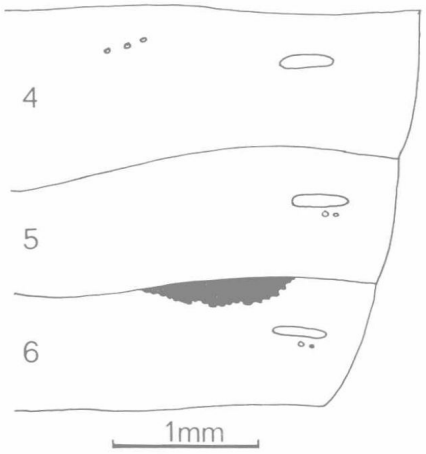

FIG. 1. An X-ray induced clone of differently coloured cells (shown in black) near the intersegmental boundary in the sixth abdominal segment of *Oncopeltus*. Note how the clone runs along, but does not cross, the boundary. Clone was produced by 100 R of X-rays delivered 18–20 h after egg–laying.

quadrant only generates cells in the same quadrant. During larval development, where the growth of clones can be observed directly, the epidermis grows evenly, cells dividing at a similar rate all over the segment. However the discontinuity at the border is associated with mitoses that have a preferred orientation, such that daughter cells come to lie side by side along the boundary. Even in unirradiated insects the border is clearly marked by a change in cell shape and pigmentation, both of which are visible in the light and electron microscopes.

STRUCTURE OF A COMPARTMENT BORDER

Serial electron microscope sections were taken of a larva in the middle of the moult cycle and reconstructions made of several epidermal cells at a boundary (Lawrence & Green, in preparation). These confirmed that the *anterior cells* of one segment are stretched out mediolaterally more than the *posterior cells* of the adjacent, more anterior, segment (Figs. 2, 3). This change occurs abruptly at the segment border. The cells are shaped rather like the hull of a yacht, oriented so that the 'deck' is near to and in the plane of the cuticle. Three types of intercellular junction link the cells:

(1) Invariably, at the extreme apex of the lateral membrane of the cell there is an attachment desmosome (Ashurst 1970). (2) Septate desmosomes (Locke

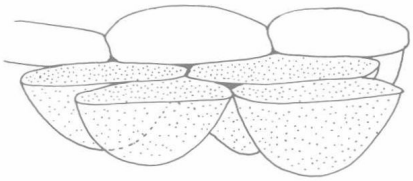

FIG. 2. Sketch of segment border to indicate cell shapes. The orange cells (anterior cells) are shaded.

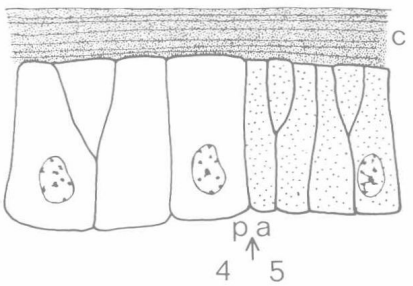

FIG. 3. Diagrammatic cross section through segment border. The arrow marks the segment boundary, separating anterior cells (a) of segment 5 from posterior cells (p) of segment 4. The cuticle (c) is indicated.

1965) and (3) gap junctions (Furschpan & Potter 1968; Hagopian 1970) were frequent and mostly confined to the apical part of the lateral membrane of the cell. All three types of junctions linked the cells of adjacent segments in a similar manner to the way they linked cells of the same segment.

When the epidermis separates from the old cuticle, preparatory to secreting the new, it becomes folded in the region of the intersegmental boundary. This is associated with a striking change of cell shape, as the posterior cells become more columnar, and the anterior cells more cuboidal; again the discontinuity of cell shape is close to the segment border itself. We need some explanation for the ability of the epidermal cells to change shape while maintaining the structural discontinuity at the border. Possibly the shape of the cells is mostly due not to some internal skeleton, but depends on whether the epidermal sheet as a whole is under pressure or tension, and the degree to which the individual cells adhere to their neighbours. The discontinuity at the border may therefore be an expression of an abrupt change in mutual adhesiveness (cf. García-Bellido 1966).

ELECTRICAL COUPLING

The presence of apparently normal gap junctions and septate desmosomes

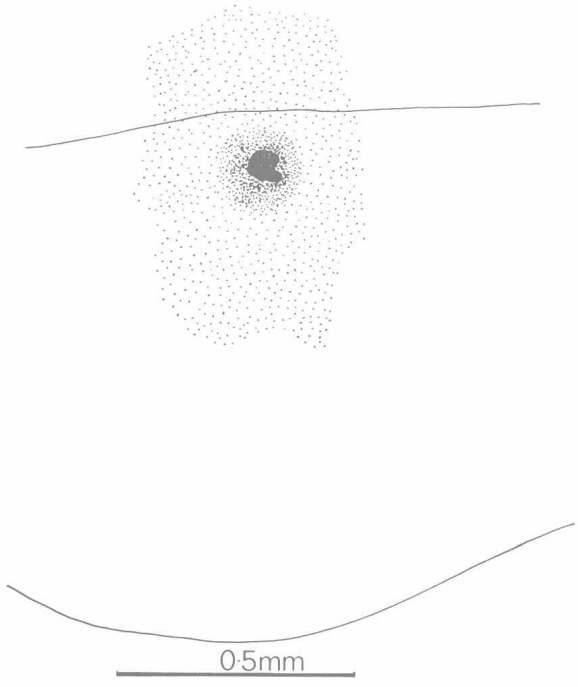

FIG. 4. Sketch to scale of small wound made near to, but not including, the intersegmental boundary. The insect was fixed and stained 76 h after the wound (the affected area is shaded). Note how the effect of the wound spreads across into the next segment. The affected area is commonly elliptical, with its long axis oriented anteroposteriorly.

linking cells of adjacent segments is consistent with our observations on electrical coupling between epidermal cells of another hemipteran, *Rhodnius*. Gap junctions are thought to be the route through which ions and small molecules pass from cell to cell (De Haan & Sachs 1972) although septate desmosomes have also been implicated (Lowenstein 1973). We observed that epidermal cells in the different segments are as well coupled to each other as to cells within the same segment (Warner & Lawrence 1973).

WOUNDING

Epidermal cells will respond to a nearby small wound by an increase in the size of the nucleoli and in the staining of the cytoplasm. They also migrate towards the site of the wound (Wigglesworth 1937). I have looked at this response vis-à-vis the intersegmental boundary. Small burns and cuts were made near the border, and cells from both sides of the boundary responded similarly (Fig. 4). Wigglesworth (1937) provided evidence that degradation products

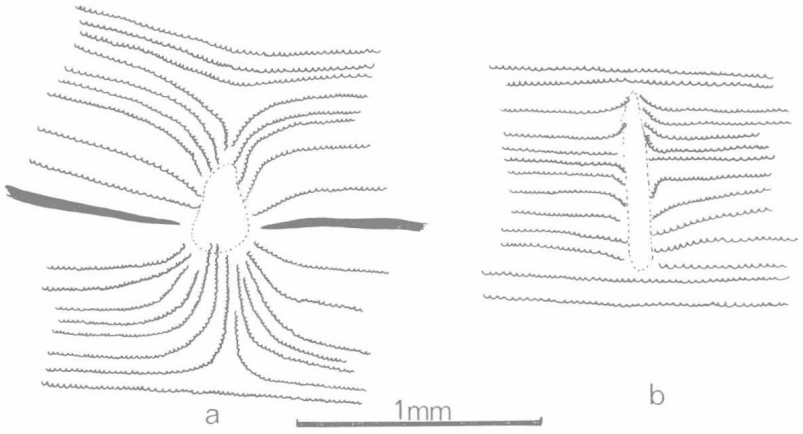

FIG. 5. Sketches of the result of two similar cuts made on 5th-stage larvae of *Rhodnius*. *(a)* The effect of a cut made across the segment boundary on the adult cuticle. *(b)* The effect of a similar cut made within the segment.

from proteins could be the stimulus for this wounding response; whatever the stimulus is, it can clearly pass across the border.

The polarity of the epidermal cells is also affected by wounding, and in this case the intersegmental boundary does seem to be an important factor. In adult *Rhodnius*, the cuticle is secreted in transversely oriented ripples (Locke 1959; Wigglesworth 1973). Cuts and burns inflicted on 5th-stage larvae in the early part (0–9 days) of the 18 day moult cycle heal completely and have no effect on the adult cuticle, and those made after 12 days leave no time for healing; between these times cuts affect the polarity of the cells as expressed in the orientation of the ripples (Lawrence *et al.* 1972). In particular, a cut oriented in the anteroposterior axis results in some nearby ripples turning parallel to it. This effect is considerably stronger when a similar cut extends across the intersegmental boundary; then many ripples turn to fan out from the cut (Fig. 5). Our explanation of this phenomenon depends at least in part on experiments on insect segmental gradients. One possible model regards the gradient as behaving like a concentration gradient of a diffusible molecule, the direction of steepest slope giving the polarity of the individual epidermal cells (Lawrence 1966*a*). This model fits the experimental data rather well, although it is certainly incomplete (Bohn 1974; Lawrence 1974; Nübler-Jung 1974). Within the terms of the model we proposed that a cut might temporarily increase the rate of diffusion of the molecule along the line of the cut. One would expect this to produce a much greater change in the concentration landscape when the cut linked anterior and posterior parts of adjacent segments,