**engrailed gene expression in the abdominal segment of Oncopeltus:**
gradients and cell states in the insect segment

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**Summary**

A monoclonal antibody that recognizes the product of the segmental gene, *engrailed* (*en*), of *Drosophila* has been used to analyse expression of the homologous gene of *Oncopeltus*. *engrailed* expression in the abdominal segment of larval *Oncopeltus* is confined to a narrow band of epidermal cells localized immediately anterior to the segment border. Expression varies in intensity during postembryonic development: no gene product is detectable in newly moulted larvae, but reappears soon after initiation of intermoult activities. One possible function of *en* in this system is revealed by a series of operations confronting cells from different anteroposterior levels in the segment. New segment borders are generated only when *en*-expressing cells confront cells from the anteriormost region of the segment. All other combinations result in intercalation of intermediate intrasegmental levels. It is therefore suggested that the most important function of *en* is the establishment of new, and presumably the maintenance of existing, segment borders.

Key words: *engrailed*, *Oncopeltus*, insect segment, gradients, pattern formation.

**Introduction**

The abdomen of *Oncopeltus* is visibly divided into segments. The interface between adjacent segments, the segment border, is characterized on the dorsal side by a row of transversely elongated cells, more darkly pigmented than surrounding cells, and by a groove in the surface of the cuticle (Lawrence, 1973a; Wright & Lawrence, 1981a; Campbell & Shelton, 1987). The traditional view of the insect abdomen holds that there is a repeating series of gradients of positional information; each gradient corresponds to a segment so that there is a discontinuity in positional value at the segment border (Locke, 1959, 1960; Stumpf, 1966; Lawrence, 1966, 1973b; Campbell & Shelton, 1987). Thus, the cells at the segment border may have special properties enabling them to stabilise such a discontinuity. The segment border is also a compartment border; the progeny of cells marked after the germ band stage are confined to a single segment (Lawrence, 1973a,c). Clonal analysis shows *Drosophila* segments to be further divided into anterior and posterior compartments (Garcia-Bellido *et al.* 1973; Crick & Lawrence, 1975; Kornberg, 1981a). Evidence for the division of *Oncopeltus* abdominal segments into anterior and posterior compartments is not conclusive (Lawrence, 1973c, 1981).

The lineage restriction at the compartment border in the imaginal discs of *Drosophila* relies upon expression of the *engrailed* gene in the posterior compartment: posterior clones of mutant *engrailed* in wild-type discs will cross the border, but this is not true for such clones in the anterior compartment (Morata & Lawrence, 1975). *engrailed* is also considered to be partly responsible for specifying the differentiated state of posterior compartment cells, because in viable mutants the posterior compartments of adults approximate to mirror-image duplications of the corresponding anterior compartment (Garcia-Bellido & Santamaria, 1972; Lawrence & Morata, 1976). Although these studies showed a continuous requirement for normal *en* function, later studies revealed its primary function to be during embryogenesis. Complete deletion of the *en* locus is lethal and results in embryos that fail to form segments properly (Nüsslein-Volhard & Wieschaus, 1980; Kornberg, 1981b). Cloning the gene and localizing transcripts by *in situ* hybridization revealed a pattern of stripes along the anteroposterior axis of the embryo (Kornberg *et al.* 1985; Fjose *et al.* 1985). Immunofluorescent staining with a polyclonal antibody against the *en* gene product gave a similar pattern of stripes and showed it to be localized in nuclei (DiNardo *et al.* 1985); the gene product is in fact a DNA-binding protein (Desplan *et al.* 1985).

The present study was undertaken initially to establish whether the abdominal segments of larval *Oncopeltus* are divided into anterior and posterior compartments. To do this a monoclonal antibody against the *en*
gene product of Drosophila was used to examine expression of the homologous gene of Oncopeltus. It is shown that the abdominal segment is in fact divided into an anterior en-negative and a posterior en-positive compartment. How this relates to gradient theory and regeneration of segment borders was investigated by a series of experiments confronting cells from different anteroposterior levels in the segment. The results of these operations are discussed in the light of the present knowledge of whether the confronted cells express en or not.

Materials and methods

(A) General
Stocks of Oncopeltus fasciatus (Lygaeidae, Hemiptera) were kept at 26°C in a D: L cycle of 12: 12 and fed sunflower seeds and water. Experimental animals were removed from the main stock within 12 h of moulting and maintained only on water. They were used within five days. These animals remain in an arrested developmental state. The intermoult/moult cycle was initiated by feeding.

(B) Immunocytochemistry
The antibody used here binds to the homeobox domain of the product of both the engrailed and invected genes (Patel, N., Martin-Blanco, E., Coleman, K. G., Poole, S., Ellis, M., Kornberg, T. & Goodman, C., submitted) and was a generous gift from Thomas Kornberg. The white body, red eye mutant of Oncopeltus was used for most of these studies because the orange pigment in the wild-type epidermal cells usually obscures staining. Integument was fixed for 15–60 min in 4% formaldehyde, 0.1 M–Pipes, 2 mM–MgSO4, 1 mM–EGTA, pH 6.95, washed for 1 h (with numerous changes) in PBS+0.5% NP-40 and then blocked for 0.5 h in this saline containing 5% FCS. The tissue was incubated overnight at 4°C in ascites fluid containing the anti-en antibody (1:100 dilution). Tissue was washed and blocked as before and incubated for 2 h in biotinylated horse anti-mouse antibody (1:200 dilution; Vector Labs). After washing for 1 h, the tissue was treated with the Vector Labs peroxidase–ABC reagent for 2 h and developed with diaminobenzidine according to instructions. Subsequent treatment in 0.02–0.2% osmium tetroxide enhanced the reaction product. The integument was dehydrated in 100% ethanol, cleared and mounted.

(C) Intracellular dye injection
This technique is described in Blennerhassett & Caveney (1984). The bathing medium used was their medium 1, TC 199.

(D) The operations
The general technique is described in Campbell & Shelton (1987). Cells from different anteroposterior levels were confronted by removing the cells in between these levels. This was done by scraping the cells from underneath the cuticle with a needle inserted through a lateral incision. The cells at the edges of the wound migrate over the cell-free space and meet in the centre.

Engrailed expression during pattern regulation was analysed in starved 4th instars, 4 days after an operation. To determine the final pattern produced by an operation, animals were wounded as 3rd instars and analysed as 5th. In order to determine the pattern of en expression in the latter, they were fixed 3 days into the moult cycle. Over half of the representatives of each category listed in Table 1 were examined for en expression.

The operations were as follows (Fig. 1).

(1) sb
This removes the cells at the 2/3 segment border and approximately 5% of both segment 2 and 3 (never more than 8% of either).

(2) sb+15ant and sb+25ant
These remove a strip of cells spanning the anterior 15 or 25% of segment 3 and the 2/3 segment border (removing less than 8% of segment 2).

(3) sb+25post
This removes a strip of cells spanning the posterior 25% of segment 3 and the 3/4 segment border (removing less than 8% of segment 4).

Results

(A) Engrailed expression in 4th instars
Temporal expression
The intermoult/moult cycle of Oncopeltus is initiated by feeding, so that larvae starved within about 8 h after ecdisis will remain in an arrested state (Nijhout, 1979). Epidermal cell activity during the intermoult/moult cycle of the 4th instar can be summarized as follows (unpublished observations). If animals are not fed after ecdisis, the epidermal cells cease many activities, including cuticle deposition, so that the cells from starved larvae are clearly inactive and do not divide. Intermoult activities commence soon after feeding: endocuticle deposition within 24 h and the first mitosis within 24–36 h. Apolysis occurs about 3 days and is followed by new cuticle secretion. The animals ecdisis after about 4–5 days.

No antibody staining was detected in whole mounts from newly moulted or starved 4th instars. In fed larvae, it first becomes detectable at 24 h; the staining becomes much stronger by 48 h. Following apolysis, staining over most of the preparations becomes faint, but at the edges of these preparations it remains intense. This variation is probably an artifact. At this stage, the basal lamina may impede antibody entry into cells; at earlier stages of the moult cycle and in newly moulted the basal lamina becomes detached when the integument is dissected from the animal (unpublished observations). Staining becomes very weak everywhere on preparations from animals just about to moult and is sometimes undetectable.

<table>
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<tr>
<th>Operation</th>
<th>New segment border</th>
<th>Band of reversed polarity</th>
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<tr>
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Table 1. The patterns resulting from the four operations
**Spatial expression**

Antibody staining is located in the nuclei of epidermal cells situated in the posterior region of the abdominal segment. The *en*-positive cells form a continuous circumferential band around the abdominal wall. The width of this band in the 3rd tergite varies from 8 to 20% of segment length, the narrowest point occurring in the midline (Fig. 2). The posterior margin of the band coincides with the segment border and is reasonably straight; the anterior margin is not as straight and accounts for the variation in width (Fig. 2). The exact site of the segment border is not easy to determine on most of these *wb, re* preparations (because cell morphology is usually obscured and at this stage the groove in the cuticle does not correspond exactly to the site of the segment border (unpublished observations)) so that it is usually impossible to be absolutely certain that *en*-expression stops exactly at this site, although it definitely does not extend into the next segment. However, on a few preparations from wild-type larvae, the exact site of the segment border can be determined because of the change in pigmentation found at this site. On these whole mounts, antibody-positive nuclei extend up to the segment border (Fig. 2C). There is no obvious spatial variation in staining, although staining at the posterior margin, i.e. just in front of the segment border, often appears slightly less intense than elsewhere in the band. There is variation between adjacent cells; this might reflect different stages of the cell cycle.

The size and location of the *en* band corresponds almost exactly to a distinct band of white pigmented cells found in the sternites of wild-type larvae (Fig. 3). On the basis of clonal analysis, Lawrence (1973c) suggested that this band might be the posterior compartment. However, it is not possible to be absolutely certain about this correspondence because it is impossible to maintain the pigmentation pattern and produce good antibody staining.

(B) Junctional permeability at the intrasegmental compartment border

Diffusion of organic tracers from cell to cell via gap junctions is impeded at the segment border (Warner & Lawrence, 1982); this appears to be caused by the presence of a special population of border cells that have reduced junctional permeability (Blennerhassett...
Fig. 2. *en*grailed expression in the abdominal segment of 4th instars. Whole mounts of dorsal, abdominal integument from normal 4th instars fixed 48 h after feeding and stained with anti-*en* antibody. (A) Centred on the 3rd tergite. (B) Same preparation at higher magnification centred on the midline. (C) A different preparation from a wild-type animal; the position of the segment border (*sb*) is clearly distinguished as the discontinuity in the pattern of pigmentation. Antibody staining is confined to nuclei in a narrow band situated just in front of the segment border (*sb*). The posterior margin of the band coincides with the segment border and is reasonably straight; the anterior margin is more irregular. There is a characteristic narrowing of the band at the midline. *A*, anterior; *sb*, segment border; *P*, posterior, 2,3,4, segment number. Bars: (A) 0.1 mm, (B) and (C) 0.05 mm.

To test whether this phenomenon is also found at the intrasegmental compartment border, carboxyfluorescein was injected into cells located close to this compartment border at the midline of the tergites of 4th instars. The margin between *en*-positive and *en*-negative cells within the segment shows none of the special properties of the segment border such as cuticular groove and cell elongation (Fig. 4), so the position of the intrasegmental compartment border in unstained preparations must be predicted on the basis of the antibody studies. This prediction is most reliable at the midline. No restriction to dye movement could be detected in the vicinity of the predicted intrasegmental border (Fig. 5); all dye diffusion patterns in this region were symmetrical with respect to the injected cell. At the segment border similar results to Blegnerhassett & Caveney (1984) were observed, i.e. diffusion of dye was impeded at the border on some, but not all, preparations. No restriction to diffusion was ever observed at the intrasegmental compartment border, even on preparations where this was observed at the segment border.
Fig. 3. The pattern of en expression coincides with the cell pigmentation pattern. Whole mounts of ventral, abdominal integument from 4th instars centred on the midline and the 4/5 segment border and fixed 48 h after feeding. (A) Stained with anti-en antibody. (B) Unstained, showing the band of white pigmented cells located in the posterior region of the segment. The white band coincides almost exactly with the band of en-positive cells in A. Anterior at left. sb, segment border; 4,5, segment number. Bar: 0.5 mm.
engrailed expression in Oncopeltus 731

Fig. 4. The intrasegmental compartment border. Whole mount of dorsal, abdominal integument from a 5th instar stained with anti-en antibody; focussed either on the cells (A) or the cuticle (B). The cells at the margin (marked) between en-positive and negative cell populations show no variation in morphology. The cuticular sculpturing at this site is also characteristic of any intrasegmental region. Anterior at left. Bar: 0.01 mm.

Fig. 5. Junctional permeability at the intrasegmental compartment border. (A) Carboxyfluorescein injected into a cell close to the intrasegmental compartment border at the midline of the 3rd tergite from a 4th instar. The predicted site of this border is indicated. The dye has spread symmetrically from the injected cell; there is no barrier to diffusion at this border. (B) Phase-contrast image of A. Posterior at left. sb, segment border. Bar: 0.02 mm.

(C) engrailed expression during pattern regulation

Confronting cells with sufficiently disparate positional values leads to localized cell division at the confrontation site, even in starved animals (Campbell & Shelton, 1987; Campbell, 1987). This reaction occurs after all four operations described here (shown for the sb+25post operation, Fig. 6A). As mentioned above, no antibody staining can normally be detected in starved larvae. However, 4 days after the sb, sb+15ant and sb+25ant operations, en-positive cells are present at the site of confrontation when the reaction is intense (the amount of cell division is variable after any operation). Only cells that would normally express it during the moult cycle are stained (Fig. 6C), so that after these three operations all the labelled cells are located anterior to the site of confrontation (this site can be detected because cell elongation is most pro-
Fig. 6. engrailed expression during pattern regulation. Whole mounts of dorsal abdominal integument from starved 4th instars fixed 4 days after an operation. (A) *sb+25post*, stained with trioxyhaematin and showing localized cell division (*m*) and cell elongation at the confrontation site. (B) Another *sb+25post* preparation, stained with anti-**en** antibody and centred on the region of cell division and elongation (this cannot be distinguished on this figure); no cells are stained. (C) *sb+15ant*, stained with anti-**en** antibody; a line of **en**-positive cells is found at the confrontation site. Although not clearly shown on this photograph, all the stained cells are located on the anterior side of the confrontation site. Anterior at top. *m*, mitotic figure. Bars: 0·05 mm.

nounced here). The *sb+25post* operation confronts non-**en**-expressing cells, and no antibody staining is found at the site of confrontation even when the reaction is very intense (Fig. 6B).

(D) Pattern two moults after the operations
The normal cuticular pattern of the tergite consists of posteriorly pointing bristles and a sculpturing pattern of irregularly packed polygons, each representing the area of cuticle secreted by a single epidermal cell (Campbell & Shelton, 1987). The polygons are polarized so that it is possible to determine individual cell polarity. This sculpturing pattern is modified at the segment border to form a characteristic array of approximately parallel lines (Campbell & Shelton, 1987). This pattern is described in more detail in Fig. 7.

These operations remove the segment border and a varying amount of tissue either side (Fig. 1). In principle, there are two possible outcomes: the segment border can regenerate or intrasegmental positional values can be intercalated forming a band of reversed polarity. In practice there is a third possibility which is a highly variable pattern consisting of rows of transversely elongated cells in which mitotic activity is high; the cuticle above these cells is smooth, but further out is distorted into an irregular array (Fig. 7C). Polarity of bristles in the surrounding region can also be disturbed, especially in the posterior region. This phenomenon is best explained as an example of aberrant pattern regulation: for whatever reason, the cells at the confrontation site of the original wound appear unable to intercalate missing positional values and continue the reaction (cell division and elongation) that is normally found only for a short period after wounding (Campbell & Shelton, 1987; Campbell, 1987). The results are summarized in Table 1.

(I) *sb*
This operation removes the 2/3 segment border confronting **en** cells with those 8%, at most, of segment
length posterior to that border (Fig. 1). In almost all cases, it results in regeneration of the border (Table 1). The new border is almost indistinguishable from an unoperated one (Fig. 7B).

(2) sb+15ant and sb+25ant
This operation confronts en cells with those slightly more posterior to the segment border than the last operation: either 15% or 25% of segment length (Fig. 1). Following over half the sb+15ant operations again the segment border regenerates, but in about a quarter a band of reversed polarity is formed (Table 1). The latter pattern is found after virtually all the sb+25ant operations (Fig. 7D); the segment border is never regenerated (Table 1). The band of reversed polarity presumably represents intercalation of intrasegmental levels, although its length, 275 ± 31 μm, is
considerably less than the width of the equivalent positional values on an unoperated segment, approximately 600 μm. The en cells only contribute to about 10% of this band suggesting that most of the intercalated region is formed from cells in the anterior region of segment 3 (Fig. 7Dii). The cuticular sculpturing pattern in this band is modified so that the normal polygonal array is compressed and the posterior margins of adjacent polygons are continuous over long distances. This is most extreme at the interface between en-positive and negative cells (Fig. 7Dii).

(3) sb+25post
This operation confronts cells located just either side of the en band (Fig. 1). After two moult, 75% have a band of reversed polarity in place of the 3/4 border (Fig. 7E, Table 1). This is even narrower (149 ± 31 μm) than that found after sb+25ant operations even though both remove the same width of cells, but the cuticular sculpturing pattern tends to be less distorted (Fig. 7Dii, Eii). No cells in this band stain with the en antibody.

Discussion

(A) engrailed expression in Oncopeltus
It is no surprise to find that Oncopeltus possesses a gene homologous to the en gene of Drosophila; homologous genes have been detected in much more distantly related animals, for example, mice (Joyner et al. 1983). The antibody used in this study also binds to the injected gene product (Patel et al. submitted). This gene appears to be translated in the same cells as en (Coleman et al. 1987) and to save repetition reference here will only be made to en, but if injected is translated, it presumably is performing a different function than en. In the abdominal segment of larval Oncopeltus, en is expressed in a band of epidermal cells located immediately anterior to the segment border. This band varies in width from 8 to 20% of segment length, the variation being due mainly to an uneven anterior margin (the intrasegmental compartment border; Fig. 2). The posterior margin is reasonably straight and corresponds almost exactly with the segment border (Fig. 2C). This pattern of expression is practically identical to that found in the embryonic segments of Drosophila (DiNardo et al. 1985). Antibody staining of the imaginal discs of Drosophila also shows engrailed to be expressed postembryonically and appears to show a spatial variation in the level of expression (Brower, 1986). Such spatial variation was not detected in Oncopeltus.

The level of en expression is not constant throughout the course of the moult cycle. No antibody staining can be detected in newly moulted or starved animals, but reappears soon after the intermoult/moult cycle has been initiated. The exact point at which expression is reduced is difficult to determine but occurs at about the time of ecdysis. The exact cause of this phenomenon is uncertain. It should be noted that the epidermal cells of Oncopeltus cease many of their activities after ecdysis and lack of antibody staining may be a reflection of this reduced activity; or it could, of course, have a more direct developmental function.

Although normally no en expression can be detected in starved larvae, this is not the case during pattern regulation. en gene product can be detected at the site of localized cell division following confrontation of disparate positional values, but only in cells that would have stained during the moult cycle (Fig. 6B,C). This may simply reflect their increased activity during regulation or again may be more developmentally significant. The pattern of cell behaviour found at such discontinuities in positional value is not dependent upon the interaction between en and non-en cells. This is clearly shown by the sb+25post scrape which confronts non-en cells and yet also results in localised cell division (Fig. 6A).

(B) Compartments in the Oncopeltus abdomen
On the basis of en expression, the abdominal segment of Oncopeltus is divided into anterior and posterior compartments. However, whether these can be directly compared to the classic lineage units of the Drosophila wing disc is not clear. The compartment border of this disc forms a very straight line and is more comparable to the segment border of Oncopeltus than to the more irregular intrasegmental compartment border (Garcia-Bellido et al. 1973; Lawrence, 1973a,c; Figs 2, 4). The lineage restriction at the segment border is probably not maintained simply by the confrontation of en with non-en cells, but more likely by the special cells located at this site. The special properties of the segment border—cuticular groove, cell elongation and reduction in junctional permeability—are not shared by the intrasegmental compartment border (Figs 4, 5).

(C) Regeneration of segment borders
The en gene is required for segmentation of the Drosophila embryo and adult (Kornberg, 1981a,b; Nüsslein-Volhard & Wieschaus, 1980). To investigate whether it is similarly required in larval Oncopeltus, different anteroposterior levels in the segment were confronted to provide a detailed picture of exactly which levels must be confronted to generate a segment border. The results of the four operations described here (Table 1) can be summarized as follows.

(a) Segment borders are generated only when en cells are confronted with non-en cells located in the anterior-most region of the segment (operation sb, Figs 7B, 8B).
(b) Confrontation of en cells with non-en cells located more than 25% of segment length posterior to the segment border results in intercalation of intermediate positional values (operation sb+25ant, Figs 7D, 8C).
(c) Confrontation of non-en cells also results in intercalation of intermediate positional values (operation sb+25post, Figs 7E, 8D).

Thus, on the basis of these results, the segment can be divided into four anteroposterior cell populations (Fig. 8A):

(i) The en cells at the posterior extreme.
(ii) A strip of cells at the anterior extreme, which will
be termed N and are characterized by their ability to form a segment border when confronted with en cells.

(iii) The remaining intrasegmental cells, here termed I.

(iv) The segment border cells characterized by their morphology, pigmentation and special junctional permeability properties (Lawrence, 1973a; Blennerhassett & Caveney, 1984; Campbell & Shelton, 1987).

This division can be compared to a model proposed by Meinhardt (1984) who also divided the segment into three transverse regions, in order to explain segmentation during embryogenesis in Drosophila. His S, A and P compartments or cell states correspond to regions N, I and en, respectively. Similarly, he suggested that the segment border formed at the interface between two of these states.

* engrailed, therefore, appears to be required for the segmentation of the Drosophila embryo and adult (Nüsslein-Volhard & Wieschaus, 1981; Kornberg, 1981a,b) and for regeneration of segment borders in Oncopeltus. However, segmentation requires more than simply confronting en-expressing cells with non-expressing cells. This is shown by the sb+25ant operation. It is also shown by the naked segmentation mutant of Drosophila. These mutants develop the characteristic en stripes during embryogenesis (although these are wider than in wild-type embryos) but do not appear to be properly segmented (Martinez Arias et al. 1988).

A previous study suggested that segment borders are intercalated in a similar fashion to intrasegmental levels so that new borders should form when cells from more than half a segment length apart are confronted (Wright & Lawrence, 1981a,b). The present results cast doubt on this, for example the sb+25ant operation confronts cells over two thirds of a segment length apart, but a

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**Fig. 8.** Modified model of the insect segment. Positional values are distributed as a non-linear gradient; the discontinuity between adjacent segments is stabilized by the cells at the segment border. The segment is also divided anteroposteriorly into three cell populations; N, I, and en; during pattern regulation, this division is only important when N and en cells are confronted. Confrontation of cells with different positional values normally results in the intercalation of missing intrasegmental values so that in C and D the normal A/P sequence of values is reversed, accounting for the band of reversed polarity found after sb+25ant and sb+25post operations. However, when en and N cells are confronted, as in B, the segment border cells are formed instead. These are polarized with reference to en and N. In B, intercalation between the new segment border and surrounding cells will restore the original pattern.
segment border is not formed (Fig. 7D). One possible criticism of the present study concerns the method of confronting cells. The assumption that cells maintain their original positional value while migrating over a wound may be invalid because previously it was demonstrated that such changes may occur (Wright & Lawrence, 1981b; Campbell & Shelton, 1987). However, this criticism is not considered justified here for the following reasons. The wounds are extremely small (less than 0.14 mm); previously it was shown that similar-sized wounds result in no cell division prior to confrontation (0.1 scrapes in Campbell & Shelton, 1987). There is no evidence in this system that positional value can change without cell division. Much larger wounds can apparently result in very little or no change in positional value during wound healing, for example, this appears to be the case following over 50% of 0-6 bord scrapes (0.4 mm in width) in Campbell & Shelton (1987).

As an alternative to the gradient model, Martinez Arias et al. (1988) suggested positional information in the insect segment may be represented as a series of cell states, with the en-expressing cells forming one of these states. If this concept holds for the Oncopeltus segment, one would expect the sb+2post operation, which confronts cells just either side of the en band, to result in intercalation of the en cell state. However, this does not happen and instead a band of reversed polarity forms (Fig. 7E), presumably representing intercalation of intrasegmental positional values (Fig. 8D). There is no evidence that during pattern regulation in this system en can be switched on in cells not normally expressing it, in contrast to the Drosophila imaginal disc in which this may happen to a limited extent (Kiehle & Schubiger, 1985).

To summarize, the results are consistent with a modified model of the insect segment (Fig. 8) in which intercalation and segment border formation are considered separate phenomena. The earlier suggestion that there is a non-linear gradient of positional values within the segment with discontinuities at the segment borders (Campbell, 1987) is maintained, but extended so that the segment is also divided into three transverse cell populations or cell states, N, I and en. Thus, cells are considered to have a positional value and a state. Confronting cells with different positional values normally result in intercalation of intermediate values, unless N and en cells are confronted; this results in the generation of a segment border.

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References


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