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Hedgehog organises the pattern and polarity of epidermal cells in the *Drosophila* abdomen

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SUMMARY

The abdomen of adult *Drosophila*, like that of other insects, is formed by a continuous epithelium spanning several segments. Each segment is subdivided into an anterior (A) and posterior (P) compartment, distinguished by activity of the selector gene *engrailed* (*en*) in P but not A compartment cells. Here we provide evidence that Hedgehog (Hh), a protein secreted by P compartment cells, spreads into each A compartment across the anterior and the posterior boundaries to form opposing concentration gradients that organize cell pattern and polarity. We find that anteriorly and posteriorly situated cells within the A compartment respond in distinct ways to Hh: they express different combinations of genes and form different cell types. They also form polarised structures that, in the anterior part, point down the Hh gradient and, in the posterior part, point up the gradient — therefore all structures point posteriorly. Finally, we show that ectopic Hh can induce cells in the middle of each A compartment to activate *en*. Where this happens, A compartment cells are transformed into an ectopic P compartment and reorganise pattern and polarity both within and around the transformed tissue. Many of these results are unexpected and lead us to reassess the role of gradients and compartments in patterning insect segments.

Key words: Hedgehog, polarity, *Drosophila*, segmentation

INTRODUCTION

The insect segment has long been a model for analyzing how pattern and cell polarity are organised. It is well established that cells within each segment make decisions about when to divide and die, what cuticular structures to differentiate, and how to orient these structures based on information reaching them from other cells (reviewed in Lawrence, 1992). This information could be local, as illustrated by the ability of bristle-forming cells to inhibit their neighbours from also becoming bristles (Wigglesworth, 1940). Or, it could be more global, as are the factors orienting cuticular structures such as ripples or hairs (Piepho, 1955; Locke, 1959, 1960; Lawrence, 1966; Stumpf, 1966), or those placing different types of cuticle within the segment (Marcus, 1962; Stumpf, 1968).

Grafting experiments on insects showed that the boundaries between segments are associated with long-range organising properties (Locke, 1960; Marcus, 1962), and this, as well as experiments in which small squares of cuticle were transplanted or reoriented, led Lawrence (1966) and Stumpf (1966) to propose that the pattern of cell types as well as the polarity of cells within each segment are controlled by gradients of diffusible morphogens; these gradients would peak at one edge of the segment and decline progressively towards the other edge. According to this model, cells at any point along the anteroposterior axis would assess their relative position by measuring the concentration of the morphogen — a scalar property — and could be polarised along the axis by detecting the local maximal slope of the gradient — a vectorial property.

This simple gradient model has been challenged by subsequent studies. For example, Lawrence et al. (1972) found evidence that the distribution of the hypothetical morphogen may not arise simply as a consequence of free diffusion. Instead, their results suggested (i) that cells behave as if they can 'hold' their level in the gradient, perhaps by secreting or absorbing morphogen themselves and (ii) that changes in the distribution of the morphogen correlate with, and may depend on, cell division. Furthermore, there is evidence that cells in different regions of the segment may be programmed to respond to the putative morphogen in different ways (Campbell and Caveney, 1989). These, and other studies (French et al., 1976; Nübler-Jung, 1977; Wright and Lawrence, 1981a,b; Campbell, 1987; Campbell and Shelton, 1987) led to discussion of 'intercalation' models, which posit that cells are patterned and polarised by a chain of local inductive interactions.

Over the last 25 years, genetic and molecular analyses of *Drosophila* have built on this earlier work and have led to three main advances.

The first was the discovery that each segment, from its

inception, is subdivided into adjacent but immiscible subpopulations, the anterior (A) and posterior (P) compartments, which form precisely defined portions of the adult pattern (Garcia-Bellido et al., 1973; Lawrence, 1973). A master 'selector' gene *engrailed* (*en*) was shown to operate in all cells of the P compartment to distinguish them from those of the A compartment (Morata and Lawrence, 1975; Kornberg, 1981; Lawrence and Struhl, 1982; Hama et al., 1990). Thus, cells within each segment exist in two states; populations of cells of the two types abut along the A/P boundary within each segment as well as along the P/A boundaries between segments. Hence, it was suggested that A/P boundaries within segments, like boundaries between segments, might serve as sources of morphogens or inducers which control growth and patterning (Crick and Lawrence, 1975; Meinhardt, 1983).

The second advance was made by Nüsslein-Volhard and Wieschaus (1980) who discovered groups of 'pair-rule' and 'segment polarity' genes responsible for defining and patterning embryonic segments (reviewed in Lawrence, 1992; Ingham and Martinez-Arias, 1992). Two of the segment polarity genes, *wingless* (*wg*) and *hedgehog* (*hh*), encode secreted proteins (reviewed in Nusse and Varmus, 1992; Ingham, 1994). During embryogenesis, these proteins serve as distinct P-to-A (Hh) and A-to-P (Wg) signals, which stabilise the boundaries between parasegments (the A/P compartment boundaries within segments) and may act at longer range to organise the pattern of cells on both sides (DiNardo et al., 1988; Martinez-Arias et al., 1988; Bejsovec and Martinez-Arias, 1991; Heemskerk et al., 1991; Heemskerk and DiNardo, 1994; Lawrence et al., 1996).

The third advance concerns the adult appendages where it has been shown that Hh crosses from P into A compartments and induces signalling molecules in the A cells such as Wg or Decapentaplegic (Dpp) (reviewed in Lawrence and Struhl, 1996). These secondary signals then behave like bona fide morphogens: they spread outwards and into both compartments where they form concentration gradients and act directly and at long range to pattern the appendage (Struhl and Basler, 1993; Zecca et al., 1995, 1996; Lecuit et al., 1996; Nellen et al., 1996).

Here, we extend the analysis of compartments, Hh signalling and the control of pattern to the adult *Drosophila* abdomen. We describe the patterns of expression of segment polarity genes. These patterns allow us to map the A and P compartments; they also suggest that Hh spreads into each A compartment from the P cells both fore and aft and forms a pair of opposing concentration gradients. We then study how ectopic Hh affects the pattern of cell types and cell polarity. Our results show that Hh can exert a long-range organizing influence on gene expression, pattern and polarity in the abdomen. However, anteriorly and posteriorly situated cells in the A compartment appear to respond to Hh in distinct ways, expressing different genes, secreting different types of cuticle, and forming structures that point either away from, or towards, Hh-secreting cells. In addition, we have found that Hh can induce cells in the middle of the A compartment to express *en* and form an ectopic P compartment. These results force us to reconsider models of gradients and patterning in insect segments.

MATERIALS AND METHODS

Genotypes

lacZ-expressing reporter genes

(i) *ci-lacZ* (*ci-D*^{plac}, Eaton and Kornberg, 1990).
(ii) *en-lacZ* (*en*^{Xho25}, Hama et al., 1990).
(iii) *wg-lacZ* (*wg*$^{1\text{-}en\text{-}11}$, Kassis et al., 1992).
(iv) *hh-lacZ* (*hh*^{P30}, Lee et al., 1992).
(v) *ptc-lacZ* (*ptc*^{AT96}; similar results were obtained with a *ptc-lacZ(III)* transgene, *ptc(10.8L)A*, inserted on chromosome III; both reporters were obtained from J. Grenier, Y. Higashi, and M. Scott, personal communication).
(vi) *dpp-lacZ* (*dpp*^{P10638}, R. Blackman, personal communication).
(vii) *slp-lacZ* (*slp*$^{A509.1F2}$, Grossniklaus et al., 1992).

Transgenes

(i) *hsp70-flp.1* (Zecca et al., 1995).
(ii) *Tubα1>y*$^{+}$*>hh* (Basler and Struhl, 1994).

Generating clones of *Tubα1>hh* cells

y hs-flp.1/y; Tubα1>y^{+}*>hh/+* larvae carrying either the *en-lacZ*, *hh-lacZ*, or *ptc-lacZ* reporter genes were heat shocked at 33°C to 34°C for 60 minutes. The heat shock induces expression of Flp causing excision of the >y^{+}> Flp-out cassette in single cells, giving rise to clones that have yellow cuticle and bristles in the adult.

X-Gal staining and preparation for microscopy

Adult abdomens were dissected and mounted in Canada Balsam dissolved in methyl salicylate, or in a 1:1 mixture of Hoyer's mountant and lactic acid. To obtain flat preparations, clones were found using the dissecting microscope, a small square of epidermis containing each clone cut out using a razor blade and the piece stretched with forceps before mounting in the Hoyer's:lactic acid mixture. For X-gal staining, abdomens were dissected in Buffer A (100 mM NaH_2PO_4, pH7.0; 150 mM NaCl; 1 mM $MgCl_2$; 0.1% Triton X-100) and then fixed in Buffer A with 1% glutaraldehyde for 15-30 minutes at room temperature. They were then rinsed in Buffer A, rinsed in Buffer B (10 mM NaH_2PO_4, pH7.0; 150 mM NaCl; 0.1% Triton X-100) and stained at room temperature in Buffer B containing 3.3 mM $K_4Fe(CN)_6$, 3.3 mM$Ke_3Fe(CN)_6$ and 0.1% X-Gal. To avoid crystals, X-Gal (dissolved in dimethylformamide) was added last to the staining solution warmed to around 70°C just prior to its addition. For staining adult abdomens, it was necessary to use them within 24 hours of eclosion, otherwise the epidermal cells stain poorly or not at all. As a consequence, the cuticles are only slightly tanned, making it difficult to see the dark pigmentation characteristic of a5 and a4 cuticle. In some cases, whole adults were fixed overnight in Buffer A with 1% glutaraldehyde, producing bloated animals with well stretched cuticles. This treatment is particularly useful for visualising P compartment regions, which otherwise remain folded underneath the A compartments.

RESULTS

Expression of segment polarity genes in the abdomen

(i) In larvae

Most of the larval cuticle is secreted by large, polyploid cells that derive directly, without cell division, from cells in the epidermis of the mature embryo. By contrast, cells destined to form the cuticle of the adult abdomen are present as clusters of small, non-dividing diploid cells (the anterior dorsal, posterior dorsal and ventral histoblast nests) located at stereo-

typed positions in the larval epidermis. We study the genes *engrailed* (*en*), *hedgehog* (*hh*), *wingless* (*wg*), *patched* (*ptc*), *cubitus interruptus* (*ci*) and *sloppy paired* (*slp*) because their products define the A and P compartment cells or are involved in the response to Hh signalling.

In general, we find that the patterns of expression of these genes in the polyploid larval cells (assayed using enhancer trap lines that drive *lacZ* expression) remain largely unchanged from those in the mature embryo (reviewed in Hooper and Scott, 1992). Thus, *en* and *hh* are expressed in bands that correspond to the P compartments (Figs 1, 2; see also Hama et al., 1990), *ptc* is expressed in thin stripes of A compartment cells flanking each P compartment and *wg* is expressed in stripes of A compartment cells positioned immediately anterior to the P compartments (Fig. 1).

Likewise in the histoblast cells (Fig. 3), *en* and *hh* are expressed in all histoblasts in the posterior dorsal nests and in a posterior subset of the cells in ventral nests, presumably the progenitors of the adult P compartments. As in the larval cells, *ptc* expression is observed only in histoblasts near or next to P compartment cells. Finally, *wg* is expressed in the same histoblasts as *ptc*, as well as in a subset of additional histoblasts located just anteriorly. Neither the *slp-lacZ* nor the *ci-lacZ* genes were expressed sufficiently strongly to be scored reliably in the larva.

(ii) In the adult

Fig. 4 is a diagram of the dorsal surface — the tergite — of a typical segment, such as the third abdominal segment (A3). We subdivide the A compartment into six types of cuticle: a1, a2, a3, a4, a5 and a6, and the P compartment into three types: p3, p2 and p1 (Fig. 5A). Note that much of the cuticle is decorated with hairs and bristles all of which point posteriorly.

We map the expression of *en*, *hh*, *ci*, and *ptc* in the tergite, all detected using *lacZ*-expressing enhancer-trap insertions into the native gene (Figs 5, 6). As in the embryo and larval epidermis, *en* and *hh* are expressed in common sets of cells, defining the P compartments (Fig. 5; see also Hama et al., 1990), and *ci* is expressed in the remaining cells, defining the A compartments (Fig. 7). Similarly, *ptc* is expressed prominantly in stripes of A cells running along both the anterior and posterior limits of the A compartments, that is where these cells are close to P cells across the boundaries (Figs 6, 7). Note that *ptc* is graded within each stripe, peaking at high level in those cells abutting Hh-secreting cells of the P compartment and declining progressively in cells further away. Thus, each A compartment contains opposing gradients of *ptc* expression: one declining into the A compartment from the anterior edge and the other from the posterior edge (Figs 6, 7). *wg-lacZ* expression is most intense along the posterior edge, like *ptc*, but grades away and extends further anteriorly than *ptc*, up to about two-thirds of the A compartment (Shirras and Couso, 1996). The *slp-lacZ* line is expressed weakly: it appears to show uniform expression in the same region in which *wg* is expressed.

The ventral epidermis of each abdominal segment forms a

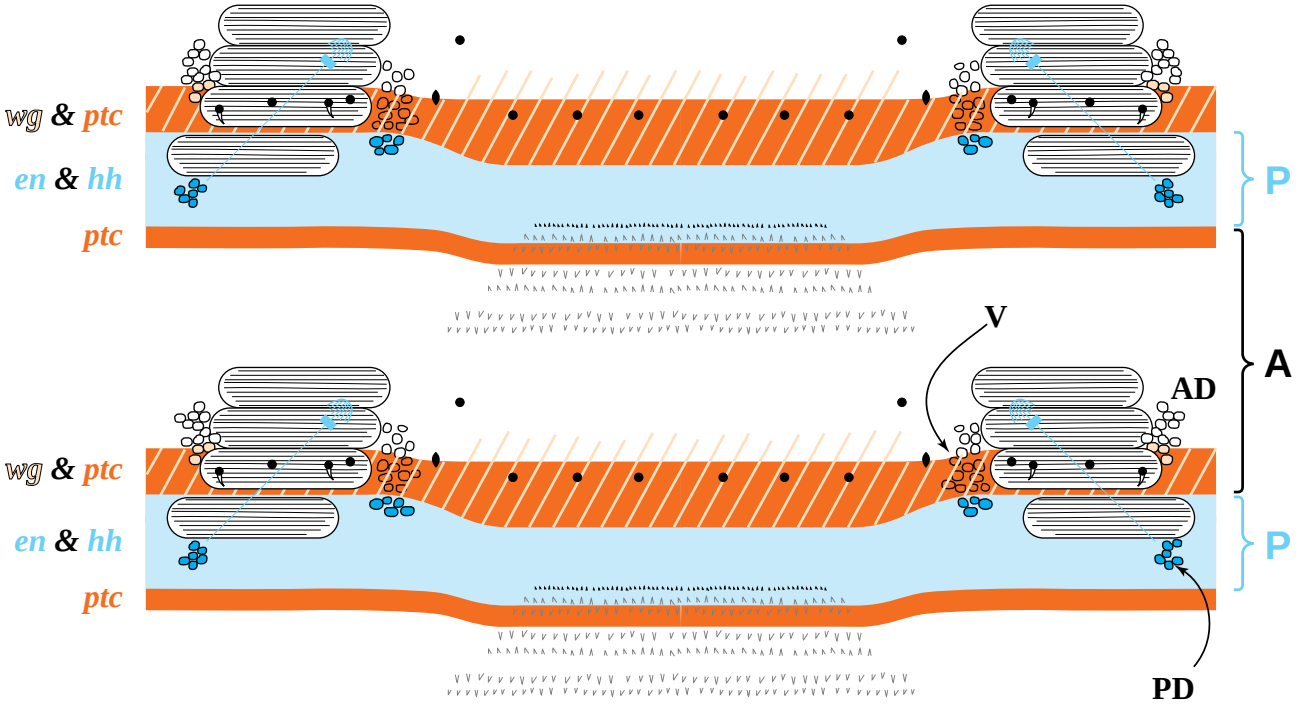


Fig. 1. Gene expression in the ventral epidermis of the 3rd-stage larva. The domains of *en* and *hh* expression, which define the P compartment, are coincident and shown in blue. The zones of *wg* (pale orange hatching) and *ptc* (red) expression are also shown. The left and right halves of each abdominal segment each contain three small groups of diploid cells which will form most of the adult epidermis: these are the ventral (V), anterodorsal (AD) and posterodorsal (PD) histoblast nests; *en*, *hh*, *wg* and *ptc* are expressed in these cells by the colour indicated. Note that, ventrally, the zone expressing *wg* extends further anteriorly than *ptc*, both in the larval epidermis and the V histoblast nest, and, dorsally, only *wg* but not *ptc* is expressed in cells of the AD nest. Also shown are the patterns of ventral hairs (denticles) secreted by the larval cells, sensilla (black symbols) and muscle groups, all of which serve as landmarks.

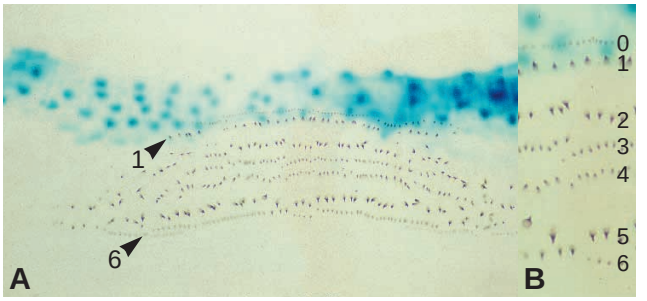


Fig. 2. *en* expression in ventral larval cells. (A) Expression of *en-lacZ* in a 3rd-stage larva. The denticle band of the fourth abdominal (A4) segment and *en-lacZ*-expressing cells of A3 are apparent. There are 7 rows of denticles (when compared with the first instar larva, a new row of fine denticles has been added in front of row 1; this row (row 0) and row 1 itself are secreted by cells that stain blue and therefore belong to the P compartment; see also Dougan and DiNardo (1992). Note that the denticles in rows 0, 1 and 4 point anteriorly, whereas those in the remaining rows point posteriorly (detailed in B; see also Fig. 1).

flexible cuticle, the pleura, with a small plate of sclerotised cuticle, the sternite, centered on the ventral midline. The pleura is covered with a uniform lawn of hairs, all pointing posteriorly, whereas the sternite contains a stereotyped pattern of bristles. *en*, *hh*, *ci* and *ptc* are all expressed in both the pleura and sternite in similar patterns as in the tergite (Fig. 7). However, *wg* is expressed ventrally in the sternite but not in the pleura. In the pleura, *decapentaplegic* (*dpp*), another gene induced by Hh in the imaginal discs, is expressed along the posterior edge of the A compartment, as if in place of *wg* (Fig. 7D). This situation is reminiscent of the mutually exclusive expression of *dpp* and *wg* in the leg imaginal discs (Brook and Cohen, 1996; Jiang and Struhl, 1996; Theisen et al., 1996)

Fig. 3. *en* expression in the ventral histoblast nest. Expression of *en-lacZ* in the ventral histoblast nest of a 3rd-stage larva stained for β-galactosidase. The histoblasts have small diploid nuclei (arrowheads), which are distinct from the large polyploid epidermal nuclei (e). This nest has about 14 histoblasts, of which 4 label with *en-lacZ* and will make the ventral derivatives (sternite and pleura) of the P compartment of a single abdominal segment.

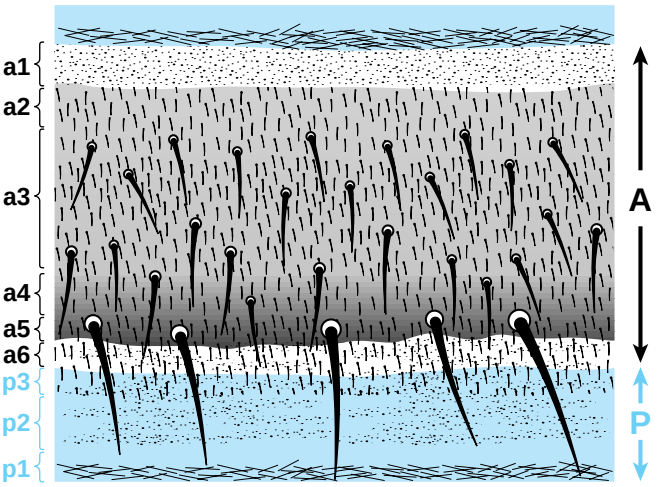


Fig. 4. Compartments and cell types in the tergite of a typical abdominal segment (A3) The cuticle of the A compartment consists of an anterior-to-posterior progression of six types distinguished by ornamentation and pigmentation as follows: a1 = unpigmented, without hairs; a2 = lightly pigmented with hairs; a3 = lightly pigmented with hairs and bristles of moderate size; a4 = darkly pigmented with hairs and bristles of moderate size; a5 = darkly pigmented with hairs and bristles of large size; a6 = unpigmented with hairs but no bristles. The P compartment (blue), in which *en* and *hh* are expressed, shows three types of cuticle (p3 = unpigmented with hairs; p2 = unpigmented without hairs, and p1 = unpigmented but tessellated). Note that several hairs are secreted per cell and that all bristles and hairs have a common polarity, pointing posteriorly. Note also that the boundary between a3 and a4 tissue is not sharp; instead the intensity of the dark pigment grades out over a few cell diameters moving anteriorly from the a4 towards the a3 territory. Finally, the p2, p1 and a1 cuticles are normally folded under the remainder of the tergite and can only be seen in well-stretched preparations.

where all A cells along the A/P compartment boundary express *ptc*, but only specific subpopulations show high levels of *dpp*, the remainder expressing *wg*. Indeed, it appears that Wg specifies the choice of tergite/sternite rather than pleura (Shirras and Couso, 1996; our unpublished findings) suggesting that Wg and Dpp act in the abdomen to organize aspects of the dorso-ventral pattern.

Ectopic Hh induces high levels of *ptc* expression in A compartment cells: evidence for gradients of Hh in the A compartment

In the imaginal discs, A compartment cells near the A/P compartment boundaries and hence within range of Hh secreted by P compartment cells, express high levels of *ptc* (Phillips et al., 1990; Capdevila et al., 1994). But cells positioned further from the boundaries express only low levels of *ptc*, unless exposed to ectopic Hh (Capdevila et al., 1994; Tabata and Kornberg, 1994; Chen and Struhl, 1996). To test whether Hh also induces A compartment cells in the abdomen to up-regulate *ptc* transcription, we used the Flp-out technique (Struhl and Basler, 1993) to generate marked clones of Hh-secreting cells and then assayed for expression of a *ptc-lacZ* reporter gene. Larvae carrying three transgenes, *Tubα1>y⁺>hh*, *hsp70-flp* and *ptc-lacZ* were subjected to a mild heat shock to generate rare *Tubα1>hh* cells by excision

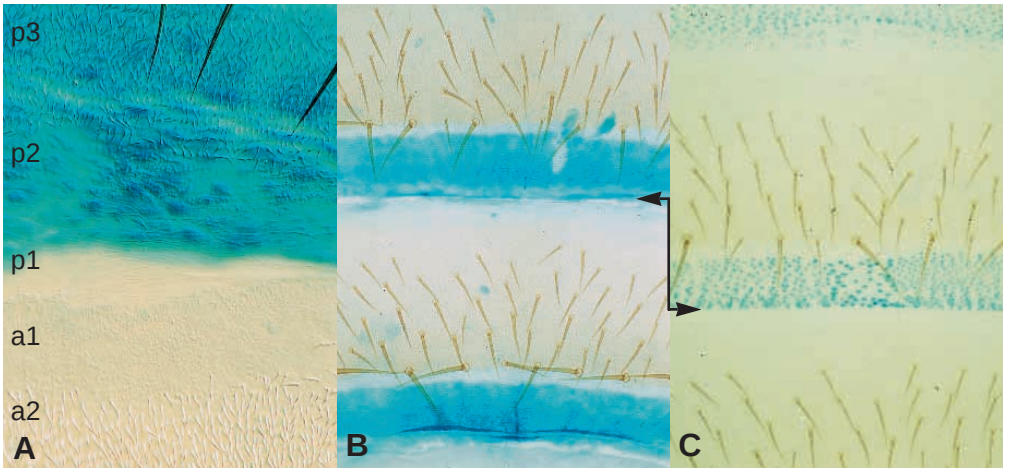


Fig. 5. *en* and *hh* expression in the A3 tergite. (A) Cuticle types near the P/A boundary between the A3 and A4 tergites (stained for *en-lacZ* expression). The boundary between blue and non-blue cells coincides approximately with the junction between the unpigmented, tessellated cuticle (p1) and cuticle that is unpigmented and unadorned apart from speckling seen under interference optics (a1). On the left, there is a gap between *en-lacZ* staining cells in the p1 territory and the a1 cells in adjacent segment: we suspect this is an artefact because the tessellated cuticle (p1) normally folds under the rest of the tergite and has to be stretched open, an action that tends to tear the columnar epithelium that is attached to this cuticle. It is our best opinion, based on many stretched preparations showing *en-lacZ*, *hh-lacZ* and *ptc-lacZ* expression, that the segment boundary coincides precisely with the p1/a1 boundary, although we are not certain of this. (B,C) *en-lacZ* (cytoplasmic) and *hh-lacZ* (nuclear) expression in the third (A3) and fourth (A4) abdominal tergites (the A3/A4 segment boundary is indicated in both micrographs); note that the patterns of expression appear identical and define the P compartment of each tergite.

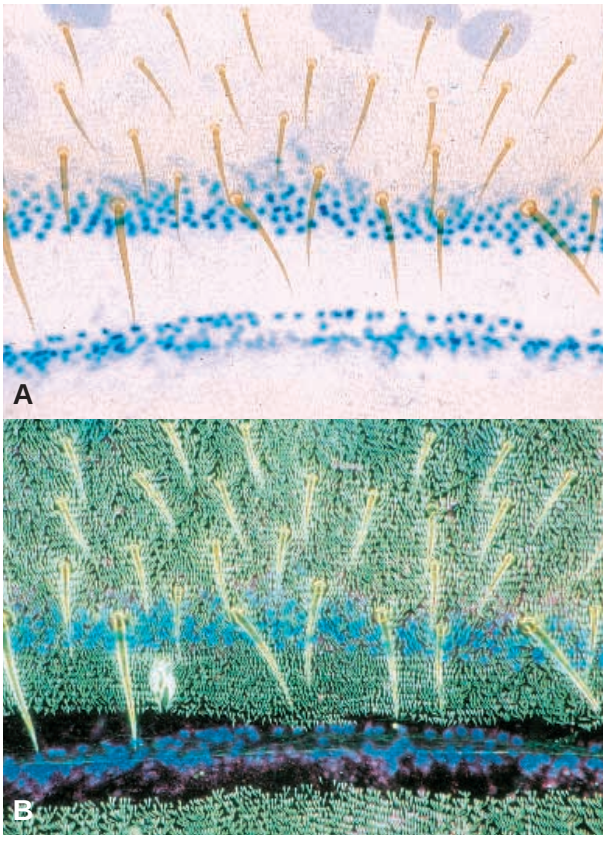
of the $>y^+>$ flp-out cassette. Because the Flp-out cassette carries the *yellow*$^+$ (y^+) gene, clones of *Tubα1>hh* cells were identified in the adult by the yellow phenotype which can be scored with single cell resolution in the bristles, though with less precision in the cuticle.

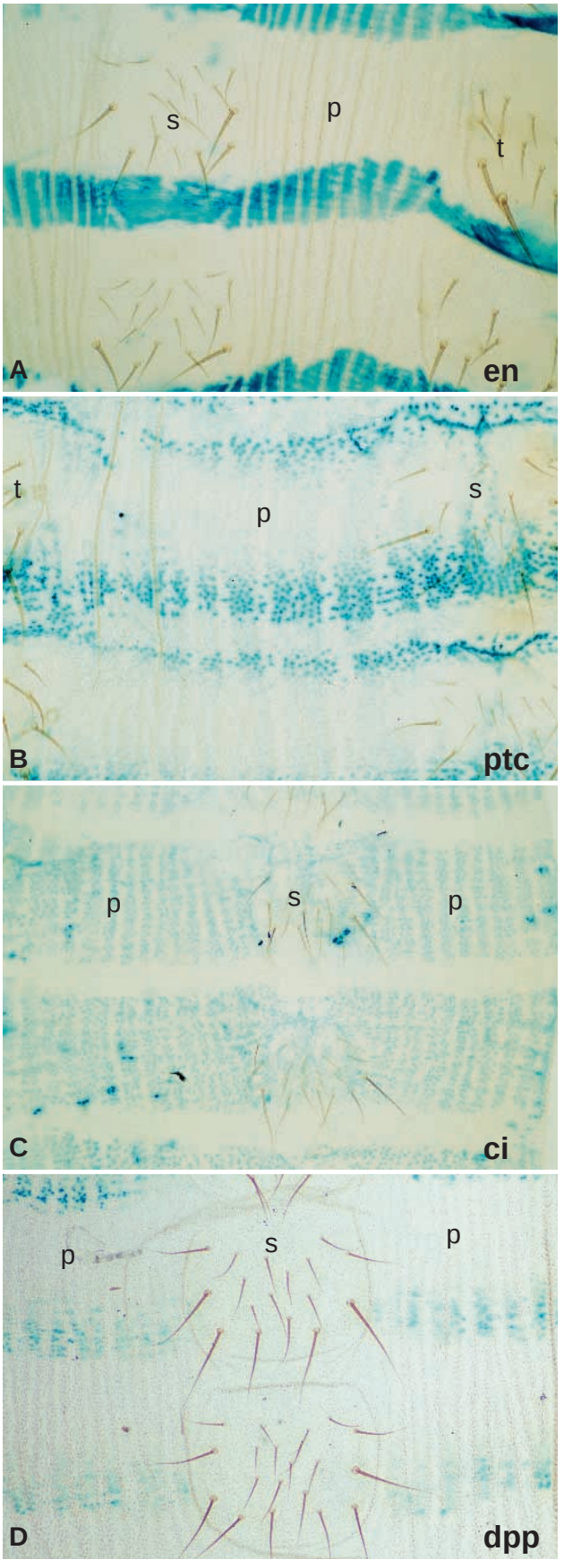
We find that A compartment clones of *Tubα1>hh* cells express *ptc-lacZ* at high level in most portions of the tergites, sternites and pleura (see Fig. 8). When such clones arise near or next to either the anterior or posterior edge of the compartment, where *ptc-lacZ* is already strongly expressed, they are associated with a broadening of the stripe (Fig. 9); when they arise at a distance from either edge, they are generally associated with an ectopic peak of *ptc-lacZ* expression, which declines in a graded fashion in surrounding wild-type cells (Figs 10, 11). Thus, as observed in the imaginal discs, *ptc-lacZ* expression in abdominal cells can serve as an in vivo assay for the distribution of Hh. Accordingly, we infer that the opposing gradients of *ptc-lacZ* expression at the anterior and posterior edges of the A compartments reflect opposing gradients of Hh secreted from the adjacent P compartments.

Fig. 6. *ptc* expression in the A3 tergite. (A,B) Bright- and dark-field images of *ptc-lacZ* expression in the A3 tergite. Expression is confined to the A compartments, beginning abruptly at the A/P and P/A borders, and being graded away from them; the dark-field image (B) allows *ptc* expression to be seen relative to the pattern of hairs and also shows the common anterior-to-posterior polarity of all the hairs and bristles. Although not visible in this picture, it appears that *ptc* is expressed throughout the A compartments at a low level, as in the wing imaginal disc (the evidence comes from a cytoplasmically expressed *ptc-lacZ* reporter gene: stained abdomens carrying this reporter clearly show that the P compartments are white, when compared to the middle of the A compartments, which are uniformly pale blue (not shown)). Note that the cuticle around the A3/A4 segment boundary is not fully stretched; some of the p2 and p3 cuticle is folded underneath the rest of the A3 tergite and not visible.

Ectopic Hh can reorganise the pattern and polarity of A compartment cells in the tergites

In the imaginal discs, clones of cells that ectopically express Hh stimulate cell proliferation and reorganise pattern (Basler and Struhl, 1994; Zecca et al., 1995). We therefore asked whether *Tubα1>hh* clones in the developing abdomen can affect pattern or cell polarity. As summarised in Fig. 8, we dis-





tinguish four classes of clones, each associated with a different phenotype.

The first class of clones are close to the A/P compartment boundary. Cells in this position are normally exposed to high levels of Hh secreted by the neighbouring P compartment and form a6 and a5 cuticle. Hence, not surprisingly, *Tubα1>hh* clones in this position develop normally, even when there is a modest broadening of the stripe of *ptc-lacZ* expression.

The second class of clones are also found near or next to the A/P boundary but extend further anteriorly than clones in the first class. Such clones cause a broadening of the stripe of *ptc-lacZ* expression and, in some cases, a second peak (Fig. 10); the cells of the clone and wild-type cells nearby form ectopic a5 and a4 cuticle, as indicated by the dusky pigmentation and the larger bristles. However, these bristles, as well as the surrounding hairs, all show normal polarity, pointing posteriorly.

The third class comprises clones that are found further anteriorly, in the region normally forming a3 cuticle. These clones induce an island of *ptc-lacZ* expression, which grades out in surrounding wild-type tissue. They also form ectopic a5 type cuticle (e.g., as indicated by the presence of large *y* bristles) and induce surrounding wild-type cells to form ectopic a5 and a4 cuticle (Fig. 11). Finally, they cause wild-type cells positioned laterally and posteriorly to secrete hairs and bristles which point centripetally towards the clone, as if pointing up the gradient of ectopic *ptc-lacZ* (Fig. 11). Thus, these clones demonstrate that ectopic Hh can reorganise and repolarise cells to make a pattern similar to that normally found near the A/P compartment boundary. These results suggest that P compartment cells normally pattern the neighbouring A compartment tissue by secreting Hh.

The fourth class of *Tubα1>hh* clones extend even further anteriorly than the third class. As we describe below, they have exceptional properties indicating that some A compartment cells have been transformed into P cells.

Ectopic Hh can transform A compartment cells into ectopic P compartment tissue

Tubα1>hh clones of the fourth class have three unique features (Figs 8, 12, 13). First, unlike clones of the other classes, they are associated with the formation of ectopic P as well as A compartment tissue. Second, this ectopic tissue is not patterned and polarised in a radially symmetric fashion relative to the clone (e.g., as in class III clones), but instead shows a progression of A and P cuticle types (a3,a4,a5,a6,p3,p2,p1) in which the sequence, as well as the polarity of hairs and bristles, are opposite to that of the normal segment (Figs 8, 12, 13). Finally, *ptc-lacZ* is

Fig. 7. Gene expression in ventral and lateral portions of the adult abdomen. *en-lacZ* (A), *ptc-lac* Z (B), *ci-lacZ* (C) and *dpp-lacZ* (D) expression in the sternites (s), ventral pleura (p) and tergites (t). *en-lacZ* and *ci-lacZ* are expressed in complementary domains defining the P and A compartments and *ptc-lacZ* is expressed in stripes of A compartment cells abutting P compartment cells along both the anterior and posterior boundaries of the compartment. Note that *dpp-lacZ* is expressed only in the ventral pleura in those A compartment cells neighbouring P compartment cells within the same segment. For both *dpp-lacZ* and *ptc-lacZ*, expression peaks along the interface with P compartment cells and declines in a graded fashion in cells further away. *wg-lacZ* expression resembles *dpp-lacZ* expression except that it is found in the tergites and sternites, rather than in the ventral pleura (Shirras and Couso, 1996; data not shown).

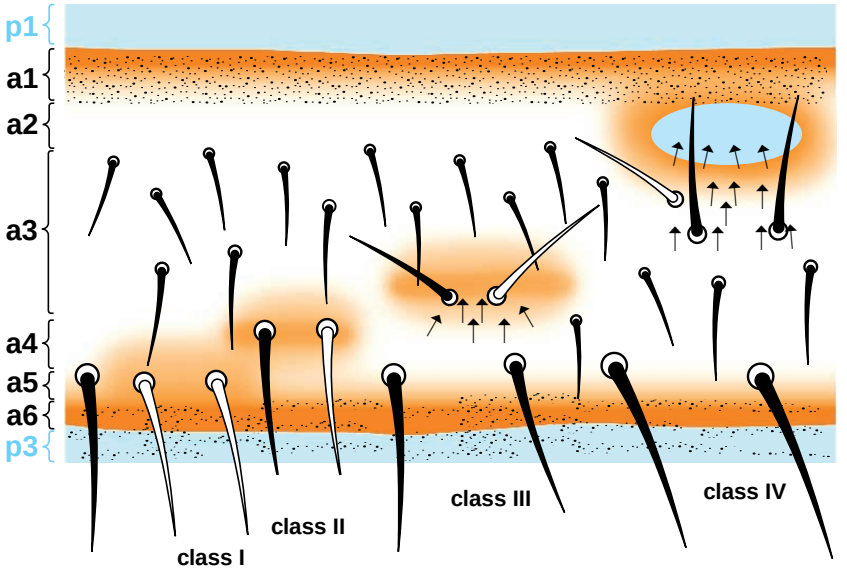


Fig. 8. Summary of the 4 classes of clones that express Hh ectopically. Clones of A compartment cells that express Hh are marked by the loss of the y^+ gene, which affects bristle and cuticle colour: wild-type (y^+) bristles are darkly pigmented (shown as black); y^- bristles belonging to the clone are shown as white. Cells of the A compartment respond to Hh by expressing the *ptc-lacZ* gene (red). All P compartment cells express the *en-lacZ* gene (shown in blue), as do A cells associated with class IV clones that are transformed into ectopic P cells. Hair polarity is normal unless arrows indicate otherwise. Examples of each class of clone are shown in Figs 9-13.

expressed asymmetrically, showing a sharp edge that coincides with the interface between ectopic a6 and p3 cuticle and declines away from that edge. Anterior to the ectopic P cells, there is usually an intact stripe of *ptc-lacZ* along the front edge of the A compartment (Fig. 12): in some cases, the anterior *ptc-lacZ* stripe is abnormally broad, or even split into two stripes as if these cells are exposed to Hh secreted both by the ectopic P cells within the segment as well as by neighbouring P cells in the next segment. We infer that class IV clones are associated with the induction of an ectopic P 'compartment' within the A compartment, bounded posteriorly by an ectopic A/P compartment boundary and anteriorly by an ectopic P/A segment boundary. This compartment, as well as neighbouring A compartment tissue, has opposite polarity relative to the normal segment.

To test whether these *Tubα1>hh* clones have induced A compartment cells to undergo an A-to-P transformation, we have monitored both *en-lacZ* and *hh-lacZ* expression (Materials and Methods). We find that the apparent P cuticle associated with class IV clones is invariably associated with the ectopic expression of both *en* (Fig. 13) and *hh*, confirming that an A-to-P transformation has occurred. We note that some clones appear to be intermediate between class III and class IV: they express only a low level of *en* and form cuticle patterns that can be interpreted as partial transformations towards a P state (Fig. 13). These clones appear to arise in more posterior positions than true class IV clones, which show a complete P transformation, but in more anterior positions than class III clones, which are associated only with reorganised A tissue.

Reorganizing activities of ectopic Hh in the sternites and pleura

The behaviour of *Tubα1>hh* clones in the sternites appears similar to that of the tergites: clones in the middle of the A compartment can reorganize the pattern and polarity of surrounding A compartment tissue, much as we observe in the tergites (not shown). Because the cells of the pleura form unpigmented cuticle covered with hairs but not bristles, we could not score for *yellow* in the *Tubα1>hh* cells. Nevertheless, we do find islands of ectopic *ptc-lacZ* expression in the pleura in animals in which *Tubα1>hh* clones have been induced. These islands, which we presume are made by clones of ectopic *hh*-expressing cells, arise in the middle of the A compartment and are associated with reversals in hair polarity in more posterior tissue (not shown).

DISCUSSION

A typical tergite of the *Drosophila* abdomen consists of at least nine types of cuticle (p1,p2,p3,a6,a5,a4,a3,a2,a1) arranged in transverse stripes. Most of these types of cuticle are decorated by hairs or bristles that point posteriorly. Thus, the *Drosophila* tergite poses two classic, and unsolved, problems in pattern formation: (i) how do cells 'choose' what structures to make based on their relative position? and (ii) how do they orient to form structures that are appropriately polarised?

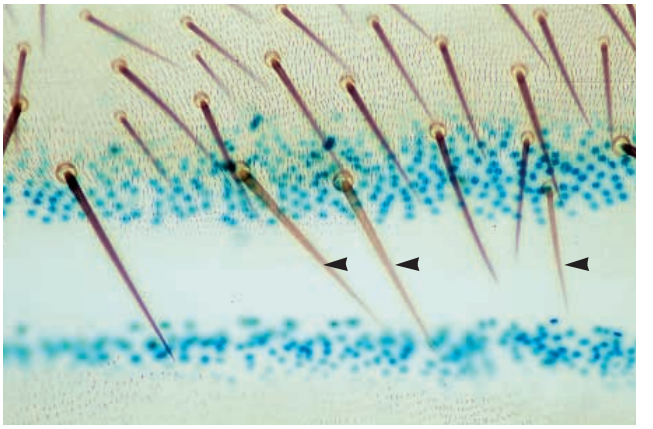


Fig. 9. Class I clone in a *ptc-lacZ* background. A clone in the region that normally forms a5 cuticle. There are three marked (y^-) bristles which are normal (arrowheads); accompanied by a slight broadening of the stripe of *ptc-lacZ* expression (compare with Fig. 6).

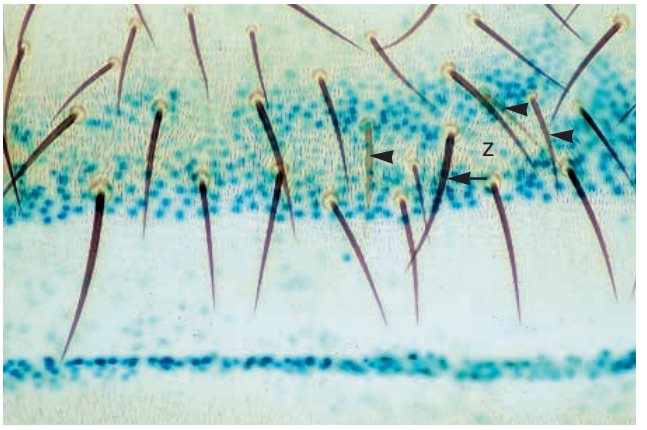


Fig. 10. Class II clone in a *ptc-lacZ* background. A clone in the region normally forming a4 cuticle. Three bristles, marked by y^- (arrowheads), derive from the clone. Note that the clone is associated with an ectopic peak of *ptc-lacZ* expression. Between this peak and the endogenous *ptc-lacZ* strip behind it is a zone with much lower *ptc* expression (z). There are also ectopic a5-type bristles (arrow), which are y^+ and thus derived from neighbouring wild-type tissue. Nevertheless, bristle and hairs show normal polarity.

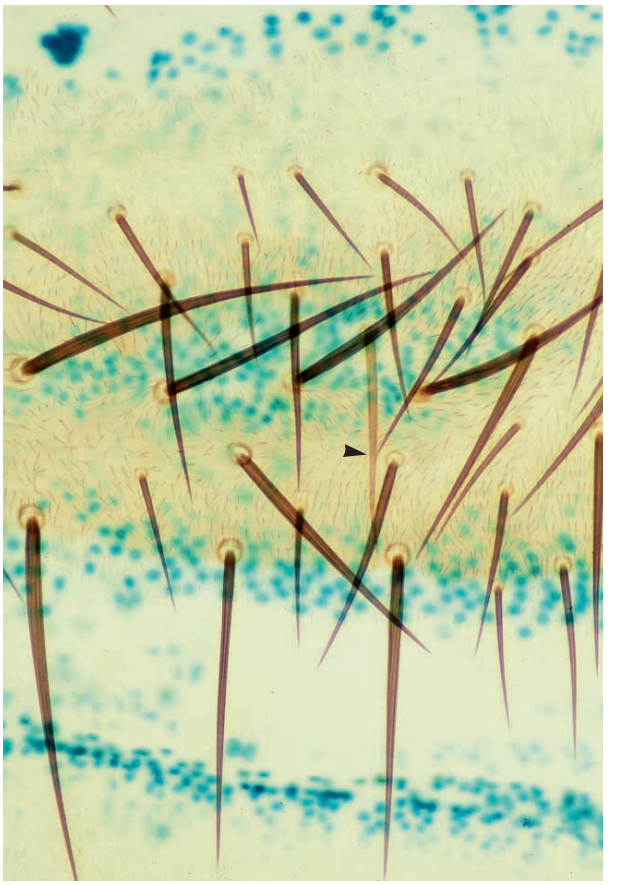


Fig. 11. Class III clone in a *ptc-lacZ* background. A clone in the posterior portion of the region normally forming a3 cuticle (marked by the presence of a single y^- bristle (arrowhead)). Note that the clone is associated with an island of ectopic *ptc-lacZ* expression and that bristles and hairs posterior to the marked bristle show reversed polarity. All of the bristles with reversed polarity are y^+, indicating that they derive from neighbouring wild-type tissue; they are also large a5-type bristles. We infer that some of the hairs with reversed polarity are also formed by wild-type cells outside of the clone because they express little or no *ptc-lacZ*, indicating that they are not themselves expressing Hh.

As reviewed in the Introduction, these questions were previously addressed by grafting experiments, most performed before the discovery of developmental compartments and segment polarity genes and without the benefit of molecular tools for assaying and manipulating gene expression. Here, we apply these new findings and approaches to the adult abdomen of *Drosophila*. We describe the system in molecular terms and provide evidence that Hh, a protein secreted by P compartment cells, accumulates as gradients in neighbouring A compartment tissue and can organize the pattern and polarity of cells in the A compartment. In the accompanying paper (Struhl et al., 1997), we examine Hh further and conclude that it controls cell type and polarity by distinct gradient and signal-relay mechanisms.

Hh organises cell pattern and polarity within the A compartment

Our evidence that Hh controls cell type and polarity in the abdominal epidermis is as follows. First, using the up-regulation of *ptc-lacZ* expression as an assay, we deduce that Hh normally accumulates in a U-shaped distribution in each A compartment, peaking at the anterior and posterior boundaries. Second, we show that clones that express Hh in the A compartment can induce ectopic peaks of *ptc-lacZ* expression and reorganise both cell type and polarity in surrounding, wild-type cells. Finally, we find that both the level and grade of ectopic Hh, as monitored by *ptc-lacZ* expression, correlates with changes in cell type and polarity in surrounding tissue, following the rules normally observed near the compartment boundary.

Hh could pattern the A compartment by a simple gradient mechanism: the concentration of Hh would be read as a scalar to determine the type of cuticle secreted. This mechanism is supported by experiments presented in the accompanying paper (Struhl et al., 1997). We note that the patterns of cuticle types in each abdominal segments are distinct, varying in the width, position and even the presence of the six types of A compartment cuticle (a1-a6). An extreme example is the fifth abdominal segment of the adult male, which differs from the more anterior segments in forming heavily pigmented a4-like cuticle in place of a3 cuticle. These differences depend on at least two groups of genes, those determining sex (pigmentation and cuticle types being sexually dimorphic) and those controlling segment type (such as genes of the Bithorax Complex; Lewis, 1978). Because the patterns of *en*, *hh* and *ptc* expression in all the abdominal segments are conserved, we think it unlikely that one level of Hh specifies one particular type of cuticle, say a4, in every segment. Instead, we infer that the particular combinations of BX-C and sex determining genes active in each segment program the cells to ‘interpret’ the same concentration of Hh in different ways (e.g., to form different cuticle types).

In principle, Hh could also polarise A compartment cells by a gradient mechanism if they could detect the direction of maximal change in Hh concentration (the vector) and orient structures accordingly. However, if this were the case, one would expect correspondence between the Hh gradient

landscape and cell polarity; yet, we find some instances in which the slope of the presumed concentration gradient of ectopic Hh, as revealed by *ptc-lacZ* expression, does not correspond with the orientation of hairs and bristles (e.g., as in some class II *Tubα1>hh* clones; see Fig. 10). One explanation for this disparity is that *ptc-lacZ* expression is assayed in adult cells, 24-36 hours after the final pattern is established, and hence may not reflect the exact distribution of Hh at time that the pattern was generated. For example, the endogenous Hh gradients might extend further, proportionally, into the A compartment when there are fewer cells, and hence overwhelm the ectopic source of Hh associated with class II clones at the time the cells are being polarized. Another explanation is that Hh may control cell polarity indirectly or in conjunction with other signalling molecules, possibilities that are supported by other experiments (Struhl et al., 1997).

Different responses to opposing Hh gradients within the same A compartment

It appears that anterior and posterior parts of each A compartment are organised by opposing gradients of the same signalling molecule, Hh. Yet, neither the pattern, nor the polarity, of structures formed by the compartment show mirror symmetry. Instead, anteriorly and posteriorly situated cells within the A compartment respond to Hh in different ways; they express different genes, form different cell types and secrete structures that have opposite polarity relative to the inferred gradient of Hh. For example, cells in the posterior half are induced to express *wg* and form a6, a5, and a4 cuticle, whereas cells in the anterior half do not express *wg* and are induced to form a1 and a2 cuticle (see also Struhl et al., 1997). Moreover, the posterior cuticle is adorned with hairs and bristles that point 'up' the Hh gradient, whereas anterior cuticle has hairs that point 'down' the Hh gradient.

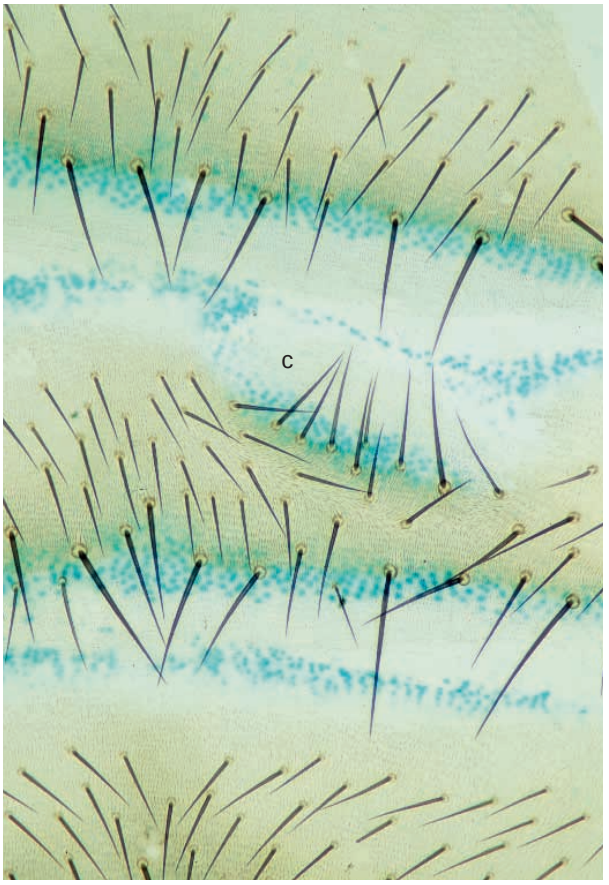


Fig. 12. Class IV clone in a *ptc-lacZ* background. A clone (c) in the anterior portion of the region normally forming a3 cuticle. Note the ectopic band of *ptc* expression, which has a sharp anterior border, but a graded posterior border. The anterior border of ectopic *ptc-lacZ* expression appears to coincide with an ectopic A/P compartment boundary and is associated with a normal progression of a4,a5,a6,p3 and p2 cuticles, which have reversed polarity (see also Fig. 13). All of the bristles falling within the ectopic a4 and a5 cuticle are wild type, and most, like the neighbouring hairs, have reversed polarity.

We do not know what factor or factors determine this difference in response, although it has been suggested, on both theoretical and experimental grounds, that A compartment cells may be subdivided into two subpopulations that respond differently to signals coming from neighbouring cells (Meinhardt, 1984; Campbell and Caveney, 1989; see also Nübler-Jung, 1979). In *Drosophila*, the two regions could be distinguished by the selective activity of a controlling gene; one candidate is *slp*, which encodes two related transcription factors that are co-expressed in the posterior, but not the anterior, portion of each A compartment. At least in the embryo, these proteins appear to program cells that are posterior in the A compartment to respond to Hh differently from anteriorly situated cells (Cadigan et al., 1994). Thus, in the adult anteriorly and posteriorly situated cells within the A compartment may secrete structures that point away or towards Hh-secreting cells because they are programmed to respond in opposite ways to Hh or a common polarizing signal induced by Hh. We note that any such hypothesis demands that each of the two distinct cell populations must coincide exactly with the realm of action of the polarising signal appropriate to it. Otherwise, some cells might receive polarising signals coming from the opposing source and make hairs that point contrarily. This demand might be weakened if cells were not able to respond individually, but did so in groups as if by consensus or interaction (Nübler-Jung, 1987).

Control of cell pattern and polarity in the P compartment

Our results do not address pattern formation within the P compartment. Nevertheless, most abdominal P compartments have three types of cuticle, and where there are hairs, they point posteriorly. In the imaginal discs, P compartment cells are insensitive to Hh itself, even though Hh signalling is required for their normal growth and patterning. This is because the P compartment, like the A compartment, is patterned indirectly by Hh, that is by Dpp and Wg secreted by those A compartment cells that receive Hh (reviewed in Lawrence and Struhl, 1996). In principle, the same mechanism might operate in the abdomen, but it seems that neither Dpp nor Wg act downstream of Hh to pattern the A compartment (Struhl et al., 1997). Hence, if Hh acts by proxy to organise patterning in the P compartment, we infer that it would do so by inducing another, as yet unknown, factor.

Ectopic Hh signalling can induce *en* expression and the formation of ectopic P cells within the A compartment

We find that clones of Hh-secreting cells can induce A com-

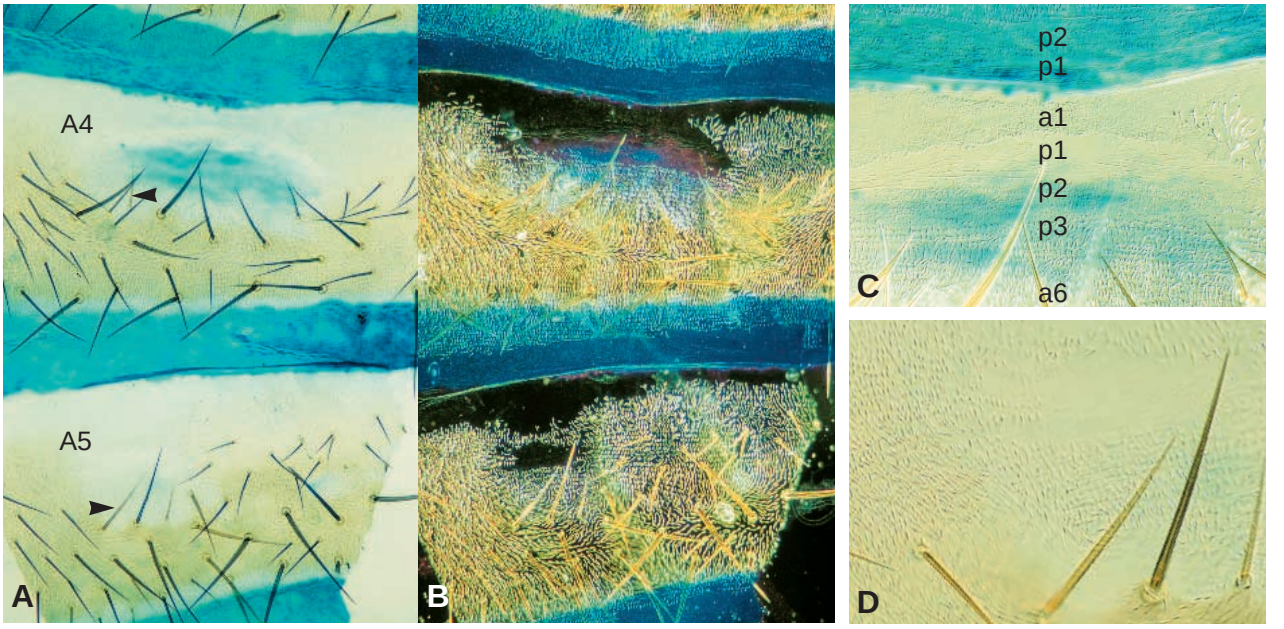


Fig. 13. Class IV clones in an *en-lacZ* background. (A,B) Bright- and dark-field images of two clones located anteriorly within the a3 region, one in the A4 and the other in the A5 segment; (C,D) details of the two clones, interference contrast. The A4 clone is marked by a single y^- bristle (arrowhead) and is associated with an island of fairly strong, ectopic *en-lacZ* expression (visible in A). Hairs within and posterior to these *en-lacZ*-expressing cells have reversed polarity and, together with the bristles, show a reversed sequence of a4,a5,a6,p3,p2,p1 and a1 cuticle types (visible in B and C). The A5 clone is marked by y^- bristles (one is arrowed) and is associated with an island of weak *en-lacZ* expression (A) and ectopic a5,a6,p3 and p2 cuticle (B,D); as in the A4 clone, all hairs associated with, and anterior to, the clone have reversed polarity. Note that a region of reversed hair polarity in A5 extends far to the right of the marked bristles in A5, and is associated with several large bristles typical of a5 cuticle (A,B); we infer that there are additional ectopic Hh-expressing cells in this region (belonging to the same or possibly a second clone) and these cause a class III phenotype.

partment cells to express *en* and to behave like bona fide P compartment cells. This A-to-P transformation is surprising for at least two reasons.

First, shortly after *en* expression is initiated in P compartment cells in early embryos, the *en* gene becomes heritably silenced in A cells. This silencing depends on the *Polycomb-Group* of genes (Busturia and Morata, 1988; Moazed and O'Farrell, 1992), the same system that silences homeotic genes (reviewed in Bienz and Müller, 1995). Nevertheless, ectopic Hh appears to relieve silencing selectively, allowing *en*, but not the homeotic genes, to escape.

Second, only a subset of A compartment cells respond to Hh

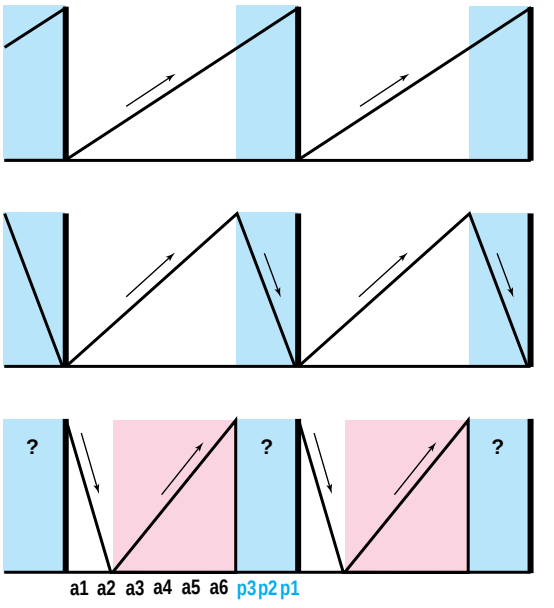


Fig. 14. Old and new models of positional information in the insect segment. Three models are shown. The upper model is the traditional one and was based on grafting experiments (Lawrence, 1966; Stumpf, 1966); each segment boundary is thought to coincide with the source of a morphogen, which is generated at the boundary and spreads away from it towards the middle of the segment to form a gradient. The middle model is based on results in the imaginal disc which show that the A/P compartment boundary can act as the source of a gradient morphogen (e.g. Dpp) to pattern both compartments (Basler and Struhl, 1994; Zecca et al., 1995; Lecuit et al., 1996; Nellen et al., 1996): Here the model is applied to the embryonic segment where the morphogen is probably Wg (Lawrence et al., 1996; Lawrence and Struhl, 1996). The lower model is based on findings in this paper, it has two opposing gradients per A compartment. These two gradients are interpreted in different ways, generating different patterns of gene expression and cell type and directing cells to form structures that point up or down the gradient. We do not know what factors govern cell pattern and polarity in the P compartment (shown in blue); it could be that P cells are also exposed to organizing signals spreading across both the anterior and posterior edges of the compartment. The arrows indicate the polarities of cuticular structures such as hairs and bristles. The pink domains may correspond to the expression pattern of the *slp* gene; it is possible that *slp* causes cells to respond differently to the Hh morphogen.

by activating *en* and becoming P cells; these cells occur in the middle of the A compartment, and therefore are not normally exposed to Hh. Just behind these the effect of Hh is to turn on *en* less completely. We find it paradoxical that only A compartment cells in a particular position can be transformed into P cells — this is because our other evidence (here and in Struhl et al., 1997) suggests that the cells only know where they are by means of Hh itself. It may be relevant that *en* is activated near to where *slp*-expressing and non-*slp*-expressing cells meet: in embryos, ectopic Hh can induce *en* expression in A compartment cells positioned just anterior to that meeting place (Tabata and Kornberg, 1994; see also DiNardo et al., 1988; Martinez-Arias et al., 1988). Perhaps, additional signals are generated along the *slp*-on:*slp*-off interface that do not depend on Hh, but which affect how cells in this region respond to Hh.

Old and new models for pattern formation in insect segments

In Fig. 14, we show the conventional model of the insect segment in which a morphogen, is generated at or on one side of the segment boundary and spreads away from it to form a segmental gradient. This model is attractive because it offers a simple explanation for how both scalar and vectorial information can be provided to all the cells of a field (see Introduction). We also show an updated version of this model, based on recent studies of limb development (reviewed in Lawrence and Struhl, 1996).

In contrast to these models, we now conclude that pattern within the A compartment is controlled by Hh, which crosses over into and acts on the A compartment at both its anterior and posterior limits, forming a U-shaped gradient. These, and other results (Struhl et al., 1997), are incompatible with models in which a single organising system, whether asymmetric or reflexed, controls the pattern of the segment as a whole. Hence, we present a third model in which we postulate two organising systems per segment, each generated across interfaces between and A and P cells. Although this model provides a better fit with our findings, it raises new questions about how a morphogen such as Hh can organize both the pattern and polarity of seemingly equivalent cells in different ways.

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