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hedgehog and *engrailed*: pattern formation and polarity in the *Drosophila* abdomen

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SUMMARY

Like the *Drosophila* embryo, the abdomen of the adult consists of alternating anterior (A) and posterior (P) compartments. However the wing is made by only part of one A and part of one P compartment. The abdomen therefore offers an opportunity to compare two compartment borders (A/P is within the segment and P/A intervenes between two segments), and ask if they act differently in pattern formation. In the embryo, abdomen and wing P compartment cells express the selector gene *engrailed* and secrete Hedgehog protein whilst A compartment cells need the *patched* and *smoothened* genes in order to respond to Hedgehog. We made clones of cells with altered activities of the *engrailed*, *patched* and *smoothened* genes. Our results confirm (1) that the state of *engrailed*, whether 'off' or 'on', determines whether a cell is of A or P type and (2) that Hedgehog signalling, coming from the adjacent P compartments across both A/P and P/A boundaries, organises the pattern of all the A cells.

We have uncovered four new aspects of compartments and *engrailed* in the abdomen. First, we show that *engrailed* acts in the A compartment: Hedgehog leaves the P cells and crosses the A/P boundary where it induces *engrailed* in a narrow band of A cells. *engrailed* causes these cells to form a special type of cuticle. No similar effect occurs when Hedgehog crosses the P/A border. Second, we look at the polarity changes induced by the clones, and build a working hypothesis that polarity is organised, in both compartments, by molecule(s) emanating from the A/P but not the P/A boundaries. Third, we show that both the A and P compartments are each divided into anterior and posterior subdomains. This additional stratification makes the A/P and the P/A boundaries fundamentally distinct from each other. Finally, we find that when *engrailed* is removed from P cells (of, say, segment A5) they transform not into A cells of the same segment, but into A cells of the same parasegment (segment A6).

Key words: *patched*, *smoothened*, Compartments

INTRODUCTION

Much of the epidermis of *Drosophila* develops as a chain of alternating anterior (A) and posterior (P) compartments, populations of cells that differ from each other because the selector gene *engrailed* (*en*) is active in the cells of P but not A. Studies of the wing have led to a general model of how compartments and selector genes build pattern. Early in development, the state of *en* expression is fixed in sets of cells ('on' in P and 'off' in A); the state being inherited by all the descendants of each set. During growth, the borderlines between A and P compartments act as engines to produce positional information – the mechanism depending on a secreted molecule Hedgehog (Hh) being made by all P cells. Hh crosses over the border to reach nearby A cells which are primed to receive it. These A cells then respond to Hh by becoming a line source of a diffusing morphogen (such as Dpp), to form a gradient with a peak near the compartment boundary – this gradient delivers information of position, polarity and dimension to both A and P compartments of the wing. Variations on this basic mechanism may be used to generate pattern in both insects and vertebrates (reviewed in Blair, 1995; Lawrence and Struhl, 1996).

But the wing can only tell part of the story; each appendage develops from just a small region of the segment, from a cluster of cells straddling one A/P boundary. In the abdomen, as in the embryo, the A cells in one compartment come into contact with P cells located both posteriorly at the A/P boundary, as well as anteriorly at the P/A boundary (in the next segment) (Hama et al., 1990; Kornberg, 1981a). Hh thus enters the A compartments from two directions, and we found that the two regions receiving it respond differently (Struhl et al., 1997a,b). There are other differences from the wing model; for example, in the dorsal abdomen, Hh does not induce Dpp (Kopp et al., 1997; Struhl et al., 1997b), but acts itself to pattern the A compartment (Struhl et al., 1997a).

Here, we have looked at the abdomen again, particularly at how the *en* and *hh* genes act to make pattern and influence polarity. Using mutant clones of cells we have removed and added En protein and manipulated the responses to Hh; we

describe the effects on both differentiation of cells and on planar polarity. We show that *en* has the same selector function in distinguishing A from P compartments in the abdomen as it does in the wing: if *en* is taken away from P cells they transform into A cells, and if A cells are given En protein they transform into P cells. The segmental nature of the transformation is particular – if *en* is removed from P cells of one segment (say A5) they transform to A cells of one segment back (A6). As in the wing (Blair, 1992; Hidalgo, 1994), we show that *en* has a limited function in the A compartment of the abdomen; it is needed to produce a specific type of cuticle found just anterior to the A/P boundary. We have new results on planar polarity (Nübler-Jung, 1987; reviewed in Shulman et al., 1998) which appears to depend on a factor 'X' induced by Hh (Struhl et al., 1997a,b). Finally, we find that both the A and the P compartments are each subdivided into two domains.

In the accompanying paper (Lawrence et al., 1999) we present evidence that Hh determines cell affinity.

MATERIALS AND METHODS

Mutations, insertions and transgenes

The FlyBase (http://gin.ebi.ac.uk:7081) entries of the mutations, insertions and transgenes as referred in the text are as follows:

ptc.lacZ: $lacZ^{ptcAT96}$, an enhancer trap insertion at the *ptc* locus (nuclear *lacZ*) or a third chromosome insertion of the *(10.8L)A* transgene which expresses a cytoplasmic form of β-gal under the control of the *ptc* promoter (Struhl et al., 1997b).

ptc^{-}: ptc^{IIw}, an amorphic allele of the *ptc* gene.

hh.lacZ: hh^{P30}, an enhancer trap insertion at the *hh* locus.

en.lacZ: en^{Xho25}, an enhancer trap insertion at the *en* locus.

en^{-}: $Df(R)en^{E}$, a deletion for both the *inv* and *en* genes.

smo^{-}: smo^{3}, an amorphic allele of the *smoothened* gene.

hs.FLP: $FLP1^{hs.PS}$, *S. cerevisiae* FLIP recombinase under the control of the *hsp70* promoter.

tub>y^{+}*>en*: $en^{FRT.CD2.alphaTub84B}$.

UAS.en: $en^{UAS.cGa}$.

UAS>y^{+}*>en*: $en^{FRT.CD2.UAS}$, similar to the construct $wg^{FRT.UAS.T:HA1}$ (Zecca et al., 1996) in which the *wingless* sequences has been replaced by the entire *engrailed* open reading frame.

Act>CD2>Gal4: $GAL4^{FRT.CD2.Act5C}$.

69B: $GAL4^{69B}$, an enhancer trap line driving the expression of *S. cerevisiae* GAL4 in the epidermis.

abx/ubx>f^{+}*>Gal4-lacZ*: $GAL4^{Ubx.PdC}$.

FRT42: *P{ry[+t7.2]=neoFRT}42D*.

CD2y^{+}: $CD2^{hs.PJ}$.

Mutant clones

Clones of mutant cells were generated by FLIP-mediated mitotic recombination (Golic, 1991), using heat shock to induce a transient pulse of FLIP recombinase at chosen stages of development. In all cases, the clones were genetically marked, usually by additional mutations such as *pwn* and *sha* which allow the genotype of hairs and bristles to be scored with single cell resolution.

The histoblasts, which are the progenitors of the adult abdomen, do not divide during the whole larval period but they do grow considerably (Madhavan and Schneiderman, 1977). We find that, when old larvae are heat shocked (from about 72 hours AEL) the clone frequency produced is much higher than with younger larvae. Clones can also be easily produced at the beginning of the pupal period, when the cells divide rapidly.

Flies of the following genotypes were heat shocked for 1 hour at 33 or 38°C at different times of development: 4±1 hours AEL at 25°C (blastoderm clones), 72±12 hours AEL (larval clones) and 18±6 hours APF (pupal clones):

- *y hs.FLP/ y; FRT42 pwn ptc*$^{-}$*/ FRT42 CD2y*$^{+}$.
- *y hsFLP/ y; FRT42 pwn en*$^{-}$*/ FRT42 CD2y*$^{+}$.
- *y hs.FLP/ y; FRT42 pwn ptc*$^{-}$ *en*$^{-}$*/ FRT42 CD2y*$^{+}$.
- *y hs.FLP/ y; FRT42 pwn/ FRT42 CD2y*$^{+}$.

Standard genetic methods were used to introduce different enhancer traps or y^{+} to some of these flies.

Ectopic *en*$^{+}$ expressing clones

Flies of the following genotypes were heat shocked for 30 minutes to 1 hour at 33°C:

- *tub-en* clones: flies carrying *hs.FLP* and *tub>y*$^{+}$*>en*.
- *act-en* clones: flies carrying *hs.FLP, Act>CD2>Gal4* and *UAS.en*.
- *69B-en* clones: flies carrying *y hs.FLP, 69B* and *UAS>y*$^{+}$*>en*.
- *abx/ubx-en* clones: flies carrying *hs.FLP* f^{36a}, *abx/ubx>f*$^{+}$*>GAL4-lacZ* and *UAS.en*.

β-galactosidase detection

As described by Struhl et al. (1997b). Note that the composition of buffers A and B in Struhl et al. (1997b) contains an error; it should have been: buffer A (100 mM sodium phosphate pH7.0, 1 mM $MgCl_2$, 0.1% Triton X-100) and buffer B (10 mM sodium phosphate pH7.0, 150 mM NaCl, 1 mM $MgCl_2$, 0.1% Triton X-100).

RESULTS

The tergites of the abdomen contain nine cuticle types, which we call a1-a6 and p3-p1 (Fig. 1).

We made clones of cells that alter Hh transduction or that remove or add *en* function. The *en* gene is next to a homologue called *invected* (*inv*) (Poole et al., 1985). Although *inv* can be removed from the wild type without effect (and therefore it makes minimal or even no contribution to normal development; see Tabata et al., 1995), it nevertheless has some *en*-like function in cells mutant for *en*. For our studies we have therefore removed both genes (termed *en*$^{-}$, see Materials and Methods). To investigate the action of Hh we have used mutations in *patched* (*ptc*) and *smoothened* (*smo*). The loss of *ptc* activates the Hh transduction pathway, while the loss of *smo* blocks Hh transduction (reviewed by Ingham, 1998). Mutant clones suggest that neither gene is required in the P compartment.

Clones that lack *en* in the A compartment

In most of the A compartment (that is in the a2, a3, a4 and a5 regions) and regardless of the time of induction, *en*$^{-}$ clones appear completely normal, they resemble clones that are wild type except for the markers (Fig. 2). This is no surprise as *en* is not expressed in these regions (Struhl et al., 1997b), so removing the gene from there should be of no consequence. Although clones could not be marked in a1, no abnormalities were found – we presume that many of the marked clones in a2 extend into a1 and form normal cuticle there (clones cross freely between a1 and a2; see Struhl et al., 1997a).

The a6 region is an exception: *en*$^{-}$ clones never make cuticle of the a6 type, and each and every one of the *en*$^{-}$ cells in a6 territory secretes a5 cuticle instead (Figs 2, 4A). We have determined the provenance of such clones by marking sister spots (Lawrence et al., 1999); we find that while some of these clones arise in the A compartment, others originate from the P

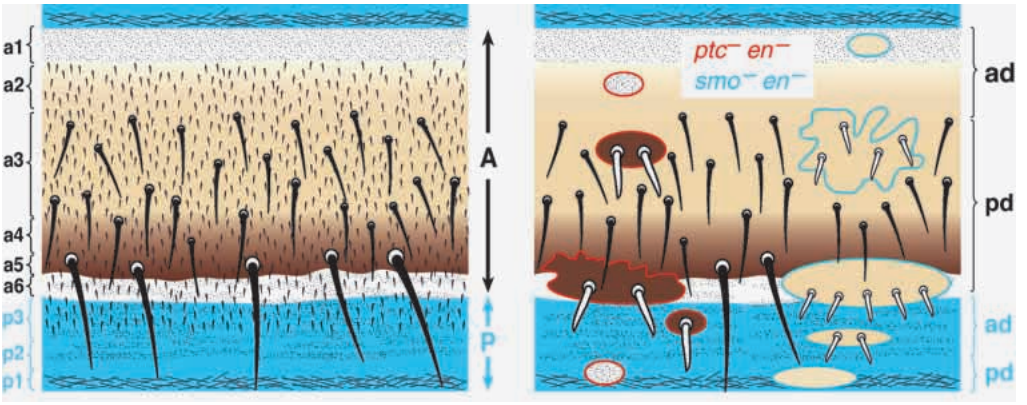

Fig. 1. A diagram of a tergite showing compartments and cuticle types (after Struhl et al., 1997b). We show an aide-mémoire of our main results with *ptc*⁻ *en*⁻ and *smo*⁻ *en*⁻ clones. The pigmentation in the A compartment is shown in brown, and the entire P compartment is blue. The identity of cells (a1, a2, a3, a4 or a5) in the clones (indicated by pigmentation, presence or absence of bristles and their size), is determined by the genotype, provenance (A or P) and position of the founding cell. The *pwn* bristles are shown as deformed and light coloured. The anterior and posterior domains in A and P are indicated (ad, pd).

and move anteriorly into the a6 region where they make a5 cuticle. Both these types of clones show that *en* is essential for making a6, even though *en.lacZ* transgenes do not stain in the a6 territory.

Clones that lack *en* in the P compartment

We find that *en*⁻ P cells are always transformed into A cells; they form A cuticle and do not express *hh.lacZ*. When *en*⁻ clones are induced at the blastoderm stage, the resulting flies do not show mutant clones in the P compartment, because they have sorted out (see below and Lawrence et al., 1999). However we do see occasional *en*⁻ clones in the back part of the A compartment, which have sister spots nearby in the P – apparently, therefore, these are originally P clones which have survived by integrating into the A compartment where they make normal A cuticle (except in the a6 region where they make a5).

When *en*⁻ clones are induced during the larval period, some remain within the P compartment. These clones are largely restricted to the anterior region of P, where they contact or are close to the adjacent A compartment. Sometimes clones form rounded excrescences upon the flat surface of the integument, or even separate completely and float as spherical vesicles within the abdomen. We believe that these mutant clones are sorting or have sorted from the P compartment because their cells have acquired different affinities (see Lawrence et al., 1999).

Over most of the P compartment the clones make cuticle of the a5 type, darkly pigmented with large bristles (Figs 1, 2, 4). This is expected, given that they are always adjacent to, or surrounded by, wild-type Hh-secreting cells of the P compartment. Hence, Hh protein should flood into the *en*⁻ clones 'telling' the mutant cells they are adjacent to an A/P boundary – at such a position they should develop as a6, but, since *en* activity is needed to make a6, the best they can do is make a5 cuticle. Because *ptc* is strongly induced by Hh, one would expect the level of *ptc.lacZ* expression in the clones to

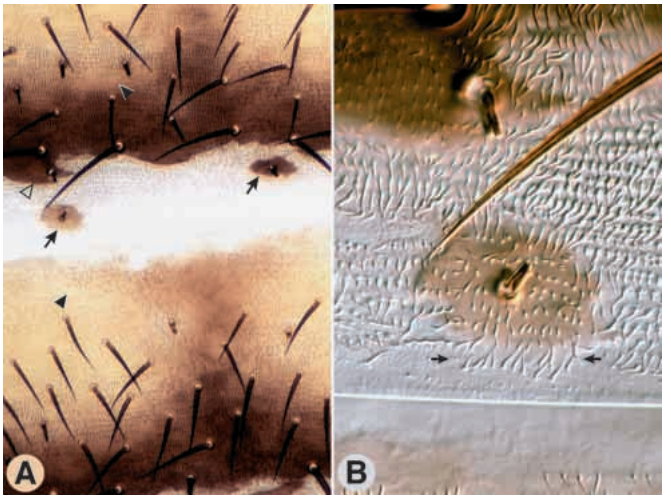

Fig. 2. Clones of *en*⁻ cells induced in the pupa. (A) There are numerous clones marked with *pwn* which affects hairs and bristles (arrows). The clones in the A compartment (arrowheads) are normal except at the posterior edge (outlined arrowhead); here no *pwn* territory ever makes clear a6 cuticle, the *pwn* cells are always darkly pigmented as in a5. In the P compartment, the anteriormost clones (arrows) are darkly pigmented, while in the more posterior regions the pigmentation is lighter, and the polarity of bristles and hairs in such clones is reversed. (B) Detail of an A clone with reversed polarity. Note some of the *pwn*⁺ hairs behind the clone are also reversed (arrows).

Fig. 3. Clone of *en*⁻ cells in the P compartment. The clone was induced in the larva and stained for *ptc.lacZ*. The cuticle marked with *y* and *pwn* is pigmented, has a smooth boundary (arrows) and bears large bristles of the a5 type (white arrowhead). The clone is 'fusing' with the A compartment along its right side and shows cytoplasmic staining for *lacZ* that is much more intense than that found in neighbouring wild-type a6 cells.

be high and we find it is even higher than in any part of a normal A compartment (Fig. 3).

Clones of *en*$^-$ cells induced during the early pupal period, after the histoblasts begin to proliferate, give rise to small patches of mutant tissue all over the P compartment (Fig. 2). Anteriorly, mutant cells generally make a5 cuticle. Near the posterior limit, they are more difficult to identify, but they can sometimes be detected with the aid of *ptc.lacZ*; they make transparent a1 cuticle, which is subtly different from the surrounding p1 or p2 cuticle (Struhl et al., 1997a,b). These results imply that the P compartment is subdivided into distinct anterior and posterior domains. Nevertheless, all the *en*$^-$ clones in the P compartment are transformed from P to A showing that *en* is required in all P cells to specify a P as opposed to an A identity.

Two further aspects of our results with *en*$^-$ clones are notable. First, we find it entertaining that when P clones are transformed to A in the abdomen they make cuticle of the nextmost segment back. For example clones in the P compartment of segment A1, both in the sternites and tergites, are changed into the A compartment of segment A2 (Fig. 4C). Both these compartments make up parasegment 7 (consisting of the P compartment of segment A1 plus the A compartment of segment A2). Likewise, clones in the P compartment of segment A5 are transformed into cuticle of the anterior compartment of segment A6 (Figs 4B, 5B). One might regard these transformations between segments as homeotic. However, given that the main homeotic genes work in a parasegmental register (Martínez Arias and Lawrence, 1985; Struhl, 1984), it is not surprising (reviewed by Lawrence, 1992).

Second, we find that *en*$^-$ clones induced in the pupa have divergent polarities. Anteriorly in the P compartment, clones that are embedded within the compartment, as well as those contacting its anterior edge, have normal polarity. However, in approximately the posterior two-thirds of the P, the clones have reversed polarity. The areas of reversed polarity coincide almost exactly with the patches of mutant cells, although there is usually a little non-autonomy: just posterior to the clone about one row of hairs formed by wild-type cells is also reversed (Fig. 2B).

Hh signalling and cell identity in *en*$^-$ cells

It appears that the types of A cuticle made by *en*$^-$ clones, whether in A or P, depend on Hh; this is shown by clones which lack both the *smo* and *en* genes. These clones are made as twins in which the sister cell (*smo*$^+$ *en*$^+$) is also marked (see Lawrence et al., 1999). In the A compartment, the *smo*$^-$ *en*$^-$ clones behave like *smo*$^-$ clones: they transform a6, a5, a4 into a3 and anteriorly they form a2 cuticle instead of a1 (Struhl et al., 1997a).

In the P compartment, few clones of *smo*$^-$ *en*$^-$ cells induced during the blastoderm stage survive to contribute to the P compartment of the adult. In the anterior region of P they form a3 cuticle and bristles (Fig. 5A,B) – this is expected as they should be unable to read the Hh flooding into them, and will 'see' themselves as A cells remote from the compartment boundary. In the posterior region they form a2 cuticle without bristles (see Fig. 4B in Lawrence et al., 1999), arguing again that the P compartment, like the A compartment, is subdivided into distinct anterior and posterior domains.

We compared *smo*$^-$ *en*$^-$ and *en*$^-$ clones generated by heat shock in the larval period; the clones differed in phenotype, with the doubly mutant ones forming cuticle which is a mixture of a3 and a5 types; the pigmentation tended to be light as in a3, but the bristles were of two sizes. Perhaps, *smo*$^+$ activity was present when the first bristles were specified, but lost later on.

The polarities of *smo*$^-$ *en*$^-$ and *en*$^-$ clones are similar. However, because *smo*$^-$ *en*$^-$ clones make a2 cuticle that has hairs, we can see in these clones that the reversal of polarity continues right to the back of the P compartment. In some cases the clones fuse with the A compartment behind them, and the polarities of the clone and the surrounding cells become confounded: for example occasional wild-type cells may acquire the reversed polarity of the clone (see Fig. 4B in Lawrence et al., 1999).

Clones that lack *ptc* only

Hh transduction is normally repressed by Patched (Ptc) which is expressed exclusively in the A compartment (reviewed by Ingham, 1998). The absence of *ptc* is expected to cause no phenotype in the P compartment, and we detected none. However, Hh enters the A compartment from both ends and forms a U-shaped landscape of opposing gradients. Thus, any cell that lacked *ptc* would be expected to develop as if it were close to a source of Hh. We find that the cells respond to loss of *ptc* in one of two different ways, depending on which domain of the A compartment the cells are in. In the anterior domain, *ptc*$^-$ cells develop normally in the a1 region but, in the a2, are transformed to a1. In the posterior domain, they develop normally in a6 but, in the a5, a4 and a3 territories give patches of clear unpigmented cuticle with hairs, like a6/p3 cuticle. We classify these cells as a6 rather than p3 for two reasons: First, when the flies carry an *en.lacZ* transgene, which stains the normal p3 strongly, neither the normal a6 cuticle nor the *ptc*$^-$ clones stain blue (Fig. 6A). Second, there are marked a5 bristles associated with the clones (bristles are characteristic of A, not P, cuticle). These bristles seem anomalous, as a6 cuticle is not normally associated with bristles. Our preferred interpretation is that both in the most posterior region of A in the wild type and in these clones, large bristles are initiated early on by maximal amounts of Hh; they therefore exist independently of whether or not the epidermal cells become induced later to form a6 cuticle.

Some *ptc*$^-$ clones induced in the larval period are more complicated: in the anterior part of A, *ptc*$^-$ clones activate the *en* gene strongly and autonomously, and this is shown both by the phenotype (the entire clone is transformed into P and, therefore, there are usually no marked bristles), and when they are monitored with *en.lacZ*. The clones (Fig. 6B) resemble those in which either *hh* or *en* (Kopp et al., 1997; Struhl et al., 1997b; and see below, Fig. 8A,B) are misexpressed in cells of the anterior region of A; they become associated with a complete sequence of cuticle types arranged in reverse order. The cells of the clone secrete p3, p2 and p1 cuticle, while, posterior to the clone, cells are induced to make a5 and a6 cuticle. At the anterior limit of the clone there is an ectopic P/A compartment boundary (p1/a1) with a1 being made by wild-type cells. More anteriorly, outside the clone, there is sometimes a strip of a2 cuticle before the polarity appears to return to normal in another strip of a1.

There is a tendency for *ptc*$^-$ clones to sort out (Lawrence et

al., 1999), but survivors in the middle of the segment give hairs and bristles pointing in towards the centre of the clone, with a zone of reversed polarity behind the clone up to several cells wide (Fig. 6B); these polarity changes are similar to *PKA*$^-$ clones which also activate the Hh pathway (Struhl et al., 1997a).

Clones that lack *ptc* and *en*

We made these clones to test whether any of the transformations produced by *ptc*$^-$ clones depend on activation of *en* or *inv*. In the a2 region of the A compartment, the *ptc*$^-$ *en*$^-$ clones behave as *ptc*$^-$ clones and make a1 cuticle (see Fig. 2C in Lawrence et al., 1999); confirming that the development of a1 cuticle in the wild type is not dependent on *en*. Over the remaining parts of the A compartment the clones do not make clear a6 cuticle (as *ptc*$^-$ clones do) but instead make dusky a5 cuticle with large bristles (Fig. 6C). This confirms that the development of a6 cuticle, but not of a5, depends on *en*. Finally, we did not find any *ptc*$^-$ *en*$^-$ clones making P cuticle in the A compartment, arguing that the ectopic P compartments that are associated with *ptc*$^-$ clones do indeed depend on the activation of *en*.

As observed for *PKA*$^-$ clones (Struhl et al., 1997a), single *ptc*$^-$ *en*$^-$ clones in A can make both a1 and a5 cuticle showing that the different domains of A are not distinct compartments with independent lineage. Also, the effects on polarity resemble those in *PKA*$^-$ clones: hairs and bristles point in towards the centre of the clones giving reversed polarity behind. It follows that the repolarisation associated with both those types of clone does not depend on ectopic activation of *en* or *inv*.

In segment A6, the type of cuticle made by *ptc*$^-$ *en*$^-$ clones differs from that in the more anterior segments; it is lightly pigmented and resembles the cuticle found just posterior to the a5 cuticle in segment A6 (Fig. 6D); this is the same type of cuticle produced by *PKA*$^-$ clones also in segment A6 (see Fig. 4 in Struhl et al., 1997a) or by *en*$^-$ clones induced in the P compartment of segment A5 (Fig. 5B). Thus it seems that a single level of Hh response will give different outcomes in different segments (see page 2150 in Struhl et al., 1997b); the level of Hh signalling reproduced in *PKA*$^-$ and *ptc*$^-$ *en*$^-$ clones corresponds to that in both dusky cuticle in segment A4 and to light cuticle in A6.

Pupal *ptc*$^-$ *en*$^-$ clones in the P compartment appear identical to *en*$^-$ clones. In the anterior part of P they give dusky a5 pigmentation and bristles (a5), while further back the clones are lighter in colour (‘a3’). Also, at the front of the P compartment the polarity of the clones is normal, while at the back it is reversed. It seems that these positional differences in differentiation and polarity cannot be due to different levels of Hh signalling, real or perceived – for all *ptc*$^-$ cells of A identity should have their Hh response set at the maximum.

Consequences of ectopic *en* activity in A compartment cells

We have examined the effects of expressing *en* in clones of A and P compartment cells. Within the P compartment we have compared such clones with controls (similarly marked with *lacZ*, but not constitutively expressing *en*) and most resemble them. However, there are some exceptions (Fig. 7); these abnormal clones are elongated and abut the A/P border for many cell diameters from the posterior side. They make a6/p3 cuticle, although the hairs are usually slightly irregular. Two facts suggest that these clones originated in the A and then merged into the P compartment: First, we rarely find marked clones in the extreme posterior region of A, suggesting that such clones have sorted out, died or migrated away (possibly migrated into the P, to give the very clones we now discuss). By contrast, control clones are common in this region. Second, some of these clones are slightly pigmented and include marked bristles (Fig. 7), indubitably characteristic of A compartment tissue; perhaps the clones have been incompletely transformed from A to P. Control clones never show these abnormalities. These two findings do not prove our case; it remains possible that the exceptional clones originate in P and, as happens in the wing, over-expression of *en* gives them some A-like properties (Guillén et al., 1995).

Clones of ectopic *en*-expressing cells in most of the A compartment activate endogenous *en*; they are large and have reversed polarity with an ectopic P region (Fig. 8 and Kopp et al., 1997). They are similar to *ptc*$^-$ clones (Fig. 6B), to clones of ectopic *hh*-expressing cells (Struhl et al., 1997b) and also to the gain-of-function mutation *en*^{erased} (Kopp et al., 1997). The *en*-expressing cells also activate *hh* in a domain that is coextensive with the P cuticle (Fig. 8A) and induce *ptc.lacZ* in zones both in front and behind the P cuticle; indicating that new A/P and P/A borders have been formed at the anterior and posterior limits of the clone. Marked bristles are very rare, suggesting that cells belonging to these clones are largely transformed to P type. Wild-type cells behind the clones have reversed polarity. Anterior to these clones there is a strip of a1 cuticle. The sequence of cuticle types as well as the orientation of hairs and bristles shows that the polarity of cells within and near to the clone is reversed – further away, both anteriorly and posteriorly, polarity returns to normal (see Fig. 8B).

In the most posterior parts of the A compartments, larger clones are rare (as we have discussed above). Surviving clones express the marker *lacZ*; they make a cuticle that we presume is P in type and, because it is entirely hairy, is p3; the clone is surrounded by unpigmented cuticle (presumably a6) (Fig. 9D). Note that polarity correlates with the orientation of the A/P boundaries which we surmise ring the clone – thus the hairs in front of the clone are normally oriented, and behind the clone are reversed. Pupal heat shocks give small clones in the anterior domain with cuticle like p1/p2, while in the posterior domain, they produce a6/p3 cuticle with varying polarities (Fig. 9A-C). There is evidence that the transformation of these clones from A to P is incomplete: the clones sometimes make bristles, a characteristic of A cells. The clones show patchy and pale expression of *hh.lacZ*, a characteristic of P cells. But there is also some uneven expression of *ptc.lacZ* which is normally only found in A cells; yet some cells just outside the clones express *ptc.lacZ*, which might be due to Hh protein leaving any P cells in the clone and reaching nearby A cells. In segment A5, the a6 (hirsute) and the p3 cuticle (bald) are distinct and since we find that the clones are hairy, they would appear to be more like a6 than p3 (Fig. 9B). The type of cuticle is characteristic of the segment in which it is found (Fig. 9B,C).

DISCUSSION

The cells of the dorsal epidermis of the adult abdomen in

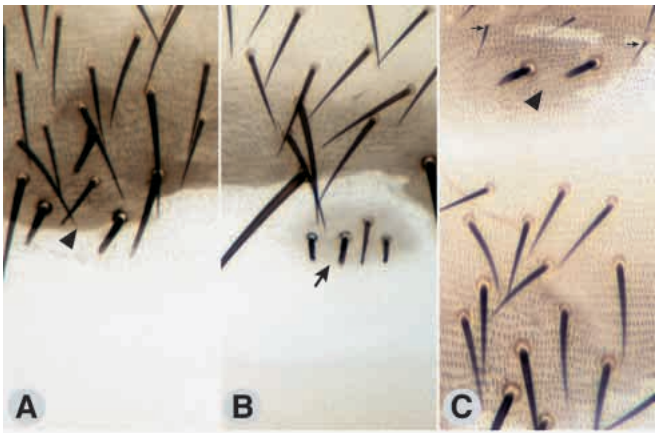

Fig. 4. Clones of *en*⁻ cells. (A,B) Clones marked with *pwn* induced in segment A5 in the pupa. (A) A clone (arrowhead) originated in the a6 region of the A compartment of A5 shows hairy cuticle with features characteristic of a5 region of the same segment. (B) A clone (arrow) presumed to be of P provenance, forms hairless cuticle characteristic of the a5 region of the A compartment of segment A6. (C) This clone (arrowhead) was induced in the P compartment of segment A1 in the larva. It makes two large bristles and pigment characteristic of segment A2. Compare with the typical bristles of A1 (small arrows).

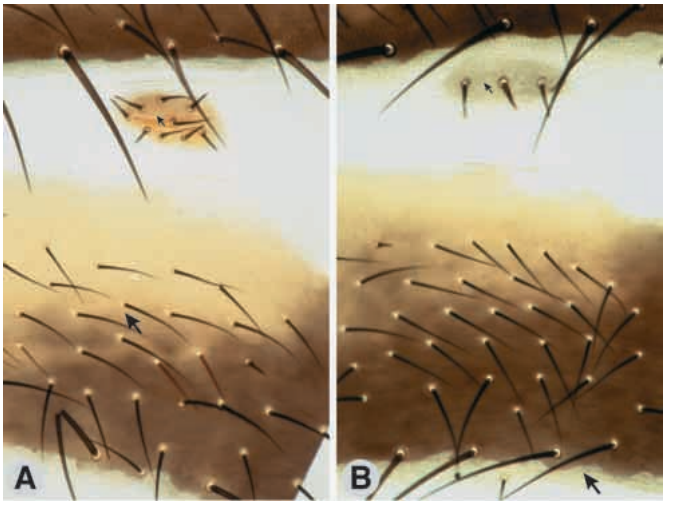

Fig. 5. Clones of *en*⁻ and *smo*⁻ *en*⁻ cells. (A) A *smo*⁻ *en*⁻ clone (marked with *pwn*) induced at the blastoderm stage in the P compartment of the A5 segment. (The Smo protein has a long perdurance, so that only after heat shocks made at blastoderm do we see the maximal phenotype). Note that the clone forms pigmented cuticle (small arrow) and small bristles typical of the a3 region of the A6 segment (arrow). (B) A comparable *en*⁻ clone induced in the larva in the same position forms the almost clear cuticle (small arrow) typical of the posterior extreme of the A compartment of A6 (arrow). Note that this clone also makes bristles of a size similar to those found in the A6 segment in positions just anterior to the light pigmented cuticle.

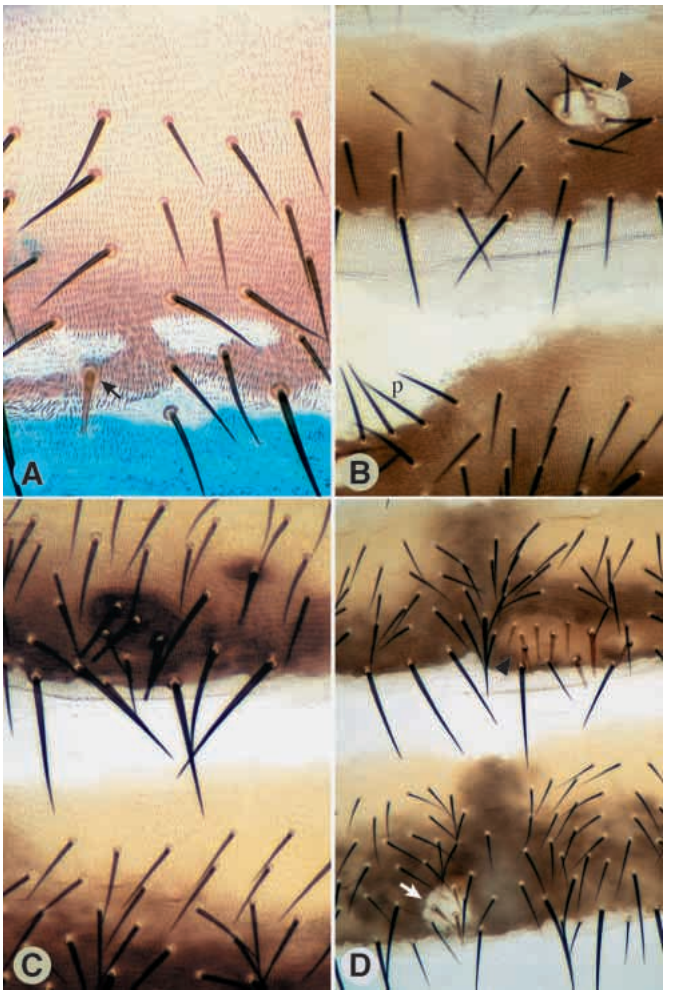

Fig. 6. Clones of *ptc*⁻ and *ptc*⁻ *en*⁻ cells induced in the larva. (A) Clones of *ptc*⁻ cells marked with *y* and *pwn* stained for *en.lacZ*; they differentiate as clear a6 cuticle. All the A surviving clones are in the posterior part of the compartment, probably because the more anterior clones tend to sort out. There are large a5-type marked bristles associated with the clones (arrow). Note that *en.lacZ* is not expressed in the *ptc*⁻ clones. (B) Clones of *ptc*⁻ cells marked with *y* and *sha;* two anterior clones are shown. One has completely reversed polarity and has induced posterior cuticle (p), the other develops a6 cuticle, is associated with misoriented hairs behind and within the clone, and it is also partially sorted out (arrowhead). (C) Clones of *ptc*⁻ *en*⁻ cells marked with *pwn*: note that in the absence of *en*, the clones make pigmented a5 cuticle, rather than clear a6 cuticle (compare with A and B). (D) Clones of *ptc*⁻ *en*⁻ cells marked with *y* and *pwn* in segments A5 and A6. Note that in A5 the clone makes pigment (arrowhead), while in A6 (white arrow) another clone develops the light pigmented cuticle typical of the posterior part of the A compartment of this segment (compare with Fig. 5B).

Drosophila exhibit two properties. The first, a scalar property, is shown by the identity of the cuticle they secrete. The second, a vectorial property, is indicated by the orientation of hairs and bristles. The key results are summarised in Fig. 1. We now discuss the scalar and vectorial properties in turn.

The scalar: the *en* selector gene is sufficient to specify P versus A cell identity

The hypothesis that developmental compartments are units of cell lineage governed by selector genes depended on two elements: first the discovery of A and P compartments in the

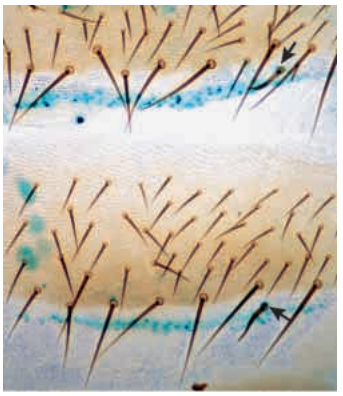

Fig. 7. Clones now located in P, in which en^+ is expressed ectopically. Two *abx/ubx-en* clones marked with *forked* and *lacZ*; they are located in the P compartment but we think they originated in the A, and have migrated backwards. Each of the clones is associated with a single marked *forked* bristle (arrows). When such clones are found in segment A1, they are associated with *forked* bristles of A2 character, which presents a conundrum that we offer to our reader(s).

adult limbs of *Drosophila* and, second, the observation that these compartments are coextensive with the realms of action of homeotic genes such as elements of the *Bithorax Complex* and *en* (Garcia-Bellido et al., 1973; Garcia-Bellido, 1975; Morata and Lawrence, 1975). The *en* gene became the cornerstone of the compartment hypothesis because it appeared to provide the cleanest evidence – in spite of the inconvenient fact that cells lacking *en* give only incomplete transformation of P cells to A identity (Kornberg, 1981b). However, at the time of Kornberg's experiments, the genotypes available to remove *en* left a sister gene *inv* intact. More recently, a deletion, $Df(2R)en^E$, which eliminates both *en* and *inv* transcripts has been used to test the complete loss of *en* activity in the wing

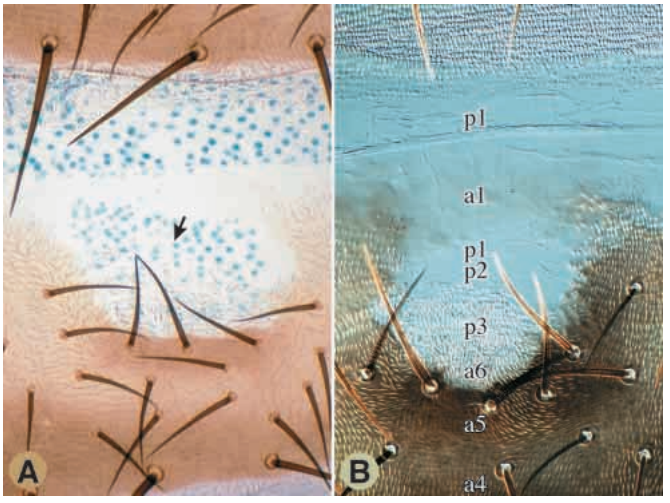

Fig. 8. Clones in which en^+ is expressed ectopically. (A) *tub-en* clone stained for *hh.lacZ*. We believe all the cells of the clone express *lacZ* (arrow). The orientation of bristles and hairs near and inside the clone as well as the sequence of cuticular types (compare with B), is reversed. (B) A similar *act-en* clone; note the sequence of cuticular types (a4, a5, a6, p3, p2, p1, a1 and p1) as indicated.

(Hidalgo, 1994; Sanicola et al., 1995; Tabata et al., 1995). Like Tabata and colleagues we find that en^- P compartment cells autonomously differentiate A cuticle and the transformation appears to be perfect. Moreover we show that en^- P clones made early in development can survive by entering and becoming subsumed into the A compartment where they develop normally. Also A clones that express *en* can become P cells. Thus, our findings in the abdomen have substantiated the compartment hypothesis, particularly with respect to *en*.

The scalar: there is a special function for *en* in the A compartment

We have found that, although *en* is inactive in most of the A compartment, the gene is needed for the specification of one A type of cuticle, a6. When *en* and *inv* are removed from cells that would have made a6, each of those cells makes a5 cuticle instead. This finding shows that the wing disc and the abdomen are similar; for in the wing disc *en* also has a local and late function at the back of the A compartment (cf Blair, 1992; Hidalgo, 1994). In the wing this late expression of *en* depends on Hh (Guillén et al., 1995; Mullor et al., 1997; Strigini and Cohen, 1997). In the abdomen, in most of the A compartment, ptc^- cells (in which Hh transduction is at the maximal level) form a6 cuticle, while ptc^- en^- cells form ectopic a5 cuticle – it follows that Hh specifies a6 identity by inducing *en*.

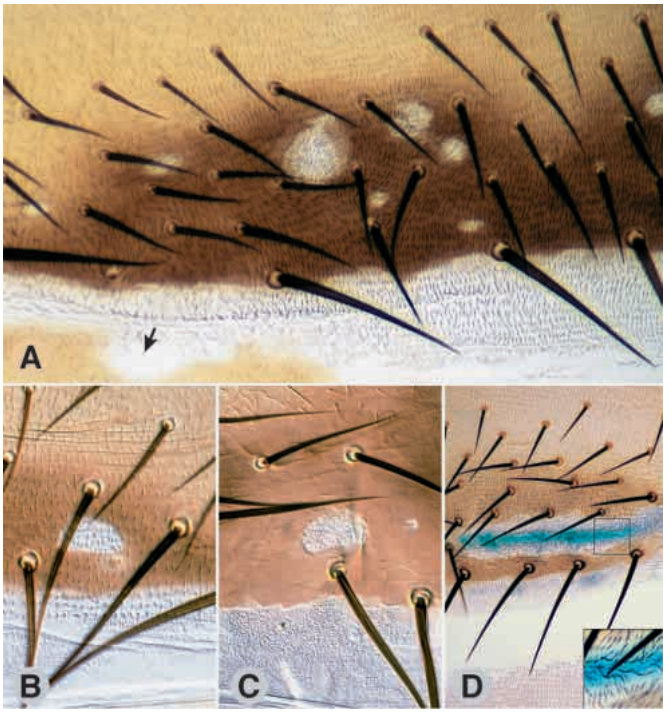

Fig. 9. Clones in which en^+ is expressed ectopically. (A-C) Clones are *69B-en* induced in pupae. (A) In the anterior region of the A compartment (as in the next segment back, arrow) the clones transform a2 into p1/ p2 cuticle. In the posterior region of A, the cuticle type of the clones is clear and hairy and there is repolarisation behind the clone. (B,C) This hairy cuticle resembles a6 and its structure is characteristic of the segment in which it is induced (segment A5 in B, and A6 in C). (D) A *abx/ubx-en* clone in the a4 region marked with *lacZ*. The clone develops as p3 cuticle (stained in blue) and induces a6 (clear) and a5 (pigmented) cuticle on both sides. The clone also causes reversed polarity posteriorly (inset).

The scalar: subdomains within both A and P compartments

We have found that *ptc*⁻ *en*⁻ cells at the front and the back of the A compartment give different transformations, confirming there are two domains in A (Struhl et al., 1997a) as shown in Fig 1. These domains correspond largely to the territories of a1, a2 (no bristles) and a3, a4, a5 cuticle (with bristles). We have independent evidence that underwrites the existence of these domains: removal of the *Notch* (*N*) gene from these two regions gives different outcomes: *N*⁻ clones in a2 make epidermal cells, while those in a3 do not (unpublished results). It follows that the cells composing a2 (non-neurogenic, see Heitzler and Simpson, 1991) and a3 (neurogenic) are fundamentally distinct.

We have found that the P compartment is also subdivided. Thus, the loss of *en* from posterior P cells converts them from making p1 cuticle to either a1 or a2, depending on whether they can receive the Hh signal. The removal of *en* from anterior P cells causes them to make either a5 or a3 cuticle, again depending on whether they can receive Hh.

Why should there be such a subdivision of the compartments? Perhaps it is connected with making a distinction between A/P and the P/A borders – for if both were simply an interface between A and P cells, they would differ only in their orientation (cf Meinhardt, 1984). We do not know what agent discriminates between the two domains in either compartment; perhaps one regulatory gene would be sufficient for both – its expression could flank the segment boundary, redefining nearby regions of the A and P compartments.

We have found that the domains are not maintained by cell lineage. Analogous domains are found in the legs, where A compartment cells respond to Hh by expressing high levels of either Decapentaplegic (Dpp) or Wingless (Wg), depending on whether they are located dorsally or ventrally in the appendage (Basler and Struhl, 1994; reviewed in Campbell and Tomlinson, 1995). This dorsoventral bias in response is established early in development, and then maintained, not by lineage, but by feedback between Wg- and Dpp-secreting cells (Brook and Cohen, 1996; Jiang and Struhl, 1996; Johnston and Schubiger, 1996; Penton and Hoffmann, 1996; Theisen et al., 1996).

The scalar: activation of *en* in response to ectopic Hh

Cells in the middle of the A compartment are normally exposed to little if any Hh, but if Hh is provided they activate *en* and produce P cells (Struhl et al., 1997b). We show here that ectopic *en* (which leads to expression of Hh), or the removal of the *ptc* gene (which activates Hh transduction) also activate *en*. In the *ptc*⁻ embryo there is a second stripe of *en* in each segment, giving adventitious A/P boundaries that are thought to cause the 'patched' cuticular phenotype. It has been proposed that this second stripe is induced by *wingless* acting non-autonomously (DiNardo et al., 1988; Martínez Arias et al., 1988). However, in the adult, we find that ectopic expression of *en* is an autonomous response to activation by Hh. Also in discs, ectopic expression of Hh can activate *en* in the A compartment (de Celis and Ruiz Gómez, 1995; Guillén et al., 1995).

The vector: dependence on Hh and 'Factor X'

Earlier we proposed a model where Hh crosses over from P to A and elicits production of a 'diffusible Factor X' that grades away anteriorly from the A/P border, and has a long range; the cells are oriented by the vector of this gradient (Struhl et al., 1997a,b). For simplicity, let's restrict this discussion to the posterior domain of the A compartments. First note that the A/P boundaries cannot be unique sources of X, for polarity changes also occur when cells from one level of A confront those from another (e.g. when a5 and a3 cells meet at the edge of *ptc*⁻ *en*⁻ clones). This suggests that, away from the compartment boundaries, cells also produce X, the quantity depending on the amount of Hh received. We therefore imagine that a gradient of X would be formed both by the graded production of X (high near the A/P boundary, low further away) and also by its further spread into territory (a3) where Hh is low or absent. Second, note that this model fits with most of our results for it makes the A/P boundaries the organisers: we find that whenever ectopic A/P boundaries are generated by the clones, their orientation correlates with the polarity of territory nearby; this is most clearly seen at the back of *en*-expressing clones (e.g. Figs 8B, 9D). Note that the line where polarity switches from normal to reversed does not occur at a fixed position in the segment (as has been previously suggested; Kopp and Duncan, 1997; Kopp et al., 1997) but rather appears to be related to the position of nearby A/P borders.

The vector: *en* and polarity in the P compartment

en⁻ clones in the P compartment make A cuticle. In the anterior part of P these clones have normal polarity. In the posterior part of P the whole clone displays reversed polarity, as do some cells outside the clone. In order to understand this (in part!), consider the behaviour of *ptc*⁻ clones in the A compartment: they behave differently depending on their distance from the A/P border, the presumed source of X. At the back of the A compartment they are near that border and have little or no effect on polarity, but when closer to the front of A, they repolarise several rows of cells in the surround. We explain this as follows: near the source of X, where the ambient level is high, limited production of X might not much affect the concentration landscape. But, far from the source, where the local concentration of X would be low, it would.

Likewise, if there were a polarising factor similar to 'X' in the P compartment, then clones of *en*⁻ cells that produce complete or partial borders might become ectopic sources of this factor – they would produce altered polarities only in an environment where the level of the factor were low. This argument suggests that a polarising factor 'Y' for the P compartment might emanate from the A/P border and spread backwards. Thus the evidence is consistent with the idea that polarising signals spread in both directions from the A/P boundaries. The P/A (segment) boundaries might act to stop these factors trespassing into the next segment, just as they appear to block the movement of Wingless protein (González et al., 1991).

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