Towards a model of the organisation of planar polarity and pattern in the
Drosophila abdomen

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SUMMARY

The abdomen of adult Drosophila consists of a chain of alternating anterior (A) and posterior (P) compartments which are themselves subdivided into stripes of different types of cuticle. Most of the cuticle is decorated with hairs and bristles that point posteriorly, indicating the planar polarity of the cells. Here we research the link between pattern and polarity.

Previously we showed that the pattern of the A compartment depends on the local concentration (the scalar) of a Hedgehog morphogen produced by cells in the P compartment. Here we present evidence that the P compartment is patterned by another morphogen, Wingless, which is induced by Hedgehog in A compartment cells and then spreads back into the P compartment. We also find that both Hedgehog and Wingless appear to specify pattern by activating the optomotor blind gene, which encodes a transcription factor.

We re-examine our working model that planar polarity is determined by the cells reading the gradient in concentration (the vector) of a morphogen ‘X’ which is produced on receipt of Hedgehog. We present evidence that Hedgehog induces X production by driving optomotor blind expression. We tried but failed to identify X and present data that X is not likely to operate through the conventional Notch, Decapentaplegic, EGF or FGF transduction pathways, or to encode a Wnt. However, we argue that Wingless may act to enhance the production or organise the distribution of X. A simple model that accommodates our results is that X forms a monotonic gradient extending from the back of the A compartment to the front of the P compartment in the next segment, a unit constituting a parasegment.

Key words: Planar polarity, Morphogen, Compartment, Hh, omb, Wnt, Drosophila melanogaster

INTRODUCTION

Planar polarity (Nübler-Jung et al., 1987) refers to the orientation of cells in an epithelium with respect to the axis of the organ (e.g. proximodistal) or to the body (e.g. anteroposterior). The mechanisms responsible for planar cell polarity are little known, yet it is an important phenomenon integral to many aspects of cell structure and function. Polarised structures such as cilia have a directed beat, and hairs and bristles (for example in plants, insects and mammals) are polarised, usually in large fields with concordant orientation. Planar polarity therefore has structural implications for most aspects of cell architecture. Separated cells are often oriented – for example, in a moving fibroblast exocytosis occurs mainly at the leading front (Bretscher, 1984) with the cytoskeleton also being polarised. For recent reviews see Drubin (Drubin, 2000).

Bonner (Bonner, 1947) first argued that, in Dictyostelium, amoebae are able to read the local slope of a concentration gradient, and experimental evidence in Hemiptera suggested that, in insects at least, this local slope (the vector) of a morphogen gradient specifies planar cell polarity (Lawrence, 1966; Stumpf, 1966). More recently it has been shown that single yeast cells, Dictyostelium cells and neutrophils are able to detect, and are polarised by, the vector of shallow gradients of substances (varying by only about 1% over the cell diameter) across a wide range of concentrations (Zigmond, 1974; Segall, 1993; Parent and Devreotes, 1999; Drubin, 2000). In this view, pattern formation in a developing field depends on a succession of events: first, gradients of morphogens are made that diffuse from localised sources and reach each cell. The concentration of a morphogen at each locale (the scalar) gives information of position that determines cell differentiation and thereby fixes pattern. The concentration landscape may also cause the graded production of other diffusible morphogens, creating secondary morphogen gradients. The vector of one of these secondary morphogens could specify planar polarity (Struhl et al., 1997a). The initial reading of this vector is then progressively elaborated within cells as proteins are localised (compare the CRAC protein in Dictyostelium) (Parent et al., 1998). One consequence of this is to place a cell hair in one part of the cell membrane – this hair then grows out in a particular direction (Eaton et al., 1996).
We use the abdomen of adult *Drosophila* because it is particularly well suited to study the global control of cell pattern and planar polarity. Most epidermal cells of the abdomen make a few hairs or a bristle, and these indicate the planar polarity. In addition, epidermal cells at different positions along the anteroposterior axis of each segment make characteristic types of cuticle to form a stratified pattern. Finally, the abdominal epidermis presents the primeval body plan of higher invertebrates, being a chain of anterior (A) and posterior (P) compartments that constitute parasegments (Martínez-Arias and Lawrence, 1985) and segments. Hedgehog (Hh), a morphogen produced by P compartment cells, is responsible for organizing both cell pattern and planar cell polarity in the neighbouring A compartments (Kopp and Duncan, 1997; Struhl et al., 1997a; Struhl et al., 1997b; Lawrence et al., 1999a).

Our main aim here is to research how polarity is determined within the context of pattern formation as a whole. We do not try to understand the mechanics of asymmetry within a single cell but instead ask how the cells of the whole epidermal segment know which way to point. For example, we do not know how the graded distribution of Hh is translated into the orientation of hairs and bristles. Hh is made in every P compartment and enters each A compartment from both anterior and posterior directions to form U-shaped concentration gradients (Struhl et al., 1997a; Struhl et al., 1997b). We have shown that Hh somehow directs planar cell polarity throughout the A compartment, causing cells to make hairs and bristles that point posteriorly towards the source. Thus a clone of cells that make Hh ectopically will reorient surrounding cells, causing them to make hairs and bristles which point towards the centre of the clone. However, when the Hh pathway is activated in cells confined to a clone, the surrounding wild-type cells are also repolarised. We argued that this repolarisation was therefore not due to Hh itself (Struhl et al., 1997a) but to something else emanating from the clone, possibly another morphogen. Here we confirm this with a new test.

Our working model is that a substance (‘X’) is produced at the back of each A compartment in response to Hh and spreads anteriorly to set up a concentration landscape of X (Struhl et al., 1997a). We then conjecture that the polarity of a cell at any point in the A compartment is specified by the local vector in this gradient landscape, in this case like an arrow that points up the steepest slope of the concentration gradient (Lawrence, 1966; Stumpf, 1966; Struhl et al., 1997a). Further, since nearly all hairs and bristles point backwards, any simple form of the model requires that the gradient of X be monotonic, decreasing consistently in one direction. We interpret all the results with respect to this hypothesis.

The model raises many questions that need to be answered by experiment. For example, which cells respond to Hh to produce X and how do they do so? How far does X move? What is the registration of the repeating X gradient relative to the chain of A and P compartments? Does X control polarity in both the A and P compartments? What is the molecular nature of X?

Our approach to these problems is to utilise those genes that have been implicated in the pathway of Hh action. We make genetically marked clones of cells in different parts of the segment that either lack a particular gene or overexpress it. We then describe the effects on polarity, both inside and outside the clone. At least within the A compartment, Hh appears to govern X production by inducing expression of optomotor blind (omb) and perhaps that of the extracellular signal Wingless (Wg) to generate a distribution of X that spreads forward. In an attempt to identify X we have tested the Decapentaplegic, Notch, EGF, FGF and, especially, the Wnt pathways, all without positive result. Hence, the identity of X remains unknown.

Another series of questions relates to the P compartment. The development of this compartment cannot be directly dependent on Hh because P cells are blind to Hh (reviewed by Lawrence and Struhl, 1996). Yet the P compartment is patterned and has oriented hairs. So what determines the scalar response in the P compartment, stratifying it into different types of cuticle? Similarly, what determines the vector, the orientation of its cells?

We present evidence that the P compartment is patterned by another morphogen that acts also through omb – omb appears to be expressed and required in the anterior region of the P compartment. Our results suggest that this morphogen is a Wnt, probably Wg itself. We discuss how the P compartment might be polarised.

**MATERIALS AND METHODS**

We manipulate the expression of genes in marked clones of cells. We use FLP recombination (Golic, 1991; Struhl and Basler, 1993), as well as the Gal4 (Fischer et al., 1988; Brand and Perrimon, 1993) and Gal80 systems (Lee and Luo, 1999).

To save space and because there are many more landmarks, we have concentrated on the tergites, which are formed by the dorsal epidermis. However, all the clone types have also been studied ventrally in the sternites and pleura – these results are given if they seem to be helpful. Unfortunately the pleura has few distinguishing marks so we could not easily determine the position of pleural clones relative to the compartmental subdivisions.

**Mutations and gene constructs**

The mutant alleles and transgenes used in this work are as follows (see also FlyBase, 1999; Lawrence et al., 1999a).

**Hh pathway**

**hh**: hhAC, a deletion removing the start of the open reading frame.

**hh.Gal4**: an enhancer trap insertion in the *hedgehog* gene which expresses Gal4 (gift from M. Calleja and G. Morata).

**hh.lacZ**: hhP10.

**Pka–**: Pka–C1E93, an amorphic allele of the cAMP-dependent protein kinase I gene. **ptc**: ptc10, an amorphic *patched* allele caused by a premature stop codon before the first transmembrane domain. **ptc52**: a hypermorphic allele. **ptc18**: an amorphic allele. **ptc.Gal4**: the insertion P[w+mW.hs=GawB]559.1, that expresses Gal4 in the ptc pattern. **ptc.lacZ**: EcoNuc1ptc–AT96, tub.ptc: puGalo81B.PCp, UAS.ptcΔloop2: the open reading frame of a form of patched lacking most of the second large extracellular loop (Briscoe et al., 2001).

**Omb**

**omb**: omb–3198, an amorphic bifid allele resulting from a premature stop codon. Kopp (Kopp and Duncan, 1997) isolated alleles of a gene (Scruffy, Scf) and suggested that this gene may act in parallel with *omb*. We have made *omb* clones in a Scf background, expecting them therefore to have a stronger phenotype, but they do not do so – they behave as *omb* clones normally do (not shown). **omb.Gal4**: an enhancer trap insertion isolated by Y. H. Sun [omb-Gal4 according
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RESULTS AND DISCUSSION

Further evidence that Hh acts through a second signal

Previously, we concluded that Hh acts indirectly via another system (a gradient of ‘X’) to effect polarity (Struhl et al., 1997a). The evidence was based on clones that lacked such downstream genes as patched (ptc) or CAMP-dependent protein kinase 1 (Pka). In the A compartments, Ptc and Pka proteins act within cells to prevent the Hh pathway from being activated inappropriately; if either protein is removed the Hh pathway becomes constitutively activated within the mutant cells themselves. With respect to the type of cuticle (the scalar output of Hh) the results fit the model; the mutant cells make the cuticle normally made by cells responding strongly to Hedgehog and all the cells outside the clone make the normal type of cuticle (a cell-autonomous effect). However, with respect to polarity (the vectorial output of Hh), the results were different; polarity was altered in the wild-type cells up to several cell diameters away from the clone (a cell non-autonomous effect). Hence, we could not prove unequivocally that low levels of ectopic Hh might be produced by Hh receipt, which is where we start our search.

Clonal analysis

Unless stated otherwise clones were induced by heat shocking at 34 or 37.5°C for 60 minutes. Either embryos at blastoderm stage or third instar larvae of the following genotypes were used.

**pter**: y w hs.FLP; FRT42D pwn ptc/FRT42D CD2y+.

**ptc** (or **arm**:): y w omb sn FRT19A/FRT19A; hs.FLP/+; hs.lacZ+/+, y w omb sn FRT19A/FRT19A; hs.FLP; ptc.lacZ.

**UAS**: y w UAS.Gal4/TM6B, UAS.Gal80; FRT42D pwn ptc/FRT42D CD2y+.

**argos** (or **arm**:): y w y w hs.FLP; FRT101/w hs.FLP FRT101; ptc.lacZ/+, y w y w hs.FLP; Dp-y Dp-syg FRT39stice FRT39.

**Pka** (or **arm**:): hs.FLP: Pka FRT39/CD2y+.

**pta** (or **arm**:): FRT42D pwn ptc/FRT42D tub.Gal80 CD2y+; tub.Gal4+.N.

**N** (or **arm**:): y w hs.FLP; FRT42D Dp-N/FRT42D pwn.

**Egfr** (or **arm**:): y w hs.FLP; FRT42D pwn Egfr/FRT42D CD2y+.

**ruga** (or **arm**:): y w hs.FLP; argos/FRT42D tub.Gal80.

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Downstream genes in the Hedgehog pathway – the A compartment

(i) optomotor blind

**omb** clones

**omb** encodes a transcription factor which is activated on receipt of high amounts of Decapentaplegic (Dpp) in both A and the P compartments of the wing and elsewhere (Lecuit et al., 1996; Nellen et al., 1996); it has been studied in the abdomen by Kopp and Duncan (Kopp and Duncan, 1997). We find it is expressed in each segment, both dorsally and ventrally, as a single stripe spanning the AP border and including the rear of the A compartment and the front region of the P (Fig. 3A). Accordingly, **omb** clones in other parts of the segment are normal – for a memorandum of **omb** clones see Fig. 2.

Within the posterior half of the A compartment, Omb is required for the normal scalar response to Hh. At the extreme back, in the a6 region, where the Hh concentration is highest, the **omb** cells develop only a little abnormally; the unpigmented cuticle of that region (a6, see Fig. 2 for nomenclature) is expanded a little anteriorly in the clone (Fig. 3B-E), but sometimes contains small ‘a3’ bristles. Note that specification of a6 cuticle normally requires *engrailed* activity, which is induced in A cells by peak levels of Hh (Lawrence et al., 1999a). However, in **omb** clones that are situated more anteriorly, in the pigmented region at the back of the A compartment (a4, a5), there is a big effect: it appears that Hh acts through **omb**, because **omb** cells never make a4 cuticle or a5 bristles (pattern elements that signal a response to Hh), and in their stead make a3 cuticle [the type of cuticle made where there is little or no response to Hh (Kopp and Duncan, 1997; Struhl et al., 1997a) (Fig. 3B-E)]. Also, Hh directly upregulates expression of *ptc*, which encodes a component of the Hh receptor (Struhl et al., 1997b) and this also occurs in **omb** clones (Fig. 3B,D). This finding indicates that Omb is not required for Hh signal transduction per se, but for the appropriate response of cells.

With regard to polarity, the clones confined to the anterior and middle part of the A compartment are normal. However, clones just behind the middle of the A compartment usually show reversal at the front, with normal polarisation at the back. More strikingly, clones confined to the very back of the A compartment, in the a6, a5 and a4 domains can be largely or entirely reversed (Kopp and Duncan, 1997) and this reversal usually extends anterior (Fig. 2, Fig. 3B,E) to the clone.

To explain these polarity changes, we suggest that Hh induces X production through the agency of Omb. It follows that little or no X can be produced within **omb** clones and therefore...
that the polarities of cells in or near such clones depend on X produced outside. Clones in the middle of the A compartment behave normally because most X is produced behind them and the gradients of X concentration are little changed. Clones located a little further back will have peaks of X both behind and in front and this can cause localised reversal at the front of the clone (Fig. 2, Fig. 4C). For a clone extending back to the AP boundary, the only source of X will be anterior to the clone, presumably because omb+ cells there will ‘see’ Hh protein that has passed through the clone. These cells should make X that spreads backwards into the clone, setting up a gradient of reversed polarity (Fig. 4B). There is corroborating evidence: in some clones there is dark pigmentation and large bristles anterior to the clone (Fig. 3C-E), confirming that Hh has indeed been received there. However, many omb- clones are associated with anterior repolarizations that occur even where there is no dark pigmentation anterior to the clone (Fig. 3B), suggesting that the level of Hh required to stimulate some X production anterior to the clone is less than that needed to make a4 pigment. It follows that, in normal flies, some X is produced by cells anterior to the a4 pigmented zone. Finally, we find that some clones, which extend nearly to the back of A, show reversed territory behind the clone (Fig. 3D), perhaps due to the domination of the X source that is anterior to the clone over any production of X behind it.

We note that the reversed polarity associated with omb- clones located at the back of the A compartment usually extends only to the AP boundary, with polarity in the P compartment being normal (Fig. 3C). This result suggests that the AP boundary coincides with a barrier to the movement or action of X. The existence of such a barrier would provide an explanation for why X normally produced in cells at the back of the A compartment does not spread posteriorly into the P compartment, reversing the polarity in P. However, in rare cases, some reversed hairs were seen in what appeared to be adjacent P compartment cells, as marked independently by ptc.lacZ staining (as in Fig. 3B; data not shown). We do not know whether these rare cases are artifactual, due to a slight posterior shift – during mounting – of the cuticle relative to the underlying epidermis, or are frank reversals of cells within the P compartment. If the reversed cells are indeed P cells, they raise a problem for the notion that the AP boundary constitutes a barrier to X movement.

omb- ptc- clones

If the production of X depends at least in part on omb, then ptc- clones, in which the Hh pathway has been constitutively activated, should produce little or no X if they also lack

Fig. 3. omb in the abdomen. (A) The expression of omb.Gal4, monitored by UAS-lacZ expression. At the front of the β-gal stripe, the boundary is graded, with staining fading out at about one third of the A compartment. Behind, the stripe ceases about half way into the P compartment. (B) A clone of omb- cells, marked with singed (yellow arrowheads) which affects the bristles: bristles often become separated from the body of the clone and hence they provide only a poor indication of the extent of the clone. The preparation is stained for ptc.lacZ which is upregulated by Hh (Struhl et al., 1997b) both inside and outside the clone. Note the omb- territory forms unpigmented (a6) cuticle at the back of the A compartment and lightly pigmented (a3) cuticle more anteriorly, in place of the normal dusky (a4) cuticle (Fig. 2). Polarity in the clone is reversed. (C) A clone of omb- cells, marked with β-gal. The clone is associated with a patch of reversed polarity which, here and there, extends both in front and behind the clone (visible in the hairs and indicated by the red arrows pointing upwards). The clone itself lacks the dark a4 pigment which is visible anterior and lateral to the clone. Inset shows detail of hair reversals in front of the clone. (D) A clone of omb- cells, marked with β-gal. This clone is near the back of the A compartment and contains largely reversed hairs; note the autonomy of the effects of omb- on pigment, and the non-autonomy of its effects on polarity. The white arrowhead indicates a patch of dusky (a4) pigment that is just anterior to the clone. Compare Fig. 4B. (E) A clone overexpressing omb, marked with β-gal. We see the hairs pointing into the centre of the clone giving reversed polarity behind it. In the middle and at the back of the A compartments, clones of this genotype give abnormal cuticle, with reduced pigmentation (not shown). Compare Fig. 4D.
omb. To test this we made clones that were both ptc− and omb−; these clones form a6 cuticle as ptc− clones do. However, in the middle of the A compartment and unlike ptc− clones in that position (Lawrence et al., 1999a) they fail to repolarise behind, but reverse polarity in front (Fig. 5A) – as omb− cells do (Fig. 2, Fig. 4B). Similarly, omb− ptc− clones situated at the back of the A compartment behave like omb− clones, the whole being reversed in polarity (and not like ptc− clones in the same location, which have normal polarity). Thus in terms of the type of the cuticle (the scalar), omb− ptc− behave as ptc− clones, but in terms of the vector they behave as omb− clones. These results confirm that Hh induces X production through the action of omb.
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omb-expressing clones

The model for X suggests that, if omb were ectopically activated in cells at the front of the A compartment, those cells could become a source of X. Indeed omb-expressing clones can repolarise the cells behind them (Fig. 3E) – as if there were a local peak in the X distribution (Fig. 4D).

(ii) smoothened

smoothened (smo), is an essential component of Hh transduction; without it the cells cannot see Hh protein (Alcedo et al., 1996; Chen and Struhl, 1996; van den Heuvel and Ingham, 1996). As regards polarity one would expect neither omb- nor smo- clones to produce X and for their phenotype to be the same. Although this is generally the case (Struhl et al., 1997a), the effects of smo- and omb- differ for clones located at the back of the A compartment. Polarity within these omb- clones is completely reversed, consistent with the model (Fig. 4C), whereas the corresponding smo- clones are reversed only within the anterior portion of the clone, polarity returning to normal at the very back of the A compartment [see fig. 7 in Struhl et al. (Struhl et al., 1997a)]. Our preferred explanation for this discrepancy is that Smo protein perdures in smo- clones, allowing partial rescue of the smo mutant phenotype, particularly at the back of the A compartment, where Hh is most abundant. This rescue could allow production of X, enough to restore normal polarity at the back of the clone, but not enough to specify a4 cuticle or to upregulate ptc.lacZ. For both smo- and omb- clones, some Hh would be expected to move forwards across the clone and induce an ectopic peak of X production in more anterior, wild-type cells, accounting for the polarity reversals that are observed in both cases

† We earlier noted polarity reversals associated with smo- clones located at the front of the A compartment, and concluded tentatively that Hh might also induce X at the front, as well as the back of the A compartment (Struhl et al., 1997b). We have looked at many more clones since then and found that most such clones have normal polarity, even though they form a2 rather than a1 cuticle as a consequence of their failure to transduce Hh. We conclude that, in the anterior region of A, Hh does not trigger X production.

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unlike smo- clones, the polarity at the back of these clones does
not return to normal. Instead, in the majority of cases, polarity
remains reversed all the way to the back edge of the clone, and
sometimes beyond, as observed for omb- clones in the same
position (Fig. 5B). These results support the perdurance
explanation for the smo- clones and are consistent with the
working model, which is based mainly on the results with omb.

What is X?
We have conjectured that X is diffusible and produced in a
graded fashion, peaking at the back of A and declining
progressively towards the front of A. We first round up the
usual suspects: these are signals transduced by the Notch, EGF,
FGF, Dpp and Wnt pathways. Briefly, we have discarded all of
these except for the Wnt pathway, because we find that
removing or overexpressing key elements of each pathway in
clones fail to perturb polarity, either inside the clone or nearby.

(1) We removed the Notch gene itself, and although clones
of this genotype fail to contribute to bristle forming
(‘proneural’) portions of the adult epidermis (the a3, a4 and a5
regions), they survive elsewhere (such as p3, a6 and a2) where
they show normal polarity.

(2) We removed the EGF receptor from clones; such clones
do alter the distribution of bristles, but the polarity is normal
(cf. Diaz-Benjumea and Garcia-Bellido, 1990). We also made
clones that remove spitz, vein, rhomboid, args and sprouty as
well as clones that overexpress Argos and activated Egfr, Raf,
Cdc42 and Rac. None of these clones showed any consistent
alterations of normal polarity.

(3) We expressed activated forms of the Breathless and
Heartless receptor for FGF in clones, without any effect on
polarity.

(4) We removed thickveins (tkv), the receptor for Dpp, or
overexpressed activated Tkv. These clones had no effects on
polarity in the tergites.

There is already circumstantial evidence suggesting that X
might be a Wnt (Adler et al., 1997; Struhl et al., 1997a; Shulman
et al., 1998). In particular, Wg can be transduced by either of
two Wnt receptors (Bhanot et al., 1996; Bhanot et al., 1999;
Chen and Struhl, 1999), Frizzled and Frizzled2 (Fz, Fz2), and
Fz is somehow involved in polarity (Gubb and Garcia-Bellido,
1982; Vinson and Adler, 1987). Also, Wg is expressed as a
gradient as we imagine for X. However, we had shown earlier
(Struhl et al., 1997a) that Pka+ clones that are also mutant for
wg can repolarize neighbouring cells, indicating that they can
still serve as ectopic sources of X. Consequently, a simple
model in which Wg is X is not tenable. Nevertheless, more
complicated scenarios remain. For example, X might be another
Wnt, or perhaps, several Wnts might act redundantly as X. We
have subjected this hypothesis to several tests.

(1) Removing Wnt genes
Wg, Wnt4, 6, and 10 are all elided by the deficiency, Df(2L)RF
(FlyBase, 1999; Janson et al., 2001). We therefore made
marked clones that are homozygous for Df(2L)RF and Pka+.
These clones appear to reverse the polarity of wild-type cells
as well as Pka+ controls do (Fig. 6A,B), implying that they are
still sources of X, in spite of lacking all four Wnt genes. Of the
remaining Wnt genes, Wnt2, 5 and 8, we have been able to
examine only the effects of removing Wnt2, as mutations that
reduce or abolish Wnt5 and 8 activity are not available. Flies

with null mutations for Wnt2 (we studied Wnt2EMSO, Wnt299
and Df(2R)11) are viable and have normal abdomens.

(2) Overexpressing Wnt genes
If we were to produce sufficient X in clones, or in defined
subdomains of the segment, the resulting ectopic peaks of X
might cause repolarizations, particularly of cells located
progressively towards the peak. We performed such experiments for Wg
and the other Wnts defined by both genetics and the Genome
Project (Adams et al., 2000). We start with Wnts 5, 6, 8 and
5: when they are expressed in clones with a strong Gal4
driver, or under the control of a ptc.Gal4 driver, which should
create an ectopic peak of Wnt expression at the front of the A
compartment, none of these Wnts cause any changes of polarity or had other effects anywhere in the abdomen (we
examined the A and P compartments of both dorsal and ventral
cuticle). Similar results were obtained when either Wg or a
membrane-tethered form of Wg, Nrt::Wg, were expressed in
clones, except that such clones caused a transformation of
ventral pleura to tergite (Shirras and Couso, 1996; Kopp et al.,
1999). Animals expressing either form of Wg under ptc.Gal4
control do not survive to adults.

Wnt4 also failed to cause any consistent changes of polarity
in the abdomen when expressed either in clones or under
ptc.Gal4 control. However, we did find that expressing Wnt4
at high temperature under ptc.Gal4 control (Gieseler et al.,
2001) occasionally altered wing patterning. Further we
observed effects on polarity when Wnt4 was driven in the P
compartment of the wing with an en.Gal4 driver: within the A
compartment the hairs posterior to vein II tended to turn
clockwise to point posteriorly, as if they were aiming towards
an ectopic source of the Wnt4 protein emanating from P behind
them. However in what ought to be a better test, marked clones
expressing Wnt4, driven by tub.Gal4, failed to affect wing
polarity. Note that the results with ectopic Wnt4 expression in
the wing are equivocal and run counter both to the results of
the same experiments in the abdomen, and to experiments in
which the gene is eliminated from Pka+ clones (see above).
Hence, we tentatively discard Wnt4 as a candidate.

Flies carrying clones expressing Wnt2 did not emerge from
the puparium and ptc.Gal4 UAS.Wnt2 flies were lethal.
However, we examined the abdomens of pharate adults
carrying numerous clones expressing Wnt2 and they had
undisturbed polarity.

All of these tests argue that neither wg nor any of the other
Wnts is X. However, they do not eliminate the possibilities that
some combination of Wnts might function together to
constitute X, or that X might be a broadly expressed Wnt that
is converted from an inert to an active form after transcription.

(3) Activating the Wnt pathway
In apparent contrast to the above results, activating the Wnt
pathway, rather than the Wnts themselves, did produce effects
on polarity; however, these could also be attributed to
unintended effects on the Hh pathway. Clones of cells mutant
for the gene shaggy (sgg) constitutively activate the Wg
pathway (Wodarz and Nusse, 1998). In the tergites, sgg- clones
are abnormally round in shape and have higher than normal
bristle densities. In addition, they cause polarity reversals
similar to those associated with Pka+ clones: hairs and bristles
at the back of these clones are reversed (Fig. 6C). However, we
also observed that sgg− clones stain blue when the flies carry ptc.lacZ, indicating that the loss of Sgg leads to ectopic activation of the Hh pathway (Fig. 6C). Under our model this would suffice to cause ectopic production of X in the sgg− clones, which would reverse hairs behind, regardless of whether or not X is a Wnt.

Less easy to understand is the observation that sgg− clones can transform a1 cuticle into a3 cuticle (Fig. 6D) – this appears to be a change of cell identity from the anterior to the posterior subdomain of the A compartment (Lawrence et al., 1999a), perhaps implicating Wg in the definition or determination of these two subdomains.

(4) Blocking the Wnt pathway
We made clones that were mutant for arm or arrow: the Wg pathway in these two types of clones should be blocked (Peifer and Wieschaus, 1990; Wodarz and Nusse, 1998; Wehrli et al., 2000).

There were two effects. The first is that clones in the dorsal epidermis differentiated cuticle characteristic of the ventral epidermis: they made pleural hairs, and patches of sternite with bristles (Fig. 6F). Clones in all portions of the tergite, in both the A and P compartments, were so transformed, indicating a general requirement for Wnt signalling to specify dorsal as opposed to ventral structures. Thus, in the wild type, all dorsal cells are probably exposed to at least low levels of Wg or some other Wnt protein.

The second is that such clones affect polarity: in the tergites, the mutant clones were normal at the rear of the clone but reversed in the front, with reversal extending outside the clone (Fig. 6F). One explanation for these polarity changes could be that, in the tergites, Wg normally acts to enhance the production of X. Thus cells deficient in the Wnt pathway would produce less X than normal, giving a dip in the concentration landscape for X, causing reversed polarity at the front of the clone. In the eye, both arm− and arrow− clones cause equivalent polarity reversals and a similar resolution has been offered: it was suggested that Wg might regulate the production of a secondary polarising factor also dubbed X (Wehrli and Tomlinson, 1998).

Thus, we propose that Wg helps to produce X, but that Wg itself is not X. If Wg were X, both arm− and arrow− clones should not be able to transduce it, and hence, should have random polarity within the clone. Moreover, the effects on polarity should be cell autonomous. Yet, as we have seen, these clones behave as if they have caused an altered distribution of X, rather than any failure to transduce X. Similar arguments apply to sgg− clones. In this case, the Wg pathway should be constitutively activated in all cells within the clone, preventing them from detecting a gradient of Wg protein. However such clones are not randomly polarized, indicating that they can still respond to graded X activity.

It is useful to compare the roles of Omb and Wg on X production. Omb is apparently essential for X production: omb− clones at the back of A show reversed polarity that extends all the way to the posterior edge of the compartment (Fig. 3B,C). By contrast, in arm− and arrow− clones, reversal occurs only in the anterior portions of such clones. Thus, we infer that arm− and arrow− cells located at the back of A can produce some X, even though they cannot activate the canonical Wnt pathway. Thus, it could be that Hh drives X production mainly through Omb, but also adds to the level of X produced through the induction and action of Wg. The combination of both Omb and Wg activity might extend the reach of the X gradient to encompass the whole A compartment, and possibly also further forwards into the neighbouring P compartment.

**Downstream genes in the Hedgehog pathway – the P compartment**

None of our previous studies has helped us understand how the P compartment is patterned or how its cells are polarised. smo− clones have no phenotype in the P compartment, confirming that Hh has no function there. In the embryo and imaginal discs, Hh crossing over from the P compartment induces the expression of Wg and Dpp in line sources along the back of

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1In the pleura but not in the sternites, Hh induces Dpp rather than Wg (Struhl et al., 1997b; Kopp et al., 1999). We imagine that in the pleura, Dpp replaces Wg and, like Wg in the tergites, enhances the production of X.
A. Both proteins then spread back into the P compartment where they act as gradient morphogens to control P growth and pattern (reviewed by Lawrence and Struhl, 1996). Wg and Dpp are also produced at the back of the A compartment in each abdominal segment (albeit in distinct dorsal and ventral domains). Hence, by analogy with the embryo and imaginal discs, these morphogens seem to be the most likely candidates to pattern the P compartment here as well. If so, we would suppose that in the tergites, Hh induces Wg (Kopp et al., 1999; Struhl et al., 1997b) and this Wg moves posteriorly across the AP compartment boundary into the P compartment where it activates expression of omb, thus specifying the zone of hairy cuticle (p3) and distinguishing it from p2 cuticle, which is bald. We have tested this hypothesis in the following experiments.

(1) Omb
Kopp (Kopp and Duncan, 1997) found that loss-of-function omb mutants tend to lose the hairy, unpigmented cuticle characteristic of both posterior A (a6) and anterior P (p3) regions, whereas gain-of-function mutations tend to acquire it. Since we have observed that omb clones in the A compartment are able to make a6 cuticle, it seems likely that Omb is required specifically for the hairy, unpigmented cuticle (p3) that normally forms at the front of the P compartment. If so, one might expect omb clones at the front of the P compartment to transform the anterior type of cuticle (p3) into that found more posteriorly (p2). Although most omb clones were normal in this region, about one third of p3 clones lost some, but not all, of the hairs within the clone (n=94 of which 36 clones had noticeable reduction of hairs) (Fig. 7A). We wondered whether this might be an artefact due, for example to our method of detecting these clones which uses the tub.Gal4 driver to activate UAS.lacZ expression, but control clones (n=47) in sister flies always gave normal hair patterns. Thus it appears that omb may be required in the p3 territory, as it is in the a5 and a4 territories, to specify the type of cuticle secreted.

(2) The Wnt pathway
If Wg activates omb in anterior regions of the P compartment, blocking the Wnt pathway in cells in the P compartment should block expression of omb. We therefore monitored expression of omb in arrow− clones. This experiment proved difficult to do, but we found that omb was sometimes, but not always, turned off autonomously in the clone (Fig. 7B,C). Conversely, ectopic activation of the Wnt pathway should transform bald cuticle (p2) at the back of P into hairy cuticle (p3) normally found at the front of P. Indeed, some clones lacking the sgg gene become hairy if situated in the bald areas of P, apparently causing a transformation from p2 to p3 cuticle (Fig. 6E). But, clones expressing either tethered Wg or activated Arm, which should behave similarly, had no clear effects. Even so the positive results with arrow and sgg give support to the hypothesis that Wg stratifies the P compartment by working through Omb.

Fig. 8. Working model for patterning the chain of A and P compartments. The P compartments are shown in blue. The model applies to the dorsal epidermis of the abdomen, where Hh induces Wg, but can be generalized to the ventral pleura where Wg is replaced by Dpp, both proteins probably performing the equivalent function. In the first step (at top) Hh is produced in the P compartment and spreads into adjacent A cells, generating a U-shaped gradient. In the A compartment, the concentration of Hh at any point provides a scalar which dictates the type of cuticle formed (a1 to a6). Cells in the anterior and posterior regions of the A compartment respond differently to Hh (Struhl et al., 1997b). In the posterior region, peak levels of Hh induce engrailed, wg and omb expression and specify a6, intermediate levels induce only wg and omb and specify a5 and a4, and low levels or no Hh specify a3. In the anterior region, Hh does not induce engrailed, wg and omb, but high levels induce a1, with a2 being specified by low levels or no Hh. In the P compartment, the scalar is provided by Wg, which is produced by cells at the rear of the A compartment and moves across the AP compartment boundary into the P compartment. Peak levels of Wg induce Omb and thereby specify p3; lower levels or no Wg specify p2 and p1. Planar polarity is controlled by a polarizing morphogen ‘X’, produced largely in posterior A cells by Hh acting through Omb. Wg/Wnt helps X production, apparently to ensure that peak levels are generated in response to Hh. In the model shown X then spreads forward, forming a concentration gradient that extends through the entire compartment and possibly into the P compartment in front. The maximal slope of X at any given position provides a vector which specifies planar polarity. Note the model appears to demand that X cannot spread backwards into the P compartment behind the source. See conclusions for an alternative model.
We looked at the pattern of fz2.lacZ, because fz2 is thought to be repressed in cells receiving the Wnt signal (Cadigan et al., 1998). Expression is weak all over the A compartment, with a slight tendency to be stronger at the front of A. However, expression is strong at the rear of the P compartment and is graded downwards and anteriorly. Expression is not detected at the front of the P compartment. This pattern is consistent with a gradient of Wnt activity that is high at the front and low at the back of the P compartment.

We are still left with the question: what polarises cells of the P compartment? There are several possibilities. A simple one (see Fig. 8) is that X could extend anteriorly from the A compartment into the adjacent P compartment, forming a monotonic gradient that governs polarity throughout the entire parasegment.

CONCLUSIONS AND SPECULATIONS

We have used a particular model to interpret our results. The heart of this model (Fig. 8) requires that a cell’s polarity be determined by reading the local slope, the vector of a morphogen, X. Within the A compartment, it proposes that X be produced in a gradient with its peak at the back of the A compartment and its minimum at the front. Hh is the primary morphogen that patterns the A compartment, and, at the rear of this compartment, it acts through omu to produce X. X spreads further anteriorly, forming a monotonic gradient that extends from the back of the A compartment and could go as far as the front of the next P compartment, thus encompassing a parasegment. In this model there might need to be a barrier to the movement of X across the AP (parasegment) border in order to isolate the X gradients in neighbouring parasegments from each other. This model is speculative; for example we have no evidence for X spreading forward into the P compartment. In an alternative scenario, X might be made near the AP border, spreading forwards into A and backwards into P to form a reflected gradient. In that case, cells in the A and P compartments would have to make hairs which point in opposite directions relative to the vector of X, as all hairs point posteriorly.

Although we propose that X is a long range morphogen, our results do not exclude models in which polarity depends on short range interactions between cells. Recent models for planar polarity concentrate mostly on this aspect of how cells become polarized, particularly on how proteins within cells become asymmetrically localized (Usui et al., 1999; Axelrod, 2001; Bellaiche et al., 2001; Strutt, 2001; Winter et al., 2001), and how such molecular polarity might propagate from cell to cell by localised recruitment of other proteins at the abutting cell membranes (Usui et al., 1999; Strutt, 2001). These models can provide explanations for the local, non-autonomous perturbations of polarity which occur along the borders of mutant clones, but they do not readily explain the longer range effects of such clones nor how polarity is determined globally in the wild-type fly – this is what we are trying to do.

The model for X can be further elaborated, for example, polarity could depend on two cooperating morphogens, each operating in different directions. While X could emanate forwards from the back of the A compartment, another polarising gradient, ‘Y’ could be sourced from the front, or from the P compartment, and move backwards. Hairs would be subject to two separate and mutually supportive influences, pointing up the gradient of X and down the gradient of Y. More complex hypotheses of this sort have two main appeals: they might help explain how the polarity is determined across the AP border and they also might help us understand why removal of genes needed for polarity, such as fz or four-jointed still gives near-normal flies with much of their polarity unscathed (Shulman et al., 1998; Usui et al., 1999; Strutt, 2001).

Clearly, it is necessary to identify the polarising factors. We have attempted, albeit unsuccessfully, to identify X, and have evidence against most of the common signalling ligands such as those operating through the Notch, Dpp, EGF and FGF pathways, as well as all of the seven known Drosophila Wnts – none of these experiments are proofs, but they are the best we could do with the available techniques and mutations. Nevertheless, Wnt signalling does appear to be needed for the normal generation of X; hence, it may be that Wnts augment the production or activity of X induced by Hh.

Many other studies on planar polarity have used the wing. The main axes differ between the wing and the abdomen: in the wing, the hairs do not point towards the source of Hh and Dpp, but point distally. Thus, none of these two factors is likely to be directly responsible for inducing the wing equivalent of X. Similarly, omu expression in the wing is controlled directly by Dpp signalling, suggesting that it, too, is unlikely to be involved in inducing factor X. Nevertheless downstream components such as Fz are needed in the wing, eye and abdomen (Vinson and Adler, 1987; Zheng et al., 1995) (P. A. L., J. C. and G. S., unpublished) indicating that the process of polarizing cells in response to X, and possibly X itself, may be the same in all systems.

Although our focus has been on how Hh organizes both pattern (scalar) and polarity (vector) throughout the abdominal epidermis, growth must also be tightly regulated. If the postulated X gradient spans the parasegment, as diagrammed in Fig. 8, then perhaps X might also be a candidate for the factor controlling size. As argued elsewhere, proliferation and cell death might depend on a cell’s perception of the slope of the gradient responsible for polarity. In the abdomen, it is X and not Hh or Wg that conveys vectorial information, raising the possibility that the X gradient also carries information about dimension (Day and Lawrence, 2000).

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