Development 129, 2749-2760 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
DEV5002

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

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Accepted 11 March 2002

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^{-}: *hh*^{AC}, 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***: *hh*^{P30}. ***Pka***$^{-}$: *Pka-C1*^{E95}, an amorphic allele of the *cAMP-dependent protein kinase 1* gene. ***ptc***$^{-}$: *ptc*^{16}, an amorphic *patched* allele caused by a premature stop codon before the first transmembrane domain. ***ptc***^{S2}: a hypomorphic allele. ***ptc***^{18}: an amorphic allele. ***ptc.Gal4***: the insertion P{w+mW.hs=GawB}559.1, that expresses Gal4 in the *ptc* pattern. ***ptc.lacZ***: *EcoI\lacZ*$^{ptc-AT96}$. ***tub.ptc***: *ptc*$^{\alpha Tub84B.PCa}$. ***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^{-}: *bi*$^{omb-3198}$, 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^{2} according

to Kopp et al. (Kopp et al., 1997)]. ***UAS.omb***: FLP-out of $bi^{Scer\backslash FRT.Rnor\backslash CD2.UAS}$.

Wnt pathway

arm^{-}: arm^{2}, a strong *armadillo* allele resulting from a premature stop codon. ***UAS.arm****: $arm^{Delta.Scer\backslash UAS.T:Ivir\backslash HA1}$, a constitutively activated form of *armadillo*. ***arr***$^{-}$: arr^{2}, an amorphic *arrow* allele. ***Df(2L)RF***: a deletion of the chromosomal region containing the genes *Wnt4, Wnt6, Wnt10* and *wg* (Janson et al., 2001). ***sgg***$^{-}$: sgg^{32}, an amorphic *shaggy* allele. ***UAS.Wnt***: The open reading frames of *Wnt2*, *Wnt4* and *Wnt5* as well as the putative *Wnt6, 8* and *10* genes (FlyBase/BDGP annotated *Drosophila* genome sequence) (Adams et al., 2000) were amplified and cloned into the pUAST vector (Brand and Perrimon, 1993) using standard techniques, (see Llimargas and Lawrence, 2001). Two independent insertions for each Wnt gene were tested. In some experiments a different *Wnt4* construct, $Wnt4^{Scer\backslash UAS.cGa}$, was also used. Apart from *Wnt10* all the ***UAS.Wnts*** were shown to be functional in different tests (Llimargas and Lawrence, 2001). ***UAS.Nrt::wg***: $Nrt::wg^{Scer\backslash UAS.T:Ivir\backslash HA1}$, the N terminus of *wg* is fused to the C terminus of the type II transmembrane protein Nrt. ***fz2.lacZ***: the insertion P{w+mC=lacW}SB227 (P0013) which expresses β-Gal in a *fz2* pattern. (A. Sato and K. Saigo, personal communication).

EGFR, FGFR pathways

argos^{-}: $argos^{\Delta 7}$, a small deletion removing the 5′ exon and the beginning of the major open reading frame. ***Egfr***$^{-}$: $Egfr^{f2}$, an amorphic allele of *Epidermal growth factor receptor*. ***UAS.Egfr****: $Egfr::tor^{act.Scer\backslash UAS}$, an activated form of Egfr. ***rho***$^{-}$: $rho^{P\Delta 5}$, a small deletion of the *rhomboid* gene. ***spi***$^{-}$: spi^{1}, a strong *spitz* allele. ***sty***$^{-}$: sty^{S73} strong allele of *sprouty*. ***vn***$^{-}$: $vn^{DeltaP25}$, an amorphic allele of *vein* originated from a P element imprecise excision. ***UAS.argos***: $argos^{Scer\backslash UAS.cHa}$. ***tub>f^{+}y^{+}>raf****: $Hsap\backslash RAF1^{\Delta 305.aTub84B.T:Myr1}$. ***UAS.CDC42^{V12}***: $Cdc42^{V12.Scer\backslash UAS}$. ***UAS.CDC42^{N17}***: $Cdc42^{N17.Scer\backslash UAS}$. ***UAS.Rac1^{V12}***: $Rac1^{V12.Scer\backslash UAS}$. ***UAS.Rac1^{N17}***: $Rac1^{N17.Scer\backslash UAS}$ ***UAS.λ-btl***: $btl^{Scer\backslash UAS.T:I\backslash cI–DD}$. ***UAS.λ-htl***: $htl^{Scer\backslash UAS.T:I\backslash cI–DD}$.

Dpp pathway

tkv^{-}: tkv^{8}. ***UAS.tkv****: $tkv^{Q253D.Scer\backslash UAS.cNb}$.

Notch pathway

N^{-}: N^{XK11}, an amorphic allele of *Notch.*

Duplications and other transgenes

Dp-y^{+}: $Dp(1;2)sc^{19}$. ***Dp-sgg***$^{+}$: *Dp(1;2)w+70h*. ***Dp-pwn***$^{+}$: *Dp(2;3)P32*. ***Dp-N***$^{+}$: *Dp(1;2)51b*. ***tub.Gal4***: $Scer\backslash GAL4^{\alpha Tub84B.PL}$, the *S. cerevisiae* Gal4 gene is expressed under the control of the *αTub84B* promoter. ***abx/ubx>f^{+}>Gal4-lacZ***: $Scer\backslash GAL4^{Scer\backslash FRT.Ubx}$. ***tub>Gal80-y^{+}>Gal4***: Similar to $Scer\backslash GAL4^{Scer\backslash FRT.Rnor\backslash CD2.\alpha Tub84B}$ with the CD2 sequence replaced with a Gal80-y^{+} cassette (Lee and Luo, 1999). ***tub.Gal80***: $Scer\backslash GAL80^{\alpha Tub84B.PL}$, the Gal4 gene is expressed under the control of the *αTub84B* promoter. ***lacZy***$^{+}$: $Ecol\backslash lacZ^{Scer\backslash FRT.NLS.\alpha Tub84B}$. ***CD2y***$^{+}$: $Rnor\backslash CD2^{hs.PJ}$. ***PSy^{2}***: $Psn^{\alpha Tub84B.PS}$. ***UAS.lacZ^{J312}***: $Ecol\backslash lacZ^{Scer\backslash UAS.T:SV40\backslash nls2}$.

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.

ptc^{S2} hh^{-}*: y w hs.FLP; FRT42D pwn ptc*$^{-}$*/FRT42D pwn ptc*^{S2}*; FRT82B Dp-pwn*$^{+}$ *tub.ptc/FRT82B hh*$^{-}$.

omb^{-}*:* (i) *y w omb*$^{-}$ *sn FRT19A/FRT19A; hs.FLP/+; hh.lacZ/+*. (ii) *y w omb*$^{-}$ *sn FRT19A/FRT19A; hs.FLP/ptc.lacZ.* (iii) *y w omb*$^{-}$ *sn FRT19A/w hs.FLP tub.Gal80 FRT19A; tub.Gal4/UAS.nlslacZ.*

omb^{-} ***ptc***$^{-}$*: y omb*$^{-}$ *sn FRT19A/y w (tub.ptc)x2 PSy*^{2} *FRT19A; ptc*$^{-}$*/ptc*^{18} *hs.FLP.*

arr^{-}*: w omb.Gal4/y w hs.FLP; FRT42D pwn arr*$^{-}$*/FRT42D CD2y*$^{+}$*; UAS.lacZ*^{J312}*/+*.

sgg^{-} (or ***arm***$^{-}$)*:* (i) *y sgg*$^{-}$ (or *arm*$^{-}$) *FRT101/w hs.FLP FRT101; ptc.lacZ/+*. (ii) *y sgg*$^{-}$ *hs.FLP; Dp-y*$^{+}$ *Dp-sgg*$^{+}$ *FRT39/stc FRT39.*

Pka^{-}*: hs.FLP; Pka*$^{-}$ *FRT39/CD2y*$^{+}$ *FRT39.*

Pka^{-} ***Df(2L)RF****: hs.FLP; Df(2L)RF Pka*$^{-}$ *FRT39/CD2y*$^{+}$ *FRT39.*

ptc$^{\Delta loop2}$*: y w UAS.ptcΔloop2/y w hs.FLP; FRT42D pwn ptc*$^{-}$*/FRT42D Tub.Gal80 CD2y*$^{+}$*; tub.Gal4/+*.

N^{-}*: y N*$^{-}$ *hs.FLP; FRT42D Dp-N*$^{+}$*/FRT42D pwn.*

Egfr^{-}*: y w hs.FLP; FRT42D pwn Egfr*$^{-}$*/FRT42D CD2y*$^{+}$.

argos^{-}*: y w hs.FLP; argos*$^{-}$ *FRT80B/lacZy*$^{+}$ *FRT80B.*

rho^{-}*: y w hs.FLP; rho*$^{-}$ *FRT80B/lacZy*$^{+}$ *FRT80B.*

spi^{-}*: y w hs.FLP; stc spi*$^{-}$ *FRT40A/FRT40A.*

sty^{-}*: y w hs.FLP; sty*$^{-}$ *FRT80B/lacZy*$^{+}$ *FRT80B.*

vn^{-}*: y hs.FLP; vn*$^{-}$ *FRT80B/lacZy*$^{+}$ *FRT80B.*

tkv^{-}*: y w hs.FLP; tkv*$^{-}$ *stc FRT39/CD2y*$^{+}$ *FRT39.*

UAS.argos, UAS.omb, UAS.Egfr* and UAS.tkv*: *y w hs.FLP f^{36a}; abx/ubx>f^{+}>Gal4-lacZ/UAS.argos. y w hs.FLP f^{36a}; abx/ubx>f^{+}>Gal4-lacZ/+; UAS.omb/+. y w hs.FLP f[36a]; abx/ubx>f[+]>Gal4-lacZ/UAS.Egfr[*]. y w hs.FLP f^{36a}; abx/ubx>f^{+}>Gal4-lacZ/+; UAS.tkv*/+*.

tub.raf*: *y w hs.FLP; tub>f^{+}y^{+}>raf*/+*.

UAS.Wnt4*: *y w hs.FLP tub.Gal4; (UAS.Wnt4)x2/+; CD2y*$^{+}$ *trc*$^{-}$ *ri FRT2A/tub.Gal80 FRT2A* (flies reared at 29°C after clone induction).

Clones with other UAS constructs: *y w hs.FLP; tub>Gal80-y*$^{+}$*>Gal4/*Φ (where Φ represents a particular UAS construct).

Dissection and mounting of abdominal cuticles as well as detection of β-Gal4 activity were carried out according to the method of Lawrence et al. (Lawrence et al., 1999a). Images were captured using Auto-Montage (Syncroscopy, Cambridge, UK).

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) (Struhl et al., 1997a; Lawrence et al., 1999a). Although we argued that these effects were not due to Hh itself (Struhl et al., 1997a), we did not eliminate the possibility that low levels of ectopic Hh might be produced by the clone and diffuse out, being sufficient to repolarise the cells without changing the scalar. We have now disproved this by making clones that lack both effective Ptc protein and the *hh* gene. These clones still caused repolarisation in the back half of the clone and behind it (Fig. 1) arguing strongly that the Hh protein cannot be a component of 'X' and raising again the question, what is X? X should be engendered downstream of Hh receipt, which is where we start our search.

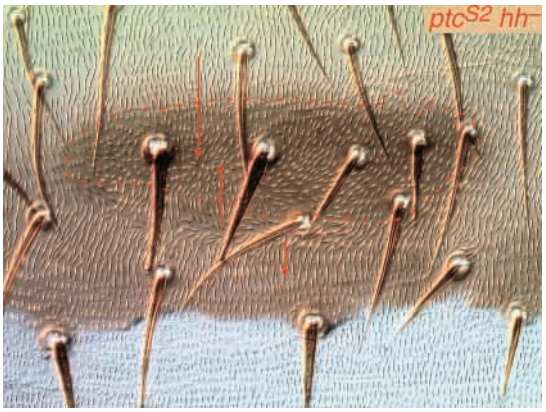

Fig. 1. Clones that have partial loss of function for *ptc* and also lack *hh*. This clone is marked with *pawn* (the mutant hairs are small and thin and the bristles are depauperate; a dotted line outlines the clone) and shows reversed polarity within the clone and behind it, and therefore eliminates the hypothesis that ectopic Hh is produced by the clone to drive the reversal. The clone makes dark a5 pigment. In this and subsequent figures anterior is upwards. Red arrows indicate the polarity.

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

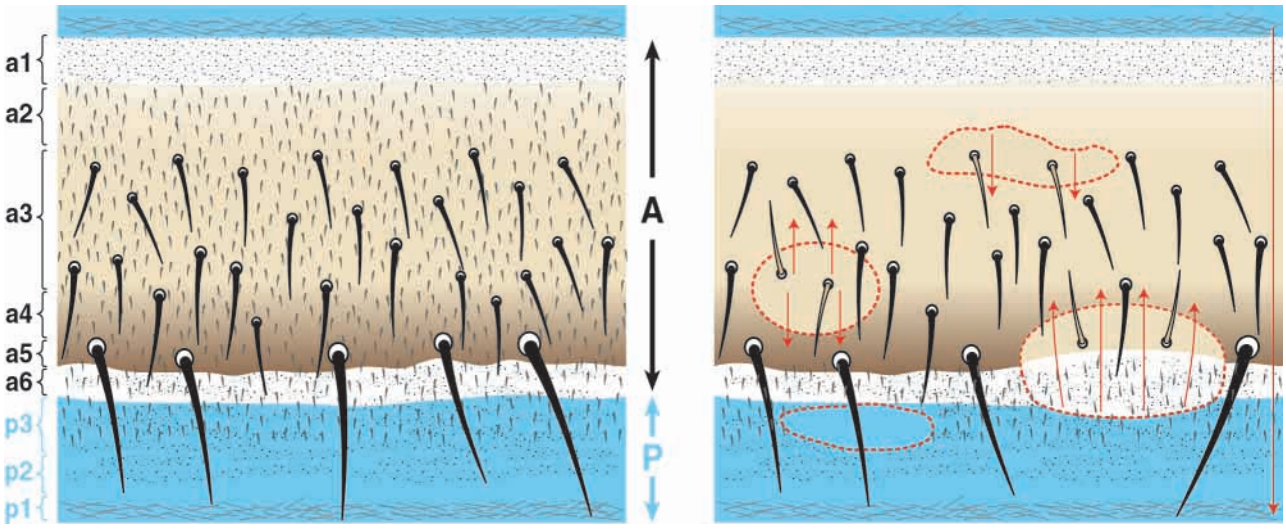

Fig. 2. An abdominal segment and effects of *omb*– clones. Left-hand panel shows a normal segment with nomenclature for the types of cuticle (Struhl et al., 1997b). Right-hand panel is a memorandum for both vectorial and scalar effects of *omb*– clones (surrounded by red dashed lines) in different positions in both the A and P compartments. The scalar is shown by the colour of the cuticle and the red arrows show the observed polarity of the hairs (which normally point posteriorly), near and within the clones. We imagine the polarity to be a consequence of the concentration landscape for X. For clarity, the hairs have been removed from part of the A compartment on the right. Compare Fig. 3 and Fig. 7.

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

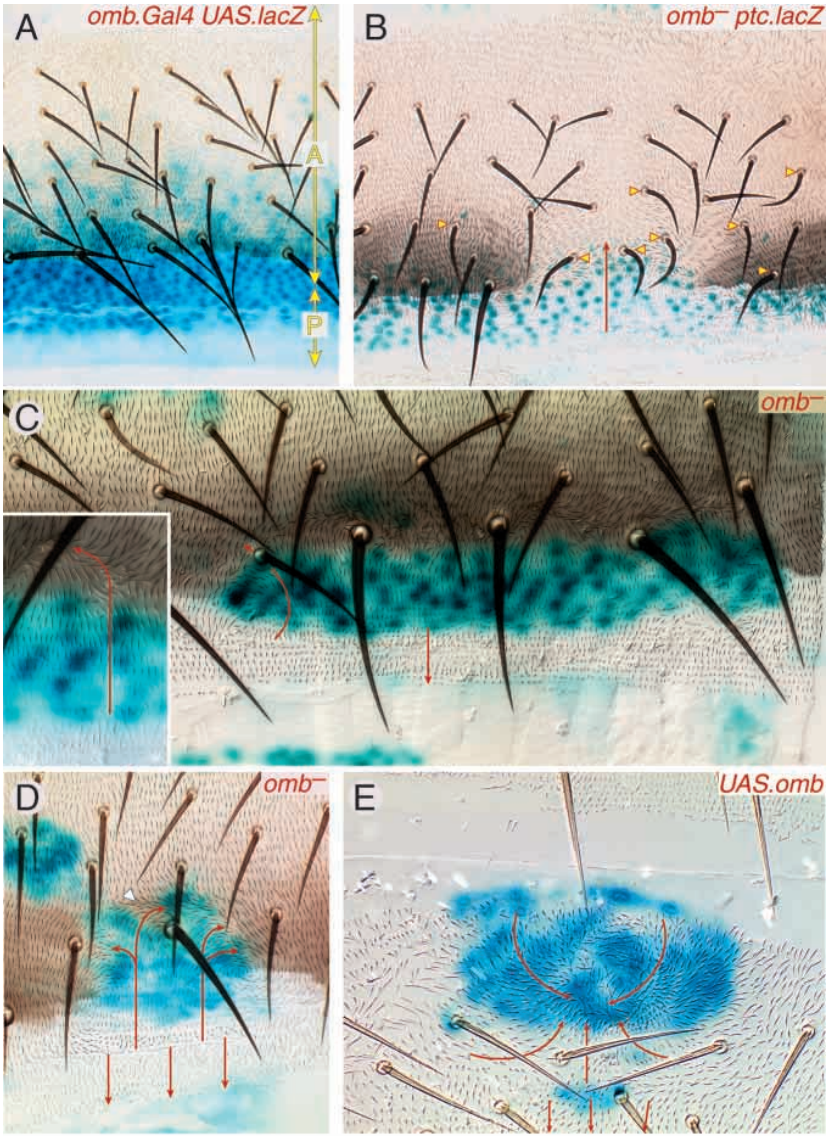

Fig. 3. *omb* in the abdomen. (A) The expression of *omb.Gal4*, monitored by UAS-lacZ expression. At the front of the β-gal stripe, the boundary is graded, with staining fading out at about one third of the A compartment. Behind, the stripe ceases about half way into the P compartment. (B) A clone of *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.

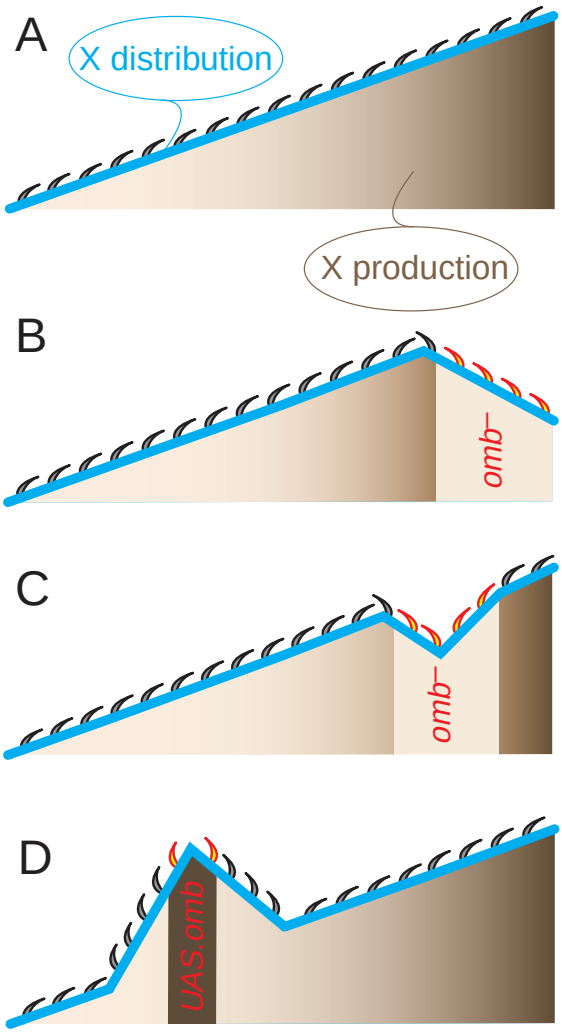

Fig. 4. Loss and overexpression of *omb*. The diagrams summarise the model. The blue line traces the actual concentration profile of X, with the brown shading indicating the strength of X production. The slope of X concentration is the vector which defines planar polarity at each point. (A) In the wild type the highest concentration of X and the highest rate of production of X coincide in cells at the back of the A compartment. (B) A clone of *omb*⁻ cells at the back of the A compartment produces little or no X, and so X spreads into it from the front, forming a reversed gradient and reversed polarity. (C) A clone of *omb*⁻ cells positioned more anteriorly than the clone in (B), X is produced both in front and behind the clone, creating two peaks and producing a reversed gradient that begins within the clone and extends anteriorly. (D) A clone over-expressing X will make a local peak of X, causing reversal within the back of the clone and behind it.

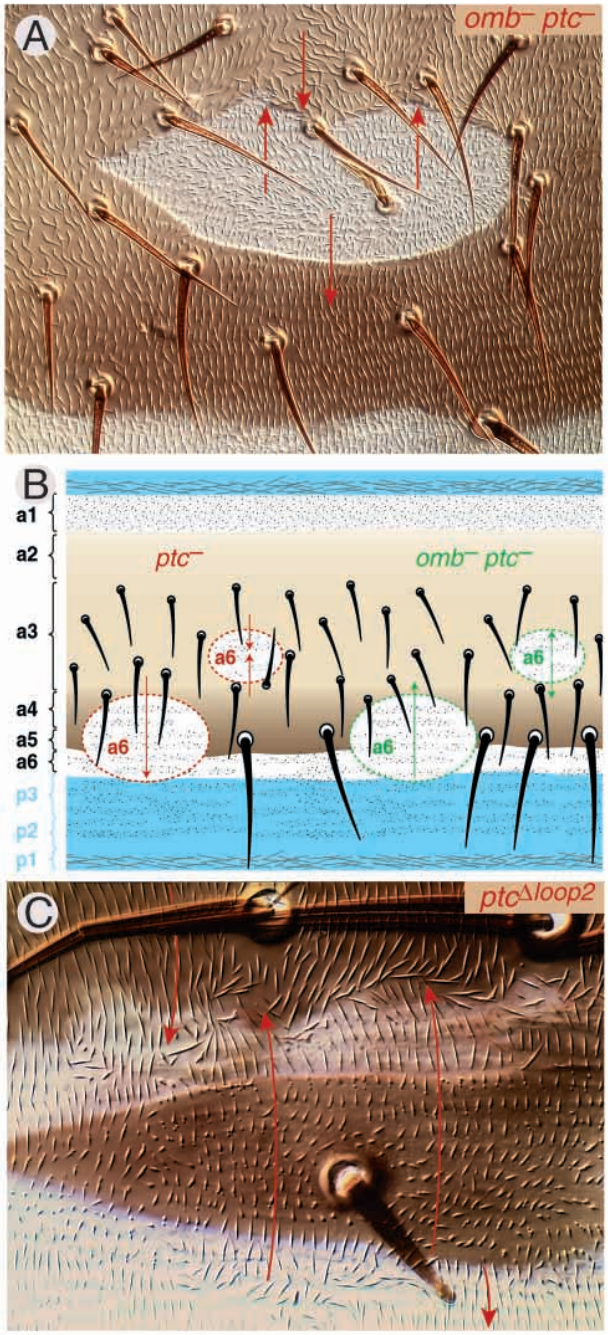

Fig. 5. The Hh pathway, *omb* and polarity. (A) A clone that lacks both *ptc* and *omb* marked with *singed*. The clone makes a6 cuticle like *ptc*⁻ clones, but reverses polarity in the front half of the clone as do *omb*⁻ clones in this position (see Fig. 2, Fig. 5B) and unlike *ptc*⁻ clones (Lawrence et al., 1999a). (B) A comparison between *ptc*⁻ and *omb*⁻ *ptc*⁻ clones, they both affect the scalar in the same way, making a6 cuticle; but they have very different effects on polarity. (C) A clone of cells, marked with *pawn* that overexpresses a form of Ptc that blocks Hh reception but not Hh movement. All the *pawn* cells with A provenance form pigmented (a3) cuticle. The polarity is largely reversed, even at the back of the clone and in some places, behind it. To the left there is a small clone of P provenance.

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*.

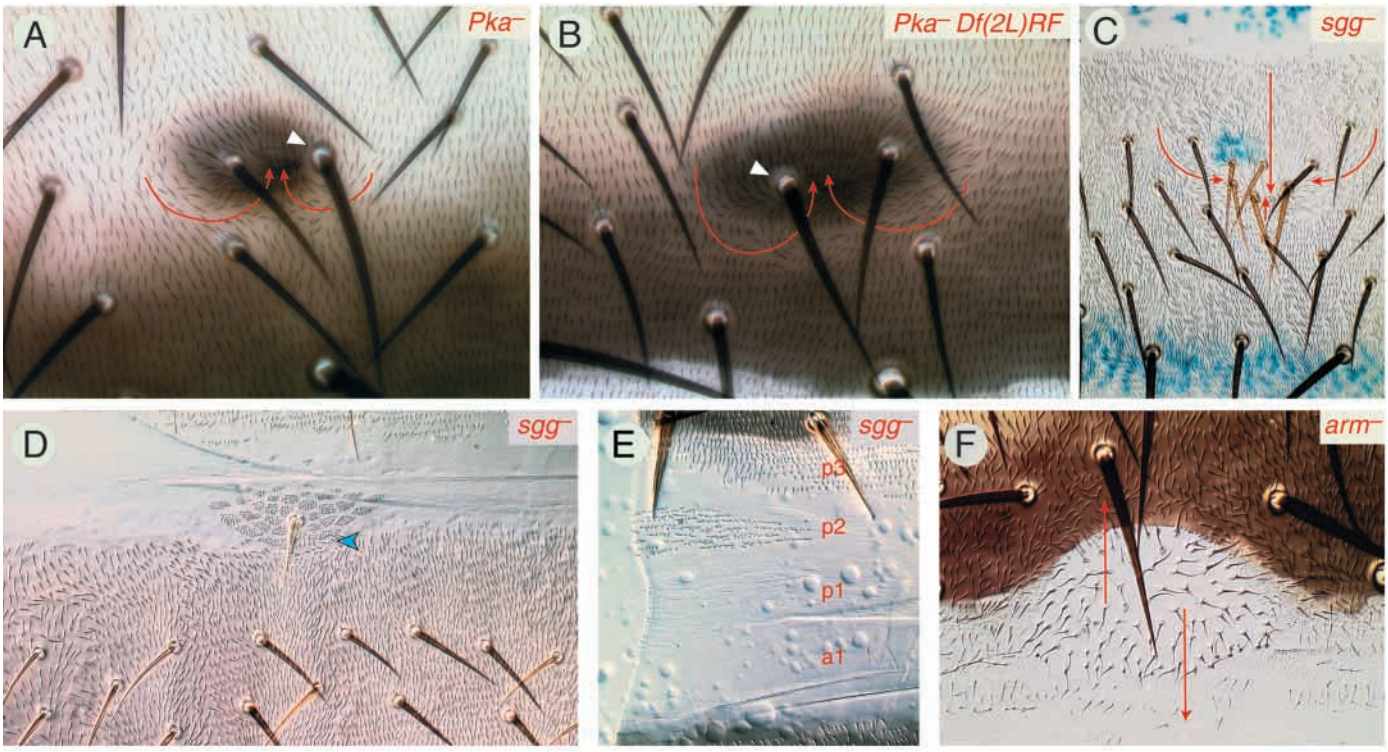

Fig. 6. The Wnt pathway and polarity. (A,B) Comparison of embryonic clones that are *Pka*⁻ as well as being otherwise wild type (A) or homozygous for *Df(2L)RF* (B). This deficiency removes *wg* as well as *Wnts* 4, 6 and 10. Both types of clones make a4 cuticle with some a5 bristles (white arrowheads) (Lawrence et al., 1999a). The polarity of hairs at the back of the clones as well as wild-type hairs behind are reversed to a similar extent in both clones (red arrows). *Pka*⁻ clones carrying the deficiency survive less often than *Pka*⁻ controls. (C) A clone of *sgg*⁻ cells, marked with *yellow* in the anterior region of the A compartment. Note the cluster of five *yellow* bristles and that the polarity is reversed behind them. The abdomen carries *ptc.lacZ* and, as in this case, these clones are usually associated with some sporadic up-regulation of *ptc*, suggesting that the Hh pathway is ectopically activated, inducing a source of X. (D) A clone of *sgg*⁻ cells marked with *yellow* and *stubby chaete* (*stc*), situated far anterior in the A compartment (*stc* causes tufts of hairs to form). The a1 cuticle in this region is apparently transformed to make a3 cuticle with hairs and bristles. Some hairs behind the clone have reversed polarity. (E) A clone of *sgg*⁻ cells marked with *stc* in the mid-region of the P compartment. The clone is transformed, making hairs characteristic of p3 cuticle. (F) A clone of cells that are mutant for *arm* in the tergites. The clone is transformed to form large pleural hairs and cuticle. It tends to sort out, forming a rounded shape (Lawrence et al., 1999b) and the polarity of the front of the clone, and of some wild-type cells anterior to the clone, is reversed.

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†.

To test this explanation we blocked Hh receipt by a different method that is not so subject to perdurance: we made a marked clone that contained no wild-type Ptc, but provided instead a mutant form of Ptc that is ineffective at transducing the Hh signal (Briscoe et al., 2001). Such clones behave like *smo*⁻ clones in most respects, including making a3 cuticle instead of a4, a5 or a6 cuticle in the back half of the A compartment, and causing polarity reversals both within and anterior to the clone. However,

†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.

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*, *argos* 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, *Wnt 4*, *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 $Wnt2^{EMSO}$, $Wnt2^{99}$ 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 posterior to 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 10: 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.

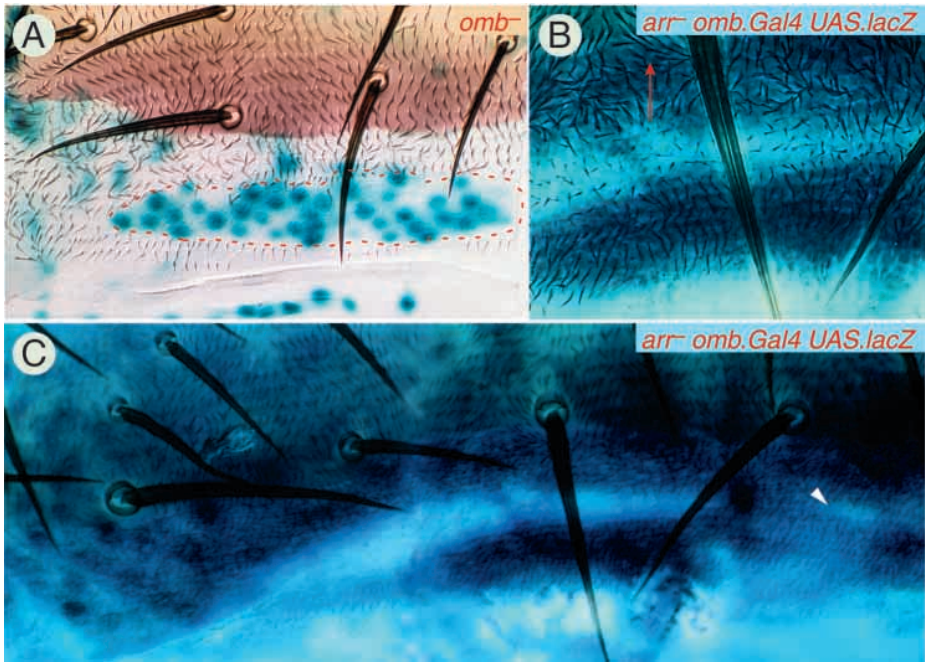

Fig. 7. *omb* in the posterior compartment. (A) A clone of *omb*⁻ cells in the P compartment; the nuclei are marked with *lacZ*. This clone removes nearly all hairs, apparently transforming the cuticle from p3 to p2 type. (B,C) A large *arrow*⁻ clone in the P compartment marked with *pawn*. The abdomen carries *omb.Gal4* and *UAS.lacZ*, and we see that, wherever the cells lack *arrow* as shown by the *pawn* marker, the β-gal staining is reduced (see detail in B). This is true of even a single *pawn* cell that is separated from the main clone (arrowhead in C). Note in B that some hairs anterior to the clone have reversed polarity (red arrow).

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

‡In 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.

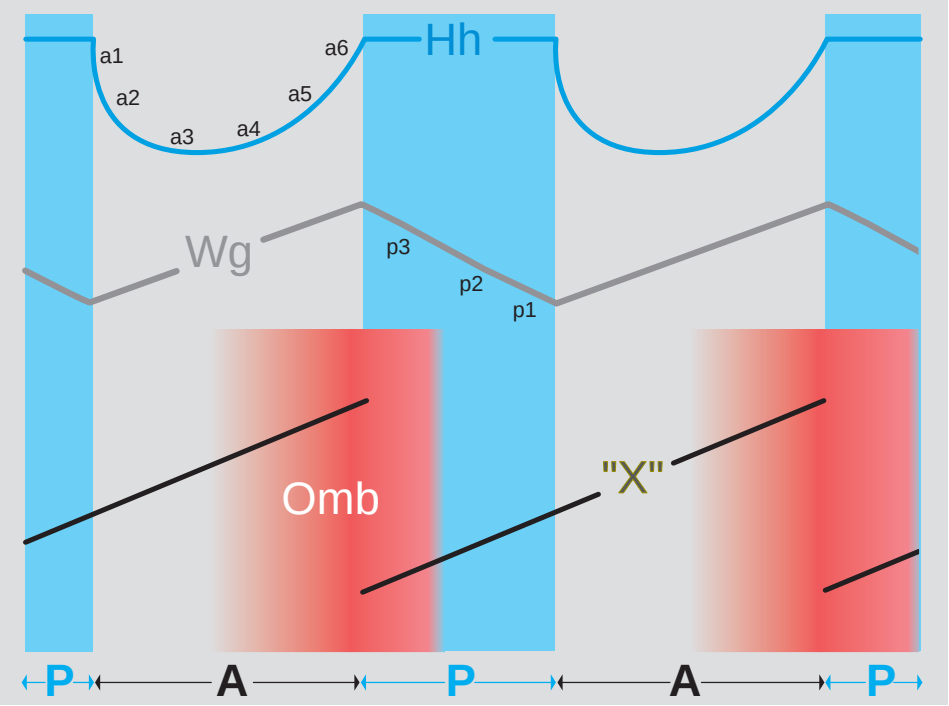

Fig. 8. Working model for patterning the chain of A and P compartments. The P compartments are shown in blue. The model applies to the dorsal epidermis of the abdomen, where Hh induces Wg, but can be generalized to the ventral pleura where Wg is replaced by Dpp, both proteins probably performing the 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.

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.

(3) fz2.lacZ

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 *omb* 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, *omb* expression in the wing is controlled directly by Dpp signaling, 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).

We thank T. Casci and J. Wasserman for making *sprouty*$^{-}$, *vein*$^{-}$ and *rhomboid*$^{-}$ clones, J. L. Mullor and J.-P. Vincent for discussion, A. Sato and A. Saigo for allowing us to use their fz2.lacZ reporter line, and M. Calleja, T. Casci, C.-M. Chen, M. Freeman, G. Morata, A. Sato, Y. H. Sun, J. Wasserman and the Bloomington Stock Center for flies. We thank Atsuko Adachi for making the UAS.Wnt transgenes. Gary Struhl is an Investigator of the Howard Hughes Medical Institute. José Casal and Peter Lawrence are supported by the MRC.

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