

Cell interactions and planar polarity in the abdominal epidermis of *Drosophila*

Peter A. Lawrence^{1,*}, José Casal¹ and Gary Struhl²

¹MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

²Howard Hughes Medical Institute, Columbia University College of Physicians and Surgeons, 701 West 168th Street, New York, NY 10032, USA

*Author for correspondence (e-mail: pal@mrc-lmb.cam.ac.uk)

Accepted 14 July 2004

Development 131, 4651–4664

Published by The Company of Biologists 2004

doi:10.1242/dev.01351

Summary

The integument of the *Drosophila* adult abdomen bears oriented hairs and bristles that indicate the planar polarity of the epidermal cells. We study four polarity genes, *frizzled* (*fz*), *prickle* (*pk*), *Van gogh/strabismus* (*Vang/stbm*) and *starry night/flamingo* (*stan/fmi*), and note what happens when these genes are either removed or overexpressed in clones of cells. The edges of the clones are interfaces between cells that carry different amounts of gene products, interfaces that can cause reversals of planar polarity in the clone and wild-type cells outside them. To explain, we present a model that builds on our earlier picture of a gradient of X, the vector of which specifies planar polarity and depends on two cadherin proteins, Dachshaus and Fat. We conjecture that the X gradient is read out, cell by cell, as a scalar value of Fz activity, and that Pk acts in this process, possibly to determine the sign of the Fz activity gradient.

We discuss evidence that cells can compare their scalar readout of the level of X with that of their neighbours and can set their own readout towards an average of those. This

averaging, when it occurs near the edges of clones, changes the scalar response of cells inside and outside the clones, leading to new vectors that change polarity. The results argue that Stan must be present in both cells being compared and acts as a conduit between them for the transfer of information. And also that Vang assists in the receipt of this information. The comparison between neighbours is crucial, because it gives the vector that orients hairs – these point towards the neighbour cell that has the lowest level of Fz activity.

Recently, it has been shown that, for a limited period shortly before hair outgrowth in the wing, the four proteins we study, as well as others, become asymmetrically localised in the cell membrane, and this process is thought to be instrumental in the acquisition of cell polarity. However, some results do not fit with this view – we suggest that these localisations may be more a consequence than a cause of planar polarity.

Key words: *frizzled*, *Van gogh*, *starry night*, *prickle*

Introduction

Polarity pervades the cell much as a magnetic field pervades space, without the help of iron filings that bring it to light

A. D. Hershey

Cells in an epithelium are polarised – both in the basal/apical axis and in the plane of the sheet. This latter or planar polarity (Nübler-Jung et al., 1987) is exemplified by the oriented outgrowth of hairs and the asymmetric action of cilia (Drubin, 2000). Planar polarity is not always so conspicuous – for example, the epithelial cells of the elongating germ band of *Drosophila* appear unpolarised, yet when they are made to express the *Slam* protein, they display planar polarity (Lecuit et al., 2002). We think of planar polarity, not as a special characteristic that occurs only when anisotropic structures are being made, but instead as an ever-present property of all or nearly all epithelial sheets, even though it is usually invisible.

How is planar polarity generated and organised? Studies of different systems [wing, eye, abdomen in *Drosophila*, and the stereocilia of the ear (Lewis and Davies, 2002) as well as convergent extension in vertebrates (Wallingford et al., 2002)]

find that many of the same genes or their homologues act in all systems, arguing that there are common elements; however there is no real understanding of the mechanism.

In the fly, polarity has been mainly studied in wing and eye, but we have chosen the abdomen. Unlike the wing (an appendage), the abdomen represents the atavistic body plan, a continuous epithelium that is subdivided into a succession of anterior (A) and posterior (P) compartments. Pattern is organized by signalling across A/P compartment boundaries and depends on the different abilities of A and P cells to send and interpret these signals (Struhl et al., 1997a; Struhl et al., 1997b; Lawrence et al., 2002).

In the anteroposterior axis, both the pattern and the gradient of cell affinity are determined by a morphogen, Hedgehog (Hh) (Struhl et al., 1997a; Struhl et al., 1997b; Lawrence et al., 1999b; Lawrence et al., 1999a). Hh, secreted by P cells, spreads as a gradient into the A compartment and specifies stripes of distinct A cell types. Hh also directs expression of a second morphogen, Wingless (Wg), which then spreads back into P, forming another gradient to specify pattern in the P compartment (Lawrence et al., 2002). Our working model is

that both these morphogens, via the activation of the transcription factor Optomotor blind (Omb), establish a different gradient, 'X' (Lawrence et al., 2002). The nature of the X gradient is unknown, it may depend on a diffusing morphogen or it may not, but in either case, the vector of X at each locale should determine the polarity of each cell (Lawrence, 1966; Stumpf, 1966; Zheng et al., 1995; Struhl et al., 1997b; Wehrli and Tomlinson, 1998). In the present model, the X gradient is reflected across compartment boundaries, giving opposing slopes in A and P (Casal et al., 2002). Evidence suggests that X is neither Hh, Dpp nor any of the seven Wnts in *Drosophila*, nor any of the fly EGF or FGF homologues, nor does it appear to depend in any way on Notch (Lawrence et al., 2002).

Polarity genes can tentatively be divided into two groups: those required for generating the X gradient, and those required for responding to it. In the first group, consisting of *four jointed* (*ff*), *dachsous* (*ds*) and *fat* (*ft*), polarity changes produced by clones of mutant cells in the A and P compartments are of *different* sign – that is like the opposing slopes of the presumed gradients of X in each compartment. For example, clones overexpressing *ff* in the A compartment reverse the polarity of cells in front of the clone, while, in the P they reverse cells behind. Building on results from the eye (Yang et al., 2002) we speculated that these three genes help form the gradient or gradients that constitute X (Casal et al., 2002).

Genes in the second group might be responsible for reading and responding to the X gradient, a process that would require cells to sense the vector of X, and to orient accordingly. Because most cells in both A and P make hairs (Struhl et al., 1997b), all of which point posteriorly, clones of cells mutant for these genes should affect polarity in the same way in both A and P. We selected four polarity genes that strongly affect polarity, *frizzled* (*fz*), *prickle* (*pk*), *Van gogh/strabismus* (*Vang/stbm*) and *starry night/flamingo* (*stan/fmi*), and show that all belong to the second group, in which mutant clones cause the *same* polarity effects in A and P. However, the properties of each gene are distinct, providing insights that allow us to build a model of planar polarity that is fundamentally different from previous ones (e.g. Adler et al., 1997; Tree et al., 2002; Ma et al., 2003). This model is based on the patterns of repolarisations seen following experiments. We conjecture that in normal development, cells align their polarity, using Stan, to detect a difference between the perceived levels of X of their neighbours (X being measured by a scalar readout related to the activity of Fz). We propose that the cells read X but also reset their own scalars towards an average of the scalar levels of their neighbours.

It has been shown in recent years that the proteins encoded by all four of these genes become asymmetrically localised in wing cells just before they make polarised hairs (reviewed by Mlodzik, 2002; Strutt, 2002). We present evidence that this localisation may not be as instrumental as is commonly assumed and may be more a consequence than a cause of planar polarity.

Materials and methods

Mutations, insertions and transgenes

The FlyBase (FlyBase, 1999) entries of the mutations, insertions and transgenes referred in the text are as follows.

Vang[−]: *Vang*^{stbm-6} is a 2 bp deletion, resulting in a frameshift.

UAS.*Vang*: *Vang*^{Scer\UAS.cWa}.

fz[−]: *fz*¹⁵ is a single nucleotide substitution; *fz*²¹ is an insertion into the first exon; *fz*³⁷ is an imprecise excision of a P element, resulting in the deletion of more than 50 kb flanking genomic sequences.

UAS.*fz*: *fz*^{Scer\UAS.cZa} and *fz*^{Scer\UAS.cSa}.

fz2[−]: *fz2*^{C1}.

stan[−]: *stan*³ and *stan*⁷¹ are hypomorphic alleles; *stan*^{E59} has a nonsense mutation in the ectodomain.

UAS.*stan*: *stan*^{Scer\UAS.cUa}.

pk[−]: *pk*^{pk-sple-13} is a small aberration affecting both *pk* and *sple* open reading frames; *pk*^{GO12} is a P-element insertion and *pk*^l is a spontaneous mutation.

UAS.*pk*: *pk*^{Scer\UAS.cGa}.

UAS.*sple*: *pk*^{sple.Scer\UAS}.

vg.*Gal4*: *Scer\GAL4^{vg.PM}*.

abx/ubx>f+>Gal4-lacZ: *Scer\Gal4^{Scer\FRT.Ubx}*.

1407.*Gal4*: *Scer\GAL4¹⁴⁰⁷*.

tub.*Gal4*: *Scer\GAL4^{alphaTub84B.PL}*.

act.*Gal4*: *Scer\GAL4^{Act5C.PP}*.

ptc.*Gal4*: *Scer\GAL4^{ptc-559.1}*.

ci.*Gal4*: a gift from Bob Holmgren.

en.*Gal4*: *Scer\GAL4^{en-e16E}*.

hh.*Gal4*: *Scer\GAL4^{hh-Gal4}*.

tub.*Gal80*: *Scer\GAL80^{alphaTub84B.PL}*.

ptc.*lacZ*: *EcoI\lacZ^{ptc-AT96}*.

hh.*lacZ*: *hh*^{P30}.

UAS.*FLP*: *Scer\FLP^{Scer\UAS.cCa}*.

hs.*FLP*: *Scer\FLP^{hs.PS}*.

UAS.*GFP*: *Avic\GFP^{Scer\UAS.T:Hsap\MYC.T:SV40\ntls2}*.

UAS.*lacZ*: *EcoI\lacZ^{Scer\UAS.T:SV40\ntls2}*.

CD2y+: *Rnor\CD2^{hs.PJ}*.

FRT18A: *P{neoFRT}18A*.

FRT42: *P{FRT(w^{hs})42D*.

FRT2A: *P{FRT(w^{hs})2A*.

FRT80: *P{neoFRT}80B*.

Experimental genotypes

(1) *tub*.*Gal4* UAS.*fz*: w/y w *hs*.*FLP*; *tub*.*Gal4*/+; UAS.*fz*/TM2.

(2) *fz*[−] clones: y w *hs*.*FLP*; *fz*¹⁵ *trc*^{C1} ri *FRT2A*/CD2y+ *hs*.*GFP* ri *FRT2A*.

(3) *abx/ubx>Gal4* UAS.*fz* clones: males w/y *hs*.*FLP*; UAS.*fz*/abx/ubx>f+>*Gal4-lacZ*.

(4) *tub*.*Gal4* UAS.*fz* clones: w/y w *hs*.*FLP*; *FRT42* pwn cn bw/*FRT42 tub*.*Gal80* CD2y+; UAS.*fz*/*tub*.*Gal4*.

(5) *act*.*Gal4* UAS.*fz* clones: y w *hs*.*FLP*; *FRT42* pwn cn bw/*FRT42 tub*.*Gal80* CD2y+; *fz*¹⁵ ri *FRT2A* *act*.*Gal4*/UAS.*fz*.

(6) *act*.*Gal4* UAS.*fz* clones in *fz*[−] flies: y w *hs*.*FLP*; *FRT42* pwn cn bw/*FRT42 tub*.*Gal80* CD2y+; *fz*¹⁵ ri *FRT2A* *Act*.*Gal4*/UAS.*fz* *fz*¹⁵ *fz2*^{C1} ri *FRT2A*.

(7) *en*.*Gal4* UAS.*fz*: y w *hs*.*FLP*; *en*.*GAL4* wg^{CX4} pr/*FRT42* pwn; UAS.*fz* *fz*¹⁵ *fz2*^{C1} *FRT2A*/+.

(8) *en*.*Gal4* UAS.*fz* in *fz*[−] flies: y w *hs*.*FLP*; *en*.*GAL4* wg^{CX4} pr/*FRT42* pwn; UAS.*fz* *fz*¹⁵ *fz2*^{C1} *FRT2A*/*fz*¹⁵ ri *FRT2A* *hh*.*lacZ*.

(9) *hh*.*Gal4* UAS.*fz*: y w *hs*.*FLP*; *FRT42* *pk*^{pk-sple-13}/CyO; *hh*.*Gal4*/UAS.*fz*.

(10) *hh*.*Gal4* UAS.*fz* in *fz*[−] flies: y w *hs*.*FLP*; *ptc*.*lacZ*/CyO; *fz*²¹ CD2y+ UAS.*GFP* *hh*.*Gal4*/UAS.*fz* *fz*²¹ *fz2*^{C1} ri *FRT2A*.

(11) *pk*[−]: *pk*^{GO12}; *hh*.*lacZ*/+.

(12) *tub*.*Gal4* UAS.*sple*: w/y w *hs*.*FLP*; *tub*.*Gal4*/If; UAS.*sple*/TM2.

(13) *en*.*Gal4* UAS.*sple*: y w/w; UAS.*sple*/+; *en*.*Gal4*/+.

(14) *hh*.*Gal4* UAS.*sple*: y w *hs*.*FLP*; UAS.*sple*/Sp; *fz*²¹ CD2y+ ri *FRT2A* *hh*.*Gal4*/MKRS.

(15) *ci*.*Gal4* UAS.*sple*: y w *hs*.*FLP*; *ci*.*Gal4*/UAS.*sple*; *fz*²¹ CD2y+ ri *FRT80*/TM6B.

- (16) *tub.Gal4 UAS.pk*: y w/y w hs.FLP; *tub.Gal4/+*; UAS.pk/TM2.
 (17) *pk⁻ fz⁻*: FRT42 *pk^{pk-sple-13}*; *fz²¹* CD2y+ ri FRT2A.
 (18) *hh.Gal4 UAS.sple* in *fz⁻* flies: y w FLP; UAS.sple/Sp; *fz²¹* CD2y+ ri FRT2A *hh.Gal4/fz²¹* CD2y+ ri FRT80.
 (19) *fz⁻* clones in *pk⁻* flies: y w hs.FLP; FRT42 *pk^{pk-sple-13}*; *fz¹⁵* *trc^{C1}* ri FRT2A/CD2y+ hs.GFP ri FRT2A.
 (20) *hh.Gal4 UAS.fz* in *pk⁻* flies: y w hs.FLP; FRT42 *pk^{pk-sple-13}*/FRT42 *pk^{pk-sple-13}* sha; *hh.Gal4/UAS.fz*.
 (21) *pk⁻*: y w hs.FLP; FRT42 *pk^{pk-sple-13}*/FRT42 *pk^{pk-sple-13}* sha; *hh.Gal4/TM2*.
 (22) *pk⁻* clones: y hs.FLP/+; FRT42 *pk¹* pwn/FRT42 CD2y+; and
 (23) y hs.FLP/+; FRT42 *pk^{pk-sple-13}*/FRT42 CD2y+; and
 (24) y hs.FLP; FRT42 *pk^{pk-sple-13}* sha/FRT42 CD2y+.
 (25) *tub.Gal4 UAS.sple* clones: w/y w hs.FLP; FRT42 pwn cn bw/FRT42 *tub.Gal80* CD2y+; UAS.sple/*tub.Gal4*.
 (26) *Vang⁻*: y hs.FLP; FRT42 *Vang⁻*.
 (27) *tub.Gal4 UAS.Vang*: w/y w hs.FLP122; UAS.Vang/*tub.Gal4*; +/TM2.
 (28) *Vang⁻* clones: y/y hs.FLP; FRT42 pwn *Vang⁻*/FRT42 CD2y+; and
 (29) y w hs.FLP; FRT42 *Vang⁻*/FRT42 CD2y+.
 (30) pwn clones: y w/+; *smo³* b FRT42 cn pwn y w; FRT42 sha; hs.FLP/+.
 (31) *tub.Gal4 UAS.Vang* clones: w/y w hs.FLP; FRT42 pwn cn bw/FRT42 *tub.Gal80* CD2y+; UAS.Vang/*tub.Gal4*.
 (32) *tub.Gal4 UAS.Vang* clones in *Vang⁻*: y w/y w hs.FLP *tub.Gal4* UAS.GFP; UAS.Vang *Vang⁻*/FRT42 *Vang⁻*; mwh jv CD2y+ FRT2A/*tub.Gal80* FRT2A.
 (33) *fz⁻* clones in *Vang⁻*: y w hs.FLP; FRT42 *Vang⁻*; *fz¹⁵* *trc^{C1}* ri FRT2A/CD2y+ hs.GFP ri FRT2A.
 (34) *tub.Gal4 UAS.fz* clones in *Vang⁻*: w/y w hs.FLP *tub.Gal4* UAS.GFP; FRT42 *Vang⁻*/FRT42 pwn *Vang⁻*; *tub.Gal4* FRT2A/UAS.fz *trc* FRT2A.
 (35) *Vang⁻ tub.Gal4 UAS.fz* clones: w/y w hs.FLP; FRT42 pwn *Vang⁻*/FRT42 *tub.Gal80* CD2y+; UAS.fz *trc* FRT2A/*tub.Gal4*.
 (36) *tub.Gal4 UAS.Vang* clones in *fz⁻*: y w hs.FLP/y w hs.FLP *tub.Gal4* UAS.GFP; FRT42 pwn/FRT42 *tub.Gal80*; UAS.Vang *fz²¹* CD2y+ ri FRT2A/*fz²¹* CD2y+ FRT80.
 (37) *Vang⁻* clones in *fz⁻*: y w hs.FLP; FRT42 pwn *Vang⁻*/FRT42; *fz²¹* CD2y+ ri FRT80/*fz²¹* ri FRT2A.
 (38) *stan⁻*: y w hs.FLP; *stan³* CD2y+/*stan^{E59}*; +/TM6B; and
 (39) *stan³*; and
 (40) y w hs.FLP; *ptc.Gal4 stan^{E59}*/FRT42 pwn *stan⁷¹*; and
 (41) y w; *stan^{E59}/stan^{E45}* 1407.Gal4; UAS.stan/+.
 (42) *tub.Gal4 UAS.stan*: y w/y w hs.FLP; UAS.stan/*tub.Gal4*; +/TM2.
 (43) *stan⁻* clones: y w hs.FLP; FRT42 *stan^{E59}*/FRT42 hs.GFPy+; *vg.Gal4 UAS.FLP*/+; and
 (44) y w FL122; FRT42 pwn *stan^{E59}*/FRT42 CD2y+ *RpL19*; *vg.Gal4/UAS.FLP*/+; and
 (45) y w/y hs.FLP; FRT42 pwn *stan^{E59}*/FRT42 CD2y+.
 (46) *stan⁻ tub.Gal4 UAS.fz* clones: w/y w hs.FLP; FRT42 pwn *stan^{E59}*/FRT42 *tub.Gal80* CD2y+; UAS.fz/*tub.Gal4*.
 (47) *fz⁻* clones in *stan⁻*: y w hs.FLP; *stan³*; *fz¹⁵* *trc^{C1}* ri FRT2A/hs.CD2y+ hs.GFP ri FRT2A; and
 (48) y w hs.FLP; *stan³*; *fz¹⁵* ri FRT2A/CD2y+ hs.GFP ri FRT2A.
 (49) *ptc.Gal4 UAS.fz* in *stan⁻*: y w hs.FLP; *ptc.Gal4 stan^{E59}/stan³*; UAS.fz CD2y+ ri FRT2A/+.
 (50) *tub.Gal4 UAS.stan* clones: w/y w hs.FLP; FRT42 pwn cn bw/FRT42 *tub.Gal80* CD2y+; UAS.stan/*tub.Gal4*.
 (51) *stan⁻ tub.Gal4 UAS.Vang* clones: w/y w hs.FLP; FRT42 pwn *stan^{E59}*/FRT42 *tub.Gal80* UAS.Vang/*tub.Gal4*.
 (52) *tub.Gal4 UAS.stan* clones in *fz⁻*: y w hs.FLP *tub.Gal4* UAS.GFP/y w hs.FLP; FRT42 pwn/FRT42 *tub.Gal80*; UAS.stan *fz²¹* CD2y+ ri FRT2A/*fz²¹* CD2y+ FRT80.
 (53) *ptc.Gal4 UAS.stan*: y w hs.FLP; Sp/*ptc.Gal4*; UAS.stan *fz³⁷*/TM6B.

(54) *ptc.Gal4 UAS.stan* in *fz⁻*: y w hs.FLP; Sp/*ptc.Gal4*; UAS.stan *fz³⁷*/TM6B.

Clones were induced by heat-shocking third instar larvae or pupae for 1 hour at 34, 35 or 37°C. β -galactosidase activity was developed as described by Lawrence et al. (Lawrence et al., 1999a). Abdominal cuticles were dissected, mounted in Hoyer's and images captured with Auto-Montage (Syncroscopy).

Results and Discussion

Dorsal and ventral

The dorsal surface of the abdomen includes the pigmented tergites as well as regions of flexible cuticle; it is mostly decorated with polarised hairs and bristles (Fig. 1A). From landmarks, we can estimate positions of the cells vis-à-vis the compartments and their boundaries. The ventral surface consists of the sternites and the pleura, the latter a flexible cuticle forming a featureless lawn of polarised hairs. Both dorsally and ventrally, hairs point posteriorly, indicating the polarity of the epidermal cells (Struhl et al., 1997b).

Even though the effects of experiments are concordant, the tergites and sternites are less sensitive to mutations in polarity genes than the pleura. For example, the pleurae of flies mutant for *fz* or *stan* bear hairs with randomised orientation, while, in the tergites and sternites, there are large areas with near-normal polarity – this implies that there may be some additional polarising mechanism in those parts. Therefore we use the pleura when we can, but we must use the tergites when it is important to know the exact position of the clone relative to the compartments.

We can remove or overexpress a gene or genes in marked clones of cells. These clones generate sharp disparities in gene function at their edges that cause changes in polarity. We can also overexpress the gene under *Gal4/UAS* control using *Gal4* drivers, which act in either the A (*ci.Gal4*) or in the P compartment (*en* or *hh.Gal4*) – these drivers create disparities across the A/P compartment boundaries. We can also make gradients within the A compartment (*ptc.Gal4*). We summarise the main results in Fig. 1B.

Building a model for how Fz, Pk, Vang and Stan act to polarise cells

frizzled (*fz*)

Fz protein

fz encodes one of the Fz family of transmembrane proteins (Adler et al., 1990; Park et al., 1994; Wodarz and Nusse, 1998), at least two of which, Fz and Fz2, function as redundant Wg receptors, each capable of bearing the full burden of Wg transduction (Bhanot et al., 1996; Bhanot et al., 1999; Chen and Struhl, 1999). Fz, but not Fz2, is also required for normal cell polarity (Gubb and Garcia-Bellido, 1982; Vinson and Adler, 1987; Chen and Struhl, 1999). In the pupal wing, Fz protein accumulates transiently along the distal edge of each cell, prefiguring the proximodistal outgrowth of a single hair from this position (Strutt, 2001; Strutt, 2002).

fz⁻ and UAS.fz flies

The abdomens of *fz⁻* flies do not lose organised polarity completely. Dorsally, the only obvious abnormality is dishevelment in the anterior portion of the A compartment with occasional whorls elsewhere, particularly in the front of the P

compartment. However, ventrally, the hairs of the pleura lack all anteroposterior bias; in some places they are randomly oriented, in others they point mostly laterally. General overexpression (*tub.Gal4*) of a *UAS.fz* transgene in the fly abdomen causes only a little dishevelment and, in the pleura, occasional patches of hairs of disturbed polarity (genotype 1 in Materials and methods).

Clones of *fz*⁻ and *UAS.fz* cells

Clones of *fz*⁻ cells have strong effects: hairs at the back of the clone are reversed, with reversal extending beyond and behind the clone for about 2-4 rows of cells (Fig. 2A). The hairs point into the clone, that is, towards the cells with no Fz. This phenotype occurs wherever the clones are made, dorsally or ventrally, regardless of compartment (A or P), or of the

presence of compartment boundaries. For example, *fz*⁻ clones at the back of the A or at the back of the P compartment will reverse the polarity of the cells behind, which will be the most anterior P and the most anterior A cells, respectively. *fz*⁻ clones in the pleura have randomised hairs within the clone, except for the most anterior row of cells within the clone (normal polarity) and the most posterior row of cells within the clone (reversed polarity).

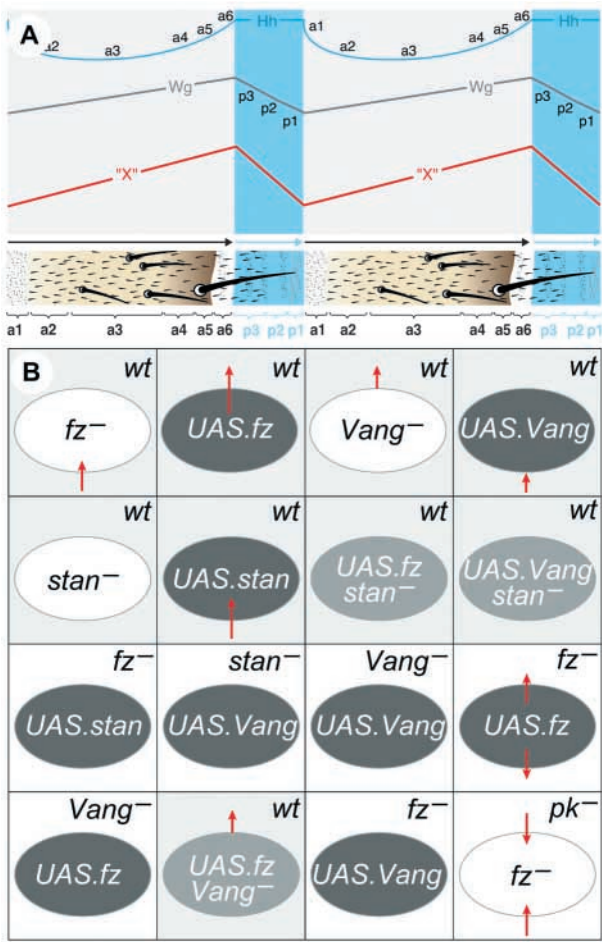


Fig. 1. Summary model and results. (A) Pattern formation in the abdomen. Two segments are shown, each with an A and a P (blue) compartment stratified into different types of cuticle (shown at bottom of the panel). The Hh and Wg gradients pattern the cuticle (Struhl et al., 1997b; Lawrence et al., 2002). The gradient of 'X' may have opposing slopes in A and P; its vector determines the orientation of the hairs in A (up the gradient) and P (down the gradient) as shown by the arrows. Anterior is to the left. (B) A field guide to the main results; the genotypes of clones are shown inside the ellipses. Genotype symbols outside the clone, say, *fz*⁻, indicate the genetic background in which the clone was induced (*fz*⁻). Red arrows mark where polarity is reversed or normalised. Anterior is up.

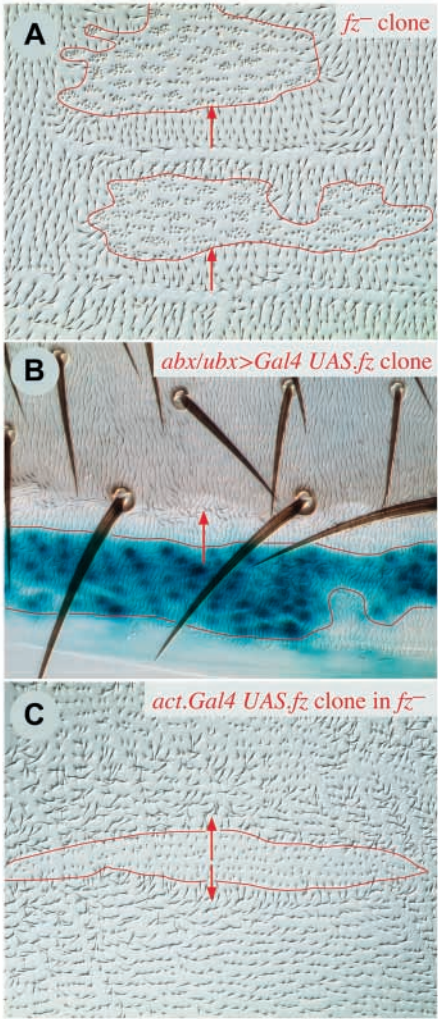


Fig. 2. Clones involving *fz*. (A) Two *fz*⁻ clones in the pleura (marked with *tricornered*, which makes each cell form a cluster of variously pointing hairs; genotype 2). Here and in most other figures, anterior is up and clones are outlined in red. Note the reversal of polarity (red arrows) for several rows of cells, behind the clones. (B) A *UAS.fz* clone reverses in front (genotype 3). This clone, stained in blue, consists of cells at the anterior limit of the P compartment. Repolarisation includes the front part of the clone and extends well into the A compartment (red arrow). Clones made with other drivers show similar phenotypes (genotypes 4 and 5). Note that here and in other figures the orientation of bristles is inconstant; this is an artefact caused by the need to flatten the preparations when mounting. (C) A *UAS.fz* clone (marked with *pawn*, which makes the hairs more tenuous and the bristles stunted) in a *fz*⁻ fly (genotype 6). The background *fz*⁻ territory has dishevelled hair polarity (compare with the left edge of A to see the wild-type pattern), and the clone imposes normal polarity for about one cell behind and reversed polarity for about one cell in front (red arrows).

UAS.fz clones cause a phenotype that is as strong, but opposite to *fz*⁻ clones. Up to several rows of cells in the anterior half of the clone, as well as beyond and in front, show reversal of polarity (Fig. 2B); hairs lateral to the clone also point outwards. Again the phenotype is the same, no matter where the clones are located; for example, clones at the anterior limit of the P compartment will reverse hairs of the A cells in front of them (Fig. 2B).

UAS.fz clones in *fz*⁻ flies

These clones behave like *UAS.fz* clones in a wild-type background, except in their capacity to repolarise surrounding cells. Inside the front part of the clone, several rows of hairs are reversed, but, outside the clone, repolarisation of the mutant cells is limited to only one cell (Fig. 2C). We also compared the effect of *en.Gal4* or *hh.Gal4* driving *UAS.fz* in the P compartment of wild-type and *fz*⁻ flies (genotypes 7–10). In *fz*⁺ flies we see reversal straddling the anterior border of the P compartment and affecting two to three cells on either side. In *fz*⁻ flies there is reversal anterior to the compartment border, but it is more limited and more difficult to define (the reversal is noisy and the hairs are somewhat dishevelled). In the pleura, imposed on the rather chaotic hairs of the *fz*⁻ territory, there are two differently sized zones of oriented hairs, the smaller pointing anteriorly near the front of the P compartment, and the larger one pointing posteriorly behind it (Fig. 3).

These results in *fz*⁻ flies show that a group of *fz*-expressing cells affects the cells both anterior and posterior to the clone, and directs hairs to point away. This makes clear that, in most other experiments, polarity changes are only seen on one side of the clone because any effects on the other side are invisible, being concordant with normal polarity. Also, they show that the *fz*-expressing cells can repolarise cells that lack Fz protein, but only if they are in direct contact.

Fz: discussion

Fz, a receptor for X?

Fz is a transmembrane receptor for Wnt genes and it has therefore been reasonably argued (Adler et al., 1997; Tomlinson et al., 1997) that, in planar polarity, it also might be a receptor for X, leading to the hypothesis that X is a Wnt. In support of this, *dishevelled* (*dsh*), which functions in the Wnt pathway, also affects planar polarity (Klingensmith et al., 1994; Theisen et al., 1994). However, tests in *Drosophila* (Lawrence et al., 2002) indicate that X is not a Wnt. Nevertheless, in addition to its function in the Wnt pathway, Fz could also be a receptor for X and, if so, *fz*⁻ cells should show randomised polarity – and this is true of the pleura and the eye (Zheng et al., 1995), as well as parts of the tergite.

Fz, polarity and the borders of compartments

All our results suggest that cells *compare* their relative levels of Fz activity and form hairs pointing away from neighbours that have a higher activity. *fz*⁻ cells can be repolarised as long as they are in direct contact with *UAS.fz* or *fz*⁺ cells: a cell with no Fz can still be compared with a cell that has some. Moreover, as observed, repolarisation should not spread further than one cell into *fz*⁻ territory because, within that territory, all cells being compared have no Fz.

In the normal fly, all cells make hairs and bristles that point posteriorly, suggesting that there is a continuous gradient of Fz

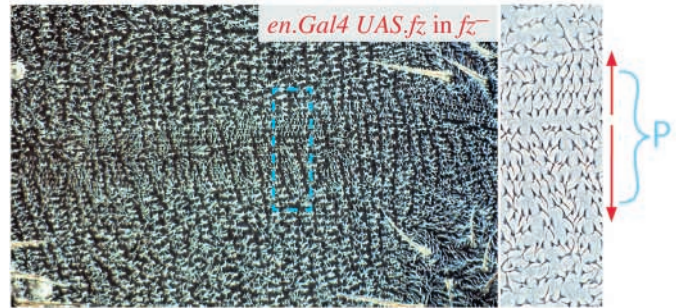


Fig. 3. Providing Fz locally to *fz*⁻ territory. *en.Gal4* driving *UAS.fz* in *fz*⁻ flies provides a band of Fz expression in the P compartment (genotype 8). This appears to have two effects: 1. The interface at the front causes a reversal of polarity around that interface that extends one cell into the *fz*⁻ territory but more cells into the P compartment. 2. The remainder of the P compartment is rescued, with the rescue extending one cell into the *fz*⁻ territory behind. Overall, we see a band of order imposed on the disorder seen in the majority of the A compartment. The detail on the right shows the two zones of organised polarity and our estimate of where the P compartment is (estimated from clones, and from other experiments where *UAS.fz* is driven by *hh.Gal4* in flies carrying *ptc.lacZ*).

activity from high to low, from anterior to posterior. Given that the pattern repeats every metamere, this would give a gradient repeating once per unit, with a precipice at either the A/P or the P/A border. Hence, if all cells secrete hairs that point towards that neighbour with the lowest Fz activity, we would expect cells flanking the A/P or P/A boundaries to point anteriorly, but this is not the case. One could postulate barriers or other special properties for the A/P and P/A boundaries that would insulate cells in different compartments. Indeed, there is some evidence that the A/P boundary may function as a barrier: *UAS.fz* clones can repolarise adjacent cells across the A/P boundary, but only in one direction (A to P, but not P to A) (Casal et al., 2002). However, *fz*⁻ and *UAS.fz* clones repolarise cells across compartment boundaries just as effectively as they do within compartments. Perhaps we could argue that such clones cause an abnormally large disparity in Fz activity, and as a consequence can override any special conditions at the boundaries. But this is not a satisfying argument.

Non-autonomy

Why do changes in polarity spread more than one cell into *fz*⁺ territory, and what limits this non-autonomy to only a few cells? These are important questions and we discuss them later.

prickle (*pk*)

Pk and Sple proteins

These are homologous proteins that derive from two transcripts constituting the *pk* locus, they are cytosolic and contain a LIM domain (Gubb et al., 1999). The *pk*⁻ mutation we use removes both transcripts. In wing cells, shortly before hair outgrowth, Pk accumulates at the proximal cell boundary, which is opposite to the site of Fz accumulation (Tree et al., 2002). In *pk*⁻ wing cells, Fz protein is still localised to the membrane of cells, but does not accumulate asymmetrically (Strutt, 2001; Tree et al., 2002). Similarly, Stan and Vang proteins fail to accumulate asymmetrically in *pk*⁻ cells (Shimada et al., 2001; Bastock et al., 2003).

pk⁻, *UAS.sple* and *UAS.pk* flies

Flies that lack *pk* are viable and have reorganised polarity in the abdomen: dorsally there is a large zone of reversed polarity near the back of the A compartment, while polarity in the rest of the segment is normal. Ventrally, the same zones are observed, but they are more regular and better demarcated (Fig. 4A). Uniform overexpression of *sple* transcript produces a near-reciprocal phenotype: both dorsally and ventrally, the polarity of the entire P compartment is reversed, while, apart from the front, the A compartment is largely unaffected (Fig. 4B). Overexpression of *sple* in the P compartment alone gives the reversed phenotype there (genotypes 13, 14), while overexpression in the A compartment (*ci.Gal4*) has relatively little effect, causing only some localised disturbances at the fronts of both A and P compartments in the tergite, but no effect

in the pleura (genotype 15). General overexpression of the *pk* transcript causes reversal of polarity in part of the A compartment, but the P compartment is unaffected (genotype 16).

pk⁻ flies that also lack *fz* do not show areas of consistently reversed polarity and, both dorsally and ventrally, have a pattern similar to *fz*⁻ flies, indicating that the *pk*⁻ phenotype is dependent on Fz (genotype 17). Similarly, if the *sple* transcript is overexpressed in the P compartment of *fz*⁻ flies, the *fz*⁻ phenotype is largely or entirely unaffected, indicating that the gain-of-function phenotype is also dependent on Fz (genotype 18).

fz⁻ clones and *UAS.fz* in *pk*⁻ flies

fz⁻ clones cause hairs around the clone to point inwards, towards

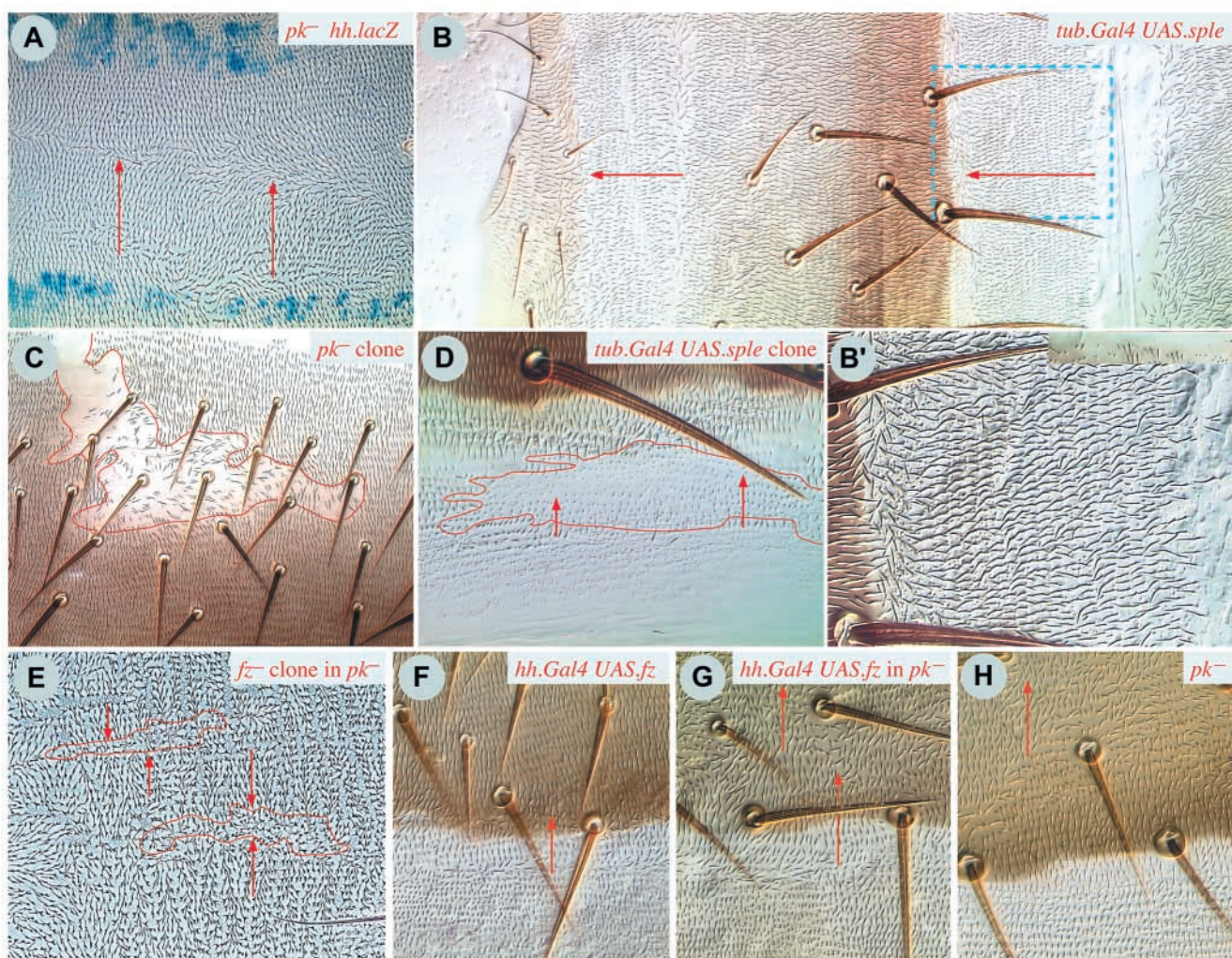


Fig. 4. Clones involving *prickle* (*pk*). (A) Part of the pleura of a fly lacking *pk* and stained for *hh.lacZ* to demarcate the P compartments (genotype 11). Note the alternating zones of polarity, with much of the back half of the A compartment having reversed hairs (red arrows). (B,B') Uniform overexpression of *sple* gives zones of reversed polarity coinciding more or less with the P compartments (genotype 12). P compartments of the first and second abdominal segments are shown, with a magnified view of part of the second (B'). Anterior to left. (C) A clone lacking *pk* (marked with *shavenoid* which removes most of the hairs). The hair orientation within the clone is whorly (genotype 24). (D) A clone expressing *sple* in the P compartment. Most of the cells in the clone, and some cells behind, have reversed polarity. The front of the clone displays normal polarity (genotype 25). (E) Two clones lacking *fz*, marked with *tricornered* in a *pk*⁻ fly (genotype 19). Note that, in spite of the varying zones of polarity in the background, the hairs point consistently into the clones. Phase image, clone outlines were ratified in a DIC image (not shown). (F-H) Expressing *fz* in the P compartment causes a zone of reversed polarity across the A/P border of wild type (F, genotype 9) and also *pk*⁻ flies (G, genotype 20). Note that in *pk*⁻ flies polarity is normal in that region (H, genotype 21).

the clones, irrespective of whether they are located in regions of normal or reversed polarity (Fig. 4E). The range appears to be longer, as also noted by Adler et al. (Adler et al., 2000) in hypomorphic *pk* mutants. Also, we used *hh.Gal4* to drive *UAS.fz* in *pk*⁻ flies; this causes a zone of reversed polarity straddling the A/P compartment boundary (compare Fig. 4G,H). The range of repolarisation is somewhat longer than that induced in wild-type flies (Fig. 4F). Thus, the loss of both Pk and Sple does not block the ability of sharp disparities in Fz activity to repolarise several rows of cells.

pk⁻, *UAS.pk* and *UAS.sple* clones

*pk*¹ and *pk*⁻ clones have disturbed polarity and usually there are whorls associated with the clones. However, the effects are almost entirely autonomous to the clone (genotypes 22, 23 and Fig. 4C).

UAS.pk clones have a strong phenotype in the wing, the hairs point towards the clone, giving some reversal of polarity of hairs distal to it (Tree et al., 2002). However, in the P compartment of the abdomen, *UAS.sple* clones are almost entirely reversed, apart from some of their most anterior cells. Immediately behind these clones, some wild-type cells are usually reversed (Fig. 4D). *UAS.sple* clones in the A compartment are mostly normal but those at the back of the A compartment reverse a zone of P cells behind them (resembling, piecemeal, the phenotype caused by uniform expression of *UAS.sple* in the A compartment).

Pk: discussion

General loss of Pk and Sple leads to a reversal of polarity in a large portion of the A compartment, the rest of the segment being unaffected. But, overexpression of Sple leads to reversal of polarity in the P compartment, without affecting the A compartment much. These phenotypes are unrelated to the scalar pattern of cell types in the abdomen, which remain normal, and, at least for the *pk*⁻ flies, are not associated with changes in either *fj* or *ds* expression (unpublished results). A clue to understanding these phenotypes may come from studying disparities in Fz activity in *pk*⁻ flies; these appear to cause polarity changes similar to those they cause in wild-type flies. Therefore, the ability to read the levels of Fz activity and to respond to it could not depend on Pk and Sple. Perhaps, in *pk*⁻ animals, many A compartment cells react to an unchanged X gradient with opposite sign? The reversal of P cells that overexpress Sple provides some support, because if such cells were to read the X gradient with opposite sign they should show reversed polarity.

This raises a related but unsolved conundrum: as discussed above, the uniform polarity of hairs in the wild type argues that the gradient of Fz activity is itself monotonic. However we previously suggested that gradients of X might have opposing slopes in the A and the P compartment, and that they might be read with different sign – to ensure that hairs in both compartments would point the same way (Casal et al., 2002). Is there no simple relationship between what we earlier called 'X' and what we now refer to as 'Fz activity'? Perhaps Pk and Sple are normally involved in rectification. And so, for example, without Sple, cells in the A compartment interpret X with the opposite sign. Likewise cells in the P compartment misinterpret X when Sple is over expressed. Clearly, we need a better understanding of how X, which we imagine is built by Ds, Ft and Fj, is linked to Fz activity.

Van Gogh (Vang)

Vang protein

This gene, also known as *strabismus*, encodes a probable transmembrane protein of type IIIa with a PDZ binding motif (Taylor et al., 1998; Wolff and Rubin, 1998; Bastock et al., 2003). Late in development, it becomes localised, like Pk, to the proximal edge of each cell in the wing, which is opposite to the site of accumulation of Fz (Bastock et al., 2003). In *Vang*⁻ wing cells, some localisation of Fz to the membrane is preserved, but it is no longer concentrated on one edge of the cell (Strutt, 2001). In such cells there is also an increase in the amount of Pk protein (Bastock et al., 2003).

Vang⁻ and *UAS.Vang* flies

Vang⁻ flies are viable. Dorsally, the tergites resemble those of *fz*⁻ flies; they are dishevelled, especially in the anterior parts of A, but have largely normal polarity elsewhere. Similarly, in the pleura, polarity is generally disordered, as in *fz*⁻, except that there is a weak tendency for the hairs to be organized into zones of alternating polarity along the anteroposterior axis. The hairs in the middle of the A compartment tend to be reversed, with the remaining hairs being more normally polarised (genotype 26). If *Vang* is universally overexpressed, the flies are viable and polarity is little affected (genotype 27).

Vang⁻ and *UAS.Vang* clones

Vang⁻ clones give consistent results both dorsally and ventrally. Within *Vang*⁻ clones marked with *pawn*, the rows of hairs are jumbled and poorly oriented; some hairs point straight upwards, especially those in clones in anterior regions of the A compartment (to see the effect of *pawn*, compare Fig. 5A and B). Thus, there is an autonomous loss of polarity. By contrast, when clones are marked with *yellow*, there is some dishevelment, but the hairs in the clone are largely oriented correctly, resembling pieces of *Vang*⁻ flies (genotype 29). This difference between *pawn* and *yellow* clones suggests there is some interaction between the *pawn* and *Vang* mutations. Nevertheless, irrespective of whether we use the *pawn* or *yellow* marker, *Vang*⁻ cells appear largely refractory to neighbouring wild-type cells.

Outside and anterior to both *pawn Vang*⁻ and *yellow Vang*⁻ clones there are polarity disturbances: these extend a few cells and are variable, usually including patches with some hairs that are reversed (Fig. 5A). This reversal anterior to the clone is more consistent and extensive in the pleura (Fig. 5C). The amount of reversal appears not to depend on the location of the clone along the A/P axis. Indeed, a clone located at the anterior extreme of the P compartment can reverse the polarity of cells across the border at the back of the A compartment.

UAS.Vang clones show two effects: (i) hairs behind the clone are usually reversed, but (ii) hairs inside and mainly at the back of the clone are dishevelled, with some disorientation within those clones that were marked with *pawn*. Hairs at the front of the clone are unaffected (Fig. 5D). Clones at the back of the A compartment can cause reversal behind, in P cells.

Thus, both *Vang*⁻ and *UAS.Vang* clones, in collaboration with *pawn*, disturb polarity within the clones; however, they have opposite effects on neighbouring cells outside the clone, causing them to make hairs which, respectively, are reversed in front of the clone or behind the clone. Note these changes are opposite in orientation to those produced by *fz*⁻ and *UAS.fz* clones.

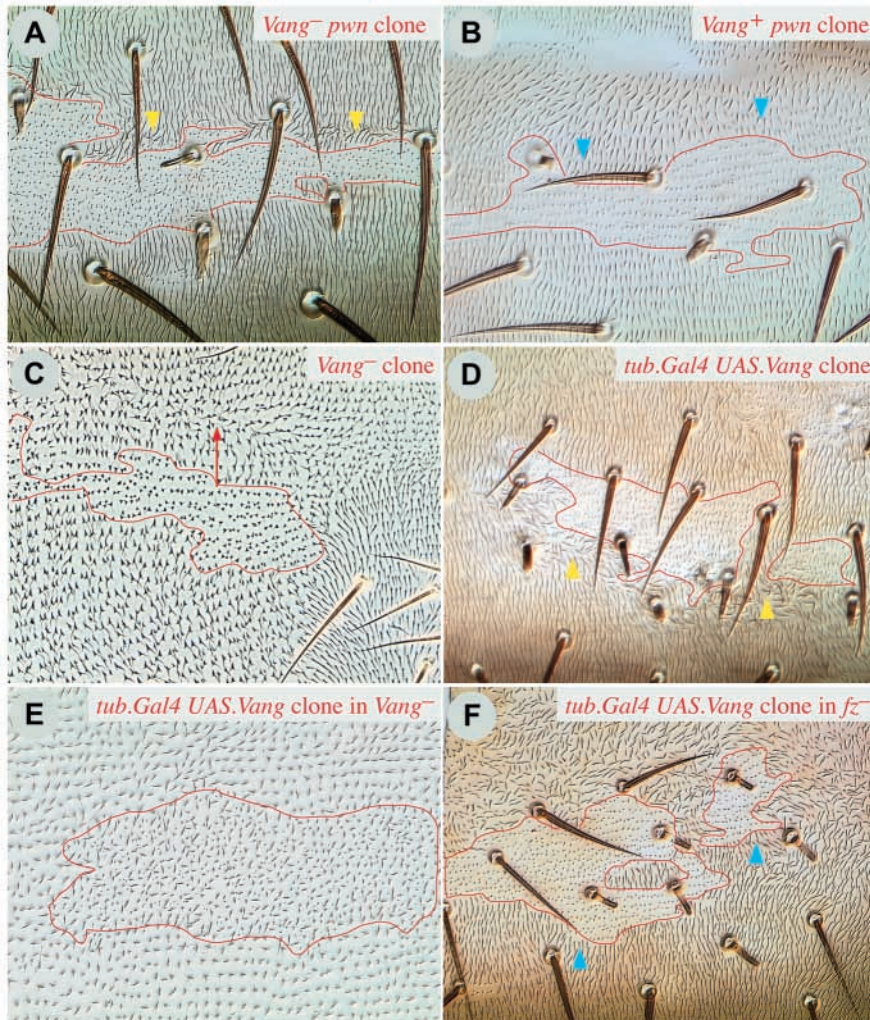


Fig. 5. Clones involving *Vang*. (A) *Vang*⁻ clone in anterior tergites marked with *pawn*. Note that the rows of hairs in the clones are disordered and the orientation is disturbed (genotype 28). Anterior to the clone, there is some reversal of hair polarity (yellow arrowheads). (B) This clone carries only the *pawn* marker and can be compared with A (genotype 30). Note that the rows of hairs are well ordered and the polarity normal (blue arrowheads). (C) *Vang*⁻ clone (marked with *pawn*) in the pleura. Hairs in the clone are dishevelled, but the hairs in front are reversed in polarity (genotype 28). (D) A clone expressing *Vang* (marked with *pawn*) showing slight disarray inside the clone, and the reversal of polarity of the hairs behind (yellow arrowheads, genotype 31). (E) A clone expressing *Vang* (marked with multiple wing hairs, in which every cell has multiple hairs that point in all directions), in a *Vang*⁻ fly. Note that the background hairs are higgledy-piggledy in orientation, but, unlike in D, they are not affected by the clone (genotype 32). (F) A clone expressing *Vang* (marked with *pawn*), in a *fz*⁻ fly. Note that the hairs in front of the clone are dishevelled because *fz*⁻ affects that region of the A compartment, but behind they are normal, and, unlike in Fig. 5D, they are unaltered by the clone (blue arrowheads, genotype 36).

UAS.Vang clones in *Vang*⁻ flies

We have studied *UAS.Vang* clones marked with *pawn* in *Vang*⁻ flies. These clones appear to have no effect on the surrounding *Vang*⁻ cells, indicating that *Vang*⁻ cells cannot be repolarised by adjacent *Vang*-expressing cells (Fig. 5E).

fz⁻ and *UAS.fz* clones in *Vang*⁻ flies

Can *Vang*⁻ cells respond to discontinuities in Fz activity between neighbouring cells? Both *fz*⁻ and *UAS.fz* clones fail to affect the polarity of *Vang*⁻ adjacent cells (genotypes 33, 34), suggesting that they cannot (similar results for *fz*⁻ clones were described in the wing) (Taylor et al., 1998). To check that the failure is specifically due to the *responding* cells, we gave those cells *Vang*, and took it away from the cells in the clone: thus we made *UAS.fz* clones that are *Vang*⁻ in otherwise wild-type animals. These clones do repolarise the cells in front (genotype 35). Hence, it appears that *Vang*⁻ cells can communicate disparities in Fz activity to their neighbours, provided that those neighbours have wild-type *Vang* activity.

Vang⁻ and *UAS.Vang* clones in *fz*⁻ flies

The repolarising effects of *Vang*⁻ and *UAS.Vang* clones are reciprocal to those of *fz*⁻ and *UAS.fz* clones; perhaps Fz activity might be abnormally elevated in *Vang*⁻ cells and suppressed in

UAS.Vang cells? Consistent with this possibility, we have examined *UAS.Vang* clones in *fz*⁻ flies, and found that these clones do not repolarise hairs immediately behind the clones (Fig. 5F). Likewise, *Vang*⁻ clones fail to reverse hair polarity in front when they were made in *fz*⁻ flies (genotype 37).

Vang: discussion

Abrupt disparities in the amount of *Vang* activity, like those in Fz, are sufficient to trigger repolarisation, which can progress a few cell diameters into wild-type territory. As *UAS.Vang* clones resemble a loss of function of *fz*, and *Vang*⁻ clones partially mimic *UAS.fz*, it could be that *Vang* acts by suppressing Fz activity. However, the properties of these two proteins differ: we have found no situation in which the polarity of *Vang*⁻ cells can be reversed, whereas *fz*⁻ cells can be repolarised. It seems that *Vang*⁻ cells can send information about their level of Fz activity to neighbouring cells, but cannot receive, or respond to, this information in return. *Vang* thus appears to be required for a subset of the functions performed by *Stan*, as described below.

starry night (stan)

Stan protein

stan, also known as *flamingo*, encodes a protein with a large

cadherin-like extracellular region and seven receptor-like transmembrane domains (Chae et al., 1999; Usui et al., 1999). Stan becomes localised transiently along both the distal and proximal edges of each wing cell shortly before hair formation (Usui et al., 1999). In *stan*⁻ cells, Fz, Pk, Vang and Dsh all fail to accumulate in the membrane, remaining largely cytoplasmic (Shimada et al., 2001; Strutt, 2001; Tree et al., 2002; Bastock et al., 2003). Usui and colleagues made the important observation that only if Stan is present in *both* adjacent cells will it accumulate along the apical membranes where these two cells abut, suggesting Stan makes homodimers that form a bridge between adjacent cell membranes (Usui et al., 1999).

stan mutant flies

Homozygous *stan*⁻ zygotes die as embryos. However, hypomorphic *stan* embryos survive to adults, as do *stan*⁻ zygotes that are rescued by neural expression of the *UAS.stan* transgene (Lu et al., 1999; Usui et al., 1999). For both the dorsal and ventral abdomen, the polarity phenotypes of hypomorphic flies (genotypes 38–40) and rescued *stan*⁻ flies (genotype 41) are the same as *fz*⁻ flies. When *UAS.stan* is generally and strongly overexpressed (*tub.Gal4*), the flies do not emerge as adults (genotype 42).

Clones of *stan*⁻ cells

It was reported that, in the wing (Usui et al., 1999), *stan*⁻ cells (marked with *pawn*) make polarised hairs with randomised

orientation, but we do not find this to be usually so. Although some clones are dishevelled, others have near-normal polarity. We found that large *stan*⁻ clones marked with *yellow* (genotype 43) are even more normal, and similar to the equivalent parts of wings that are mutant for *stan*. This suggests that the *pawn* marker itself might be contributing to the disarray and indeed, in the wing, *pawn* clones that are otherwise wild type are sometimes dishevelled. We made wings that were largely *pawn*⁻ *stan*⁻, with only small patches of *stan*⁺ cells. These wild-type patches most often have normal polarity, indicating that small clusters of *stan*⁺ cells can respond autonomously, and correctly, to the X gradient even when isolated in a sea of mutant tissue (genotype 44).

In the abdomen, *pawn* itself does not noticeably alter polarity (Fig. 5B) and *stan*⁻ clones marked with *yellow* resemble pieces of the pattern of *stan* mutant flies (normal in some areas, abnormal in others). However, *stan*⁻ clones marked with *pawn* are dishevelled, both in orientation and in the orderliness of the hair rows (Fig. 6A). Within the clone, they thus resemble patches of *pawn Vang*⁻ cells. However, unlike *pawn Vang*⁻ clones, *pawn stan*⁻ clones have little effect on surrounding wild-type cells, except for rare, local disturbances just outside and mostly in front of the clones. In the pleura of *stan* mutant flies polarity is lateralised/randomised, and *stan*⁻ clones, both marked with *pawn* and unmarked, appear to show this phenotype autonomously (the frequency, size and shape of the unmarked patches indicate that they are clones). These

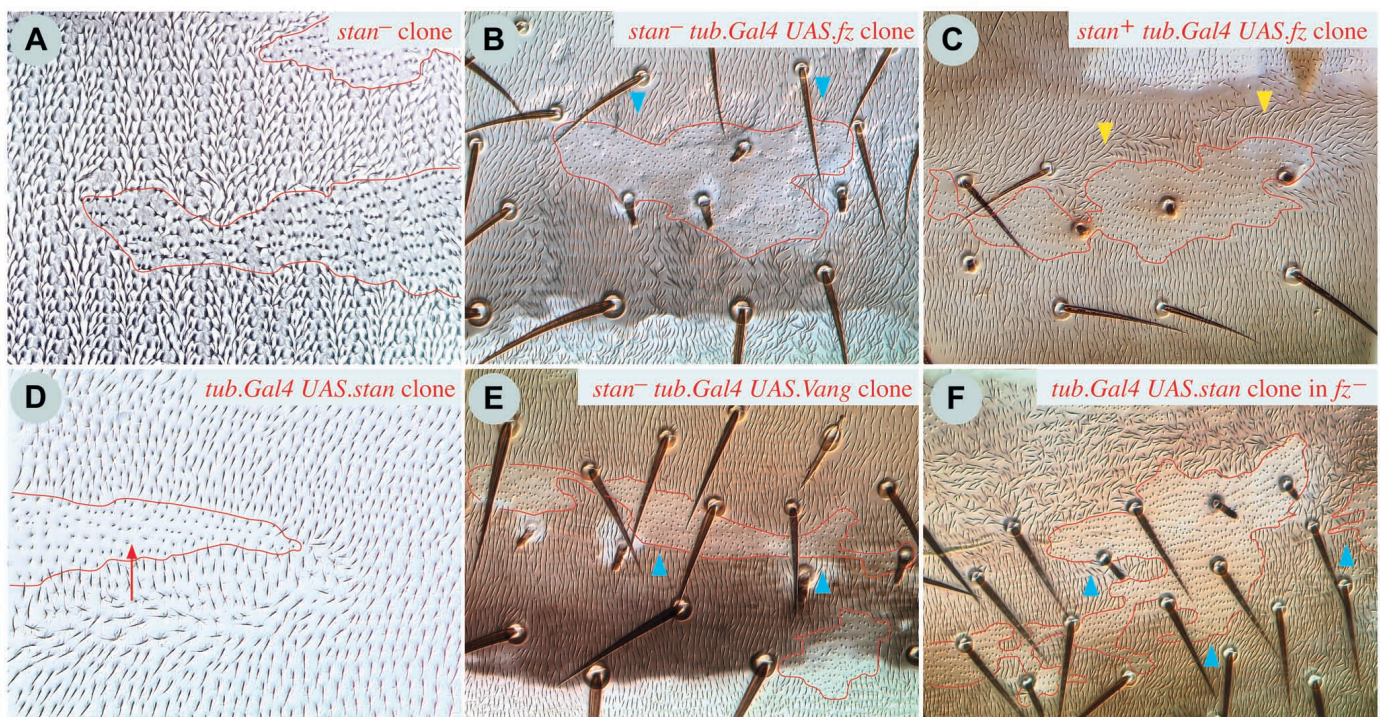


Fig. 6. Clones involving *starry night* (*stan*). (A) *stan*⁻ clones marked with *pawn*; inside the clones the polarity is dishevelled but there are no consistent effects on neighbouring cells outside the clone (genotype 45). (B) A *stan*⁻ clone expressing *fz* marked with *pawn*, note the dishevelled rows and orientation of hairs inside the clone but the lack of effect on polarity outside the clone (blue arrowheads, genotype 46). Compare with (C) a *stan*⁺ clone overexpressing *fz*; the clonal hairs are well-ordered but there is reversed polarity within the front and anterior to the clone (yellow arrowheads, genotype 4). (D) Part of a clone overexpressing *stan* marked with *pawn* in the pleura, note the substantial reversal at the back of the clone and behind (red arrow, genotype 50). (E) Two *stan*⁻ clones expressing *Vang* marked with *pawn*; note the disarray in the clones and the lack of reversal behind them (blue arrowheads, genotype 51). Compare with Fig. 5D. (F) Clones overexpressing *stan* in a *fz*⁻ background fail to repolarise behind (blue arrowheads, genotype 52).

clones in the pleura do show occasional local non-autonomy, disturbing the polarity of hairs here and there in front of the clone. Thus, *stan*⁻ cells appear to be unable to receive polarising information from their wild-type neighbours, and have little, or no ability to send such information. In this respect they differ from *Vang*⁻ cells, which typically repolarise wild-type neighbours.

stan⁻ clones that express *UAS.fz* or *UAS.Vang*

Clones of *stan*⁻ cells that express *UAS.fz* show randomised polarity inside (owing to the lack of Stan in combination with *pawn*) but fail to repolarise in front of the clone (Fig. 6B), in contrast to simple *UAS.fz* clones (Fig. 6C), and to *UAS.fz Vang*⁻ clones (genotype 35), which normally do. Likewise, if *fz*⁻ clones are made in flies carrying viable mutations of *stan* (genotypes 47, 48), repolarisation both inside and outside the clone is blocked [as in the wing (Chae et al., 1999)]. Also, if *fz* is driven by *ptc.Gal4* in *stan* mutant flies, the repolarisation that occurs in a wild-type background is suppressed (genotype 49). Finally, as observed for *UAS.fz stan*⁻ clones, *UAS.Vang stan*⁻ clones show randomized polarity within the clone (marked with *pawn*), but fail to repolarise wild-type cells outside (Fig. 6E). These results indicate that *stan*⁻ cells are unable to communicate their level of Fz activity to neighbouring cells, and are unable to respond to such information communicated to them.

UAS.stan clones

Cells within *UAS.stan* clones show some dishevelment, possibly because of the presence of *pawn*. The clones induce extensive reversal behind the clone, both in the A and P compartments, and also within the clone, at the back (Fig. 6D). This suggests that an abrupt disparity in the amount of Stan protein is sufficient to polarise cells, with effects spreading outwards from the interface, providing that at least some Stan is expressed in cells on both sides of that interface.

Both *UAS.stan* and *UAS.Vang* clones cause a similar phenotype to *fz*⁻ clones, raising the possibility that overexpression of either of these proteins autonomously suppresses Fz activity. Consistent with this, *UAS.stan* clones fail to cause repolarisations in *fz*⁻ flies, even in cells along the clone border (Fig. 6F). We have also expressed *UAS.stan* at the back of the A compartment using *ptc.Gal4*, which normally results in a zone of reversed polarity at the back and behind the A compartment (genotype 53). This is expected from the clones (at the back of the A compartment there will be a sharp interface in the amount of Stan), but, again, if *stan* is expressed in the same pattern in *fz*⁻ flies, that zone is not seen (genotype 54). These findings show that Fz is required for *UAS.stan* clones to cause repolarisations.

Stan: discussion

Animals that are entirely mutant for *stan* or *fz* have similar phenotypes in the abdomen, notably in the pleura where hair polarity is randomized. Stan and Fz are seven-pass transmembrane proteins with similar structures, making them both valid candidates for receptors for X. However, unlike Fz, we find that Stan appears necessary on both sides of a clone interface (say, a clone that is overexpressing *fz*) to induce and propagate a change in polarity both into and out of the clone. It is probably important to this function that Stan may form

homodimers that act as a link between cells (Usui et al., 1999). But note that wild-type cells that are adjacent to *stan*⁻ clones, or patches of *stan*⁺ cells in a sea of *stan*⁻ cells are, usually, normally polarised, suggesting that polarity in one cell does not depend on it having such links with every neighbour.

General Discussion

Outline of a working model for cell polarity in the abdomen

There are a number of simple systems in which isolated cells orient to a polarising signal. These include the localized outgrowth, or 'schmooing' of yeast in response to mating pheromone and directed migration of *Dictyostelium* cells up a gradient of cyclic AMP (Arkowitz, 1999). Small differences (as little as 1-5%) in receptor activation across single cells are sufficient to polarise them (Zigmond, 1974; Parent and Devreotes, 1999; Drubin, 2000), a response that, in yeast and elsewhere probably depends on localised exocytosis (Bretscher, 1984; Valdez-Taubas and Pelham, 2003). It is not known whether the polarisation of single, isolated cells is a model for planar polarity of cells in an epithelium, but it is likely that they share at least some of the mechanisms.

We have proposed that, in the abdomen of *Drosophila*, morphogen gradients (Hh in the A compartment and Wg in the P compartment) organise a secondary gradient ('X'); the vector of X specifying the polarity of each cell (Struhl et al., 1997a; Struhl et al., 1997b; Lawrence et al., 2002). Although the composition of X is unknown, at least three proteins, Fj, Ds and Ft, are implicated (Casal et al., 2002; Lawrence et al., 2002; Yang et al., 2002). All three may be expressed, or be active, in bell-shaped distributions that peak near the A/P (Ds) or P/A (Fj, Ft) boundaries (Casal et al., 2002). Ds and Ft are transmembrane proteins in the cadherin superfamily; Fj probably acts in the Golgi (Strutt et al., 2004). Ds and Ft are integrated into the membrane, suggesting that the X gradient itself may not be diffusible but instead might depend on information transfer from cell to cell.

How does Hh set up the X gradient? Although changing the real or perceived level of Hh does affect polarity, many clones (for example clones that lack Smo, an essential component of Hh reception) show there is no simple correlation between Hh concentration and polarity. For instance, large *smo*⁻ clones in the centre of the A compartment are polarised normally, even though they are blind to Hh. Also, while *smo*⁻ clones in some regions of the A compartment do affect polarity, both mutant and wild-type cells are repolarised (Struhl et al., 1997a). Both these observations argue for some transfer of information about polarity between cells, a process that would be at least partly Hh independent. This paper explores this process and is concerned with four genes (*stan*, *fz*, *Vang* and *pk*) that probably act downstream of *ds*, *ft* and *fj*.

Perhaps normal cells could transfer information from one to another (this might be particularly important for nascent cells following mitosis) to help keep the readout of X as a smooth gradient? To do this they might make a comparison of their neighbours and modify this readout of X towards an average of those neighbours. X might be read by a receptor molecule and our results point to Fz being the most likely candidate. Our results indicate that the comparison itself requires the cadherin Stan. Thus, a cell would need to read and compare (using Stan)

the levels of X (recorded in the activity of Fz) in neighbouring cells. Then, in a way analogous to how a *Dictyostelium* amoeba reads the vector of a cAMP gradient, a cell would determine its polarity from the vector of Fz activity. The results suggest that Vang also acts in this step, helping cells to sense the level of Fz activity in neighbouring cells.

The working model, details and conundrums

We now discuss some of the results in terms of the model.

Non-autonomy

Clones that lack, or overexpress Fz cause local and consistent repolarisations of cells that extend from within the clone and affect normal wild-type cells outside it (Gubb and Garcia-Bellido, 1982; Vinson and Adler, 1987). Because simply removing the *fz* gene from all cells randomizes polarity in the ventral pleura, it is self-evident that these organised polarity reversals must result from an interaction between the clone and the surrounding cells. We have argued that Stan and Fz act in this process, but how? Note that *stan* and *fz* are the only mutants that have randomised hairs in the pleura, and our results indicate that neither Stan nor Fz can function properly without the other. Averaging might depend on the capacity of Stan to form homophilic dimers as bridges between neighbouring cells (Usui et al., 1999), with such Stan:Stan dimers serving as a conduit for information about the relative level of Fz activity in each cell. However, with respect to non-autonomy, the results with the two genes differ:

(i) *stan*⁻ cells cannot be repolarised by, and cannot repolarise, neighbouring cells. This shows that Stan is essential in both neighbouring cells for the transfer of information between them. Without Stan, the cells cannot compare and cannot therefore determine any vector. However, a *stan*⁺ cell, even if it is adjacent to a *stan*⁻ cell, can be polarised normally; having Stan it should be able to read the levels of all neighbouring cells except the *stan*⁻ one and, having Fz, it should be able to set its own level.

(ii) But, *fz*⁻ cells, unlike *stan*⁻ cells, can repolarise neighbouring wild-type cells. Also, a *fz*⁻ cell, again unlike a *stan*⁻ cell, can itself be repolarised. The results also show that to be repolarised, or to polarise a neighbour, a *fz*⁻ cell must be adjacent to a *stan*⁺ *fz*⁺ cell. Such a *fz*⁻ cell will accumulate Stan in that membrane which abuts the *stan*⁺ neighbour (Usui et al., 1999) so it should be able to read the level of the neighbouring wild-type cell and be polarised accordingly. Consider a *fz*⁻ cell at the outer edge of a *fz*⁻ clone (Fig. 7): lacking Fz, its activity level would be zero, but this level would be communicated by Stan to the neighbouring cells. Wild-type cells outside would obviously have a higher level (than zero). The result would be that the two cells abutting the interface, the *fz*⁻ cell inside, and a *fz*⁺ cell outside, would both make hairs that point into the centre of the clone. The nextmost interior cell would not be polarised, as all its neighbours would be cells with level zero. In contrast, the next most exterior cell would be repolarised, as its scalar level would be brought down by the averaging process.

How far does the non-autonomy spread into wild-type cells? We have simulated this process (Fig. 7, see also supplementary material). According to the model this range would depend on the value of a single adjustable parameter, *a* (see supplementary material) that relates to how much a cell's scalar is read from X. At one extreme for this parameter

(*a*=0), when the scalar of a cell depends only on X, a wild-type cell just posterior to a clone of *fz*⁻ cells would reset its scalar as it was before; there could be no averaging and only that cell and its *fz*⁻ neighbour will be repolarised. Thus the non-autonomy would be limited to one cell. At the other extreme (*a*=1), any local disturbance produced by a clone would decay rapidly because of averaging, and the repolarisation will tend to be lost altogether. In between these extremes, the non-autonomy spreads more than one cell, but over diverse values for this parameter, the range is near the amount we usually observe (2–4 cell diameters; see supplementary material).

It has been observed that *fz*⁻ clones have effects over longer range in backgrounds such as *ds*⁻ (Adler et al., 1998; Ma et al., 2003) where the X gradient might be flatter than normal. Similarly, cells are normally polarised in large *smo*⁻ clones in the middle of the A compartment, where, because there can be no input from Hh, the X gradient could also be flat. Both these results are consistent with the model, because the range affected by averaging will increase (see supplementary material).

The localisation of proteins in cells of pupal wings

Many of the proteins required for normal cell polarity, including Fz, Dsh, Dgo, Pk, Vang and Stan are found to be asymmetrically localised in the proximodistal axis of wing cells (Strutt, 2002). This localisation is restricted to a brief period of just a few hours shortly before the wing hairs grow out, but, nevertheless it is assumed to be mechanistically important to planar polarity (Strutt, 2002). For example, non-autonomy could be explained if localised proteins were components of one or more molecular complexes that propagate polarity from cell to cell (Usui et al., 1999; Axelrod, 2001; Feiguin et al., 2001; Strutt, 2001; Adler, 2002; Strutt, 2002; Tree et al., 2002; Bastock et al., 2003; Ma et al., 2003). In support of this, note that loss of any of these proteins, including the removal of both Pk and Sple, prevents the asymmetric localisation of the others (Strutt, 2002; Bastock et al., 2003).

But our results do not seem to fit with such a mechanism – mainly because they provide evidence that polarity can propagate into cells that lack, or fail to localise all of these proteins. In particular, we find that *pk*⁻ cells are normally polarised throughout the P compartment and can be repolarised in both compartments by sharp discontinuities in Fz activity even in the pleura (where polarity is randomized in *fz*⁻ and *stan*⁻ animals). At a minimum, these findings challenge the hypothesis that Pk itself is an essential component of a feedback amplification mechanism responsible for polarising cells (Tree et al., 2002). Furthermore, if we assume that the observed failure of Fz, Dsh, Vang and Stan to localize in *pk*⁻ wing cells reflects a general property, these results also challenge the idea that Fz, Dsh, Pk, Vang, Diego and Stan must be able to accumulate asymmetrically in order for cells to detect, and be polarised by, the X gradient, or by disparities in Fz activity. Indeed, Adler has already hinted that there is no convincing evidence that the asymmetric localisation of these proteins actually functions in planar polarity: “the preferential accumulation [of proteins] along the...edges of wing cells is a process that intuitively seems likely to be part of a core system...but perhaps it is not and if not...this would leave rather little in the core” (Adler, 2002; Strutt and Strutt, 2002).

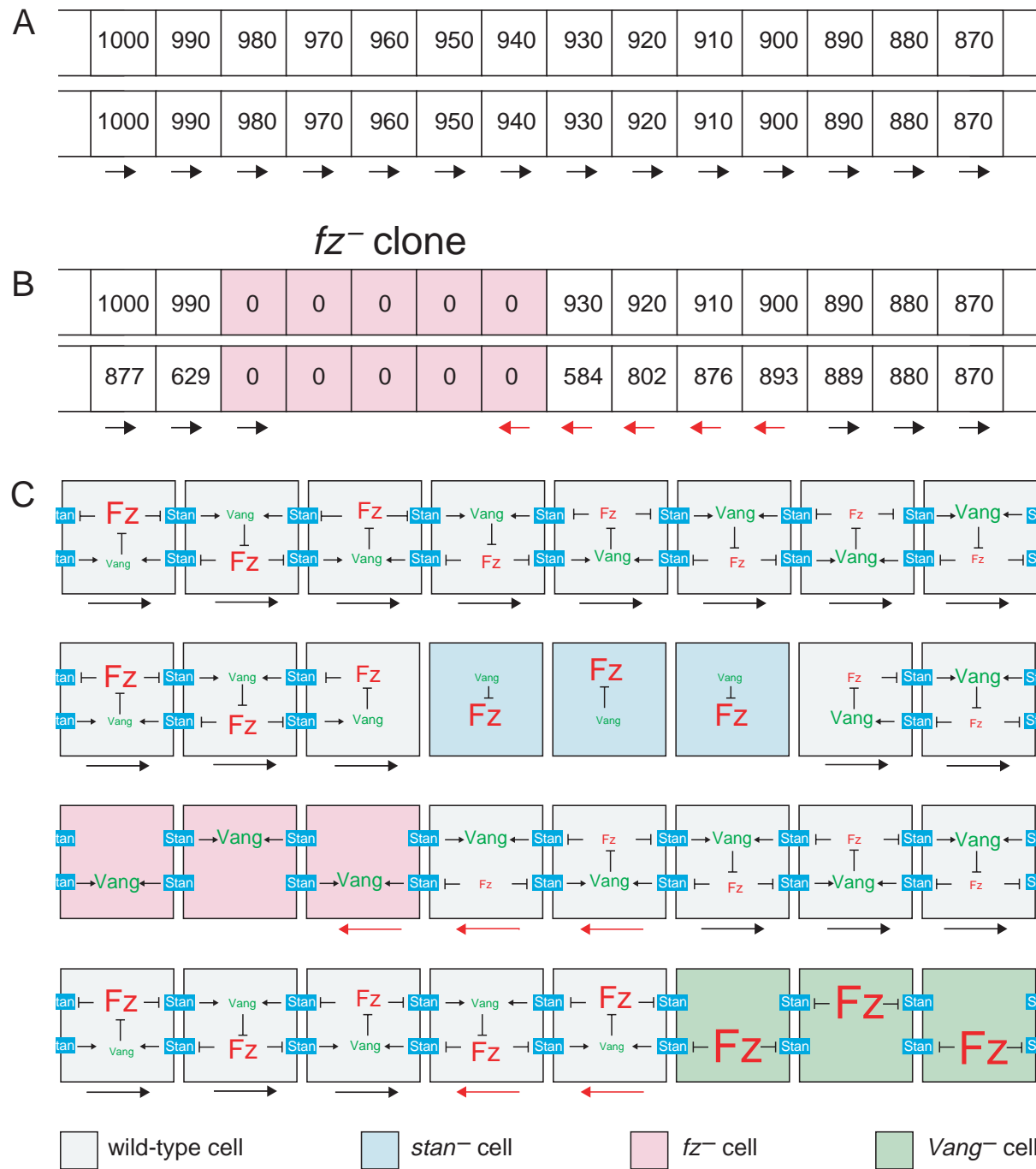


Fig. 7. The averaging model. (A) Imagine a gradient of Fz activity from 1000 downwards (see top diagram, upper row). A cell takes into account both its position, given to it by the scalar of an X gradient, but also the scalars of its neighbours. After five iterations (lower row) the gradient does not change (see supplementary material). (B) However, now introduce a *fz*⁻ clone of five cells across with an activity level of 0. At the edge of a clone, a cell (say, at level 930) now finds itself between a *fz*⁻ cell (with level 0) and its normal neighbour (level 920). After the same five iterations (lower row), the values of several of the *fz*⁺ cells have changed and, consequently, the scalars, the vectors and the polarity as shown. Red indicates a cell whose polarity has been reversed ($a=0.75$, see supplementary material). (C) A simple but speculative model of the functional interactions. This model is based on our results and helps explain them. Using Stan, each cell compares the Fz activity of adjoining cells, forming hairs that point towards the neighbour with the lowest activity. The top row shows the normal epithelium, with an activity gradient of Fz (increased activities are indicated by larger fonts). The cadherin Stan bridges from cell to cell and activates Vang. This activation is repressed by Fz activity. Within the cell, Vang, a membrane protein, represses Fz. Consider any three consecutive cells. High Fz activity in cell 1 inhibits Stan in the same cell, an effect which passes across the Stan-Stan bridge to repress Stan in cell 2, thus reducing Vang activation and increasing Fz activity in cell 2. This effect on Fz activity is counterbalanced by cell 3, which has itself lower Fz activity and hence higher Stan activity; this propagates back, via the Stan-Stan bridge, to enhance Stan activity on the opposite surface of cell 2, thus enhancing Vang activation and reducing Fz activity in cell 2. The whole field is subject to the X gradient which limits the tendency of Fz activity to rise. The effects of *stan*⁻, *fz*⁻ and *Vang*⁻ clones are shown in the bottom three rows.

Are wing cells polarised only briefly just prior to the hair outgrowth? The reason for raising this possibility is that the proteins are apparently only asymmetrically localised at that time. If this localisation were not causal, as we now suggest, it could be that the cells are polarised for all or most of development – again arguing that the ephemeral localisation of the proteins is more a consequence than a cause of polarisation.

We have had much help from the *Drosophila* community, we mention particularly David Gubb, Bob Holmgren, Xiao-Jing Qiu, David Strutt and Tanya Wolff, as well as the Bloomington Stock Center who have been generous with stocks and advice. Graeme Mitchison has given us a *nihil obstat* for the maths. We ask that author order here, as well as our four previous publications on the *Drosophila* abdomen, not be invested with significance: all three of us have contributed differently and we share responsibility. P.A.L. and J.C. are supported by the MRC; G.S. is an HHMI Investigator.

Supplementary material

Supplementary material for this article is available at <http://dev.biologists.org/cgi/content/full/131/19/4651/DC1>

References

- Adler, P. N. (2002). Planar signaling and morphogenesis in *Drosophila*. *Dev. Cell* **2**, 525-535.
- Adler, P. N., Charlton, J. and Liu, J. (1998). Mutations in the cadherin superfamily member gene *dachsous* cause a tissue polarity phenotype by altering *frizzled* signaling. *Development* **125**, 959-968.
- Adler, P. N., Krasnow, R. E. and Liu, J. (1997). Tissue polarity points from cells that have higher *Frizzled* levels towards cells that have lower *Frizzled* levels. *Curr. Biol.* **7**, 940-949.
- Adler, P. N., Taylor, J. and Charlton, J. (2000). The domineering non-autonomy of *frizzled* and *van Gogh* clones in the *Drosophila* wing is a consequence of a disruption in local signaling. *Mech. Dev.* **96**, 197-207.
- Adler, P. N., Vinson, C., Park, W. J., Conover, S. and Klein, L. (1990). Molecular structure of *frizzled*, a *Drosophila* tissue polarity gene. *Genetics* **126**, 401-416.
- Arkowitz, R. A. (1999). Responding to attraction: chemotaxis and chemotropism in *Dictyostelium* and yeast. *Trends Cell Biol.* **9**, 20-27.
- Axelrod, J. D. (2001). Unipolar membrane association of Dishevelled mediates *Frizzled* planar cell polarity signaling. *Genes Dev.* **15**, 1182-1187.
- Bastock, R., Strutt, H. and Strutt, D. (2003). Strabismus is asymmetrically localised and binds to Prickle and Dishevelled during *Drosophila* planar polarity patterning. *Development* **130**, 3007-3014.
- Bhanot, P., Brink, M., Samos, C. H., Hsieh, J. C., Wang, Y., Macke, J. P., Andrew, D., Nathans, J. and Nusse, R. (1996). A new member of the *Frizzled* family from *Drosophila* functions as a *Wingless* receptor. *Nature* **382**, 225-230.
- Bhanot, P., Fish, M., Jemison, J. A., Nusse, R., Nathans, J. and Cadigan, K. M. (1999). *Frizzled* and *Dfrizzled-2* function as redundant receptors for *Wingless* during *Drosophila* embryonic development. *Development* **126**, 4175-4186.
- Bretscher, M. S. (1984). Endocytosis: relation to capping and cell locomotion. *Science* **224**, 681-686.
- Casal, J., Struhl, G. and Lawrence, P. A. (2002). Developmental compartments and planar polarity in *Drosophila*. *Curr. Biol.* **12**, 1189-1198.
- Chae, J., Kim, M. J., Goo, J. H., Collier, S., Gubb, D., Charlton, J., Adler, P. N. and Park, W. J. (1999). The *Drosophila* tissue polarity gene *starry night* encodes a member of the protocadherin family. *Development* **126**, 5421-5429.
- Chen, C. M. and Struhl, G. (1999). *Wingless* transduction by the *Frizzled* and *Frizzled 2* proteins of *Drosophila*. *Development* **126**, 5441-5452.
- Drubin, D. (2000). *Cell Polarity*. Oxford: Oxford University Press.
- Feiguin, F., Hannus, M., Mlodzik, M. and Eaton, S. (2001). The ankyrin repeat protein Diego mediates *Frizzled*-dependent planar polarization. *Dev. Cell* **1**, 93-101.
- FlyBase (1999). The FlyBase database of the *Drosophila* genome projects and community literature. <http://flybase.bio.indiana.edu>. *Nucleic Acids Res.* **27**, 85-88.
- Gubb, D. and Garcia-Bellido, A. (1982). A genetic analysis of the determination of cuticular polarity during development in *Drosophila melanogaster*. *J. Embryol. Exp. Morphol.* **68**, 37-57.
- Gubb, D., Green, C., Huen, D., Coulson, D., Johnson, G., Tree, D., Collier, S. and Roote, J. (1999). The balance between isoforms of the prickle LIM domain protein is critical for planar polarity in *Drosophila* imaginal discs. *Genes Dev.* **13**, 2315-2327.
- Klingensmith, J., Nusse, R. and Perrimon, N. (1994). The *Drosophila* segment polarity gene *dishevelled* encodes a novel protein required for response to the *wingless* signal. *Genes Dev.* **8**, 118-130.
- Lawrence, P. A. (1966). Gradients in the insect segment: The orientation of hairs in the milkweed bug *Oncopeltus fasciatus*. *J. Exp. Biol.* **44**, 607-620.
- Lawrence, P. A., Casal, J. and Struhl, G. (1999a). *hedgehog* and *engrailed*: pattern formation and polarity in the *Drosophila* abdomen. *Development* **126**, 2431-2439.
- Lawrence, P. A., Casal, J. and Struhl, G. (1999b). The *Hedgehog* morphogen and gradients of cell affinity in the abdomen of *Drosophila*. *Development* **126**, 2441-2449.
- Lawrence, P. A., Casal, J. and Struhl, G. (2002). Towards a model of the organisation of planar polarity and pattern in the *Drosophila* abdomen. *Development* **129**, 2749-2760.
- Lecuit, T., Samanta, R. and Wieschaus, E. (2002). *slam* encodes a developmental regulator of polarized membrane growth during cleavage of the *Drosophila* embryo. *Dev. Cell* **2**, 425-436.
- Lewis, J. and Davies, A. (2002). Planar cell polarity in the inner ear: how do hair cells acquire their oriented structure? *J. Neurobiol.* **53**, 190-201.
- Lu, B., Usui, T., Uemura, T., Jan, L. and Jan, Y. N. (1999). Flamingo controls the planar polarity of sensory bristles and asymmetric division of sensory organ precursors in *Drosophila*. *Curr. Biol.* **9**, 1247-1250.
- Ma, D., Yang, C. H., McNeill, H., Simon, M. A. and Axelrod, J. D. (2003). Fidelity in planar cell polarity signalling. *Nature* **421**, 543-547.
- Mlodzik, M. (2002). Planar cell polarization: do the same mechanisms regulate *Drosophila* tissue polarity and vertebrate gastrulation? *Trends Genet.* **18**, 564-571.
- Nübler-Jung, K., Bonitz, R. and Sonnenschein, M. (1987). Cell polarity during wound healing in an insect epidermis. *Development* **100**, 163-170.
- Parent, C. A. and Devreotes, P. N. (1999). A cell's sense of direction. *Science* **284**, 765-770.
- Park, W. J., Liu, J. and Adler, P. N. (1994). The *frizzled* gene of *Drosophila* encodes a membrane protein with an odd number of transmembrane domains. *Mech. Dev.* **45**, 127-137.
- Shimada, Y., Usui, T., Yanagawa, S., Takeichi, M. and Uemura, T. (2001). Asymmetric colocalization of Flamingo, a seven-pass transmembrane cadherin, and Dishevelled in planar cell polarization. *Curr. Biol.* **11**, 859-863.
- Struhl, G., Barbash, D. A. and Lawrence, P. A. (1997a). *Hedgehog* acts by distinct gradient and signal relay mechanisms to organise cell type and cell polarity in the *Drosophila* abdomen. *Development* **124**, 2155-2165.
- Struhl, G., Barbash, D. A. and Lawrence, P. A. (1997b). *Hedgehog* organises the pattern and polarity of epidermal cells in the *Drosophila* abdomen. *Development* **124**, 2143-2154.
- Strutt, D. I. (2001). Asymmetric localization of *Frizzled* and the establishment of cell polarity in the *Drosophila* wing. *Mol. Cell* **7**, 367-375.
- Strutt, D. I. (2002). The asymmetric subcellular localisation of components of the planar polarity pathway. *Semin. Cell Dev. Biol.* **13**, 225-231.
- Strutt, H., Mundy, J., Hofstra, K. and Strutt, D. (2004). Cleavage and secretion is not required for Four-jointed function in *Drosophila* patterning. *Development* **131**, 881-890.
- Strutt, H. and Strutt, D. (2002). Nonautonomous planar polarity patterning in *Drosophila*: dishevelled-independent functions of *frizzled*. *Dev. Cell* **3**, 851-863.
- Stumpf, H. F. (1966). Mechanism by which cells estimate their location within the body. *Nature* **212**, 430-431.
- Taylor, J., Abramova, N., Charlton, J. and Adler, P. N. (1998). *Van Gogh*: a new *Drosophila* tissue polarity gene. *Genetics* **150**, 199-210.
- Theisen, H., Purcell, J., Bennett, M., Kansagara, D., Syed, A. and Marsh, J. L. (1994). *dishevelled* is required during *wingless* signaling to establish both cell polarity and cell identity. *Development* **120**, 347-360.
- Tomlinson, A., Strapps, W. R. and Heemskerk, J. (1997). Linking *Frizzled* and Wnt signaling in *Drosophila* development. *Development* **124**, 4515-4521.
- Tree, D. R., Shulman, J. M., Rousset, R., Scott, M. P., Gubb, D. and Axelrod, J. D. (2002). Prickle mediates feedback amplification to generate asymmetric planar cell polarity signaling. *Cell* **109**, 371-381.

- Usui, T., Shima, Y., Shimada, Y., Hirano, S., Burgess, R. W., Schwarz, T. L., Takeichi, M. and Uemura, T. (1999). Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. *Cell* **98**, 585-595.
- Valdez-Taubas, J. and Pelham, H. R. (2003). Slow diffusion of proteins in the yeast plasma membrane allows polarity to be maintained by endocytic cycling. *Curr. Biol.* **13**, 1636-1640.
- Vinson, C. R. and Adler, P. N. (1987). Directional non-cell autonomy and the transmission of polarity information by the *frizzled* gene of *Drosophila*. *Nature* **329**, 549-551.
- Wallingford, J. B., Fraser, S. E. and Harland, R. M. (2002). Convergent extension: the molecular control of polarized cell movement during embryonic development. *Dev. Cell* **2**, 695-706.
- Wehrli, M. and Tomlinson, A. (1998). Independent regulation of anterior/posterior and equatorial/polar polarity in the *Drosophila* eye; evidence for the involvement of Wnt signaling in the equatorial/polar axis. *Development* **125**, 1421-1432.
- Wodarz, A. and Nusse, R. (1998). Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol.* **14**, 59-88.
- Wolff, T. and Rubin, G. M. (1998). *Strabismus*, a novel gene that regulates tissue polarity and cell fate decisions in *Drosophila*. *Development* **125**, 1149-59.
- Yang, C., Axelrod, J. D. and Simon, M. A. (2002). Regulation of Frizzled by Fat-like cadherins during planar polarity signaling in the *Drosophila* compound eye. *Cell* **108**, 675-688.
- Zheng, L., Zhang, J. and Carthew, R. W. (1995). *frizzled* regulates mirror-symmetric pattern formation in the *Drosophila* eye. *Development* **121**, 3045-3055.
- Zigmond, S. H. (1974). Mechanisms of sensing chemical gradients by polymorphonuclear leukocytes. *Nature* **249**, 450-452.