Current Biology, Vol. 12, 1189–1198, July 23, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0960-9822(02)00974-0

Developmental Compartments and Planar Polarity in *Drosophila*

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Summary

Background: Planar polarity refers to the asymmetry of a cell within the plane of the epithelium; for example, cells may form hairs that point in a posterior direction, or cilia may beat in one way. This property implies that cells have information about their orientation; we wish to understand the nature of this information. Relevant also is the body plan of insects, which, in the ectoderm and somatic mesoderm, consists of a chain of alternating anterior and posterior compartments — basic units of development with independent cell lineage and subject to independent genetic control.

Results: Using the abdomen of adult *Drosophila*, we have taken genes required for normal polarity and either removed the gene or constitutively expressed it in small clones of cells and observed the effects on polarity. Hitherto, all such studies of polarity genes have not found any difference of behavior between the different compartments. We report here that the three genes, *four-jointed*, *dachsous*, and *fat*, cause opposite effects in anterior and posterior compartments. For example, in anterior compartments, clones ectopically expressing *four-jointed* reverse the polarity of cells in front of the clone, while, in posterior compartments, they reverse behind the clone. These three genes have been reported by others to be functionally linked.

Conclusions: This discovery impacts on models of how cells read polarity. At the heart of one class of models is the hypothesis that cell polarity is determined by the vector of a morphogen gradient. Here, we present evidence that cell polarity in the abdomen depends on at least two protein gradients (Fj and Ds), each of which is reflected at compartment borders. Consequently, these gradients have opposing slopes in the two compartments. Because all polarized structures made by abdominal cells point posteriorly, we surmise that cells in each compartment are programmed to interpret these protein gradients with opposite signs, pointing up the gradient in one compartment and down the gradient in the other.

Introduction

Planar polarity [1] refers to the orientation of a cell within the plane of an epithelium; for example, the cell can be oriented like an arrow and can produce a hair that points one way along the anteroposterior axis of the body. Planar polarity is usually concordant within a tissue, and this implies that cells either act together to orient themselves, or that their polarity is fixed by some external and pervasive influence.

These observations raise questions that have long been unanswered [2–5]. The questions include: how do the cells know which *way* to point? Does the planar polarity of one cell influence its neighbors, and if so, how? What are the molecular mechanisms needed to orient the cell, and what are the structural consequences? Answers to these questions relate to both the structure and function of the individual cell and the coordination between cells as they make oriented patterns.

In *Drosophila*, the approach has been genetic: genes have been identified because mutations disturb hair and bristle orientation. Several strategies have been used to study these polarity genes, and one strategy is to clone them and find the products in the cell. In some cases, it has turned out that a product is briefly concentrated in part of the membrane; for example, on the same side from which a hair will be secreted. This approach has characterized the types of proteins involved and has hinted at their association with one another. For example, products of genes such as *diego* (*dgo*), *dishevelled* (*dsh*), *flamingo* (*fmi*), and *frizzled* (*fz*) become transiently localized to part of the membrane (proximal or distal or both) of each cell [6–9].

Another approach, which we employ here, is to make clones of cells that either lack polarity genes or make excess amounts of their products and then observe the polarity effects within and around the clone. This method is better suited to assess how polarity is determined over an entire field of cells and how far changes of polarity might spread from cell to cell. For example, in the wing, clones that lack the Wnt receptor Fz induce surrounding hairs to point toward the clone [10], whereas clones that overexpress Fz cause surrounding hairs to point away from the clone [8, 11]. Hence, it appears that cells may normally be directed to point hairs from a higher toward a lower activity of Fz [11].

These experiments have led to some tentative and incomplete hypotheses to explain planar polarity; the two main hypotheses are as follows: (1) A long-range model in which polarity is fixed in a cell as a result of reading the local slope of a concentration gradient of a morphogen, "X" [2, 4]. The identity of X (if it exists!) is still unknown [12, 13]. (2) A short-range model in which polarity is fixed in a cell by molecular associations in part of its membrane; polarity could spread from cell to cell if the associations in one cell could influence those in the next [11]. For example, Fmi is a cadherin-like protein [9] and might form homophilic dimers between one cell and its neighbors. Fmi is concentrated on the membranes of wing cells, where they abut along the proximodistal axis [9]. Note that these two models are not mutually exclusive, each could be part of the answer.

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For instance, the first could explain how polarity is organized in the tissue as a whole to give all cells a common orientation; the second might explain how polarity is determined in individual cells and coordinated with neighboring cells.

We study the abdominal epidermis, a sheet of cells encompassing several segments, of adult *Drosophila*. Each segment is subdivided into an anterior (A) and a posterior (P) compartment. The secreted protein Hedgehog (Hh), which travels from P to A cells, is responsible for organizing the pattern of cell differentiation as well as planar polarity [13–18].

Results and Discussion

Four-Jointed and Polarity

Fj is a type II transmembrane glycoprotein that may be cleaved and secreted [19]. There is a vertebrate homolog [20]. Both in the fly eye and in the wing, it is expressed in a graded manner [19, 21–23]. Studying clones ectopically expressing *fj* in the wing, Zeidler and colleagues concluded that the orientation of hairs is reversed distal to some clones, and this finding suggests that neighboring cells point *toward* the ectopic source of Fj, that is *up* the presumed gradient of Fj. However, they also looked at the abdomen, where cells make hairs that point posteriorly. Here, they found that clones expressing Fj reverse the polarity of abdominal hairs anterior to the clone, as if the hairs were pointing *away* from the source of ectopic Fj and *down* the presumed gradient. The results on the wing and abdomen, therefore, appear to differ in sign [23].

Expression of a *fj* Enhancer Trap

To find where Fj is expressed relative to the A and P compartments of the abdomen, we used a *fj.lacZ* transgene [21]. In the dorsal epidermis, which forms the tergite (Figure 1A), expression is concentrated both in the bristled portion of the A compartment [22] (the a3–a5 territories; Figure 1A) and in the most anterior portion of the A compartment (a1–a2). Strong expression is observed in the sternite, which also forms bristles. However, the remainder of the ventral epidermis forms pleura, a lawn of cells that secretes only hairs, and here the pattern of expression is simple: there is a band of staining near the front of the A compartment (Figure 1D). One attractive interpretation is that the *fj.lacZ* transgene may pick up extraneous enhancers active in bristly (neurogenic) cuticle; therefore, we only see the "true" Fj pattern in the nonneurogenic pleura. This is attractive because it is consistent with the following independent data suggesting that there is a gradient of Fj activity, with its peak at the front of A.

The Abdomen in Flies Mutant for *fj*

Flies that lack *fj* activity in all cells show some effects on pattern [19]. In the abdomen, there is some disheveled of hairs and bristles, but only in the anterior portion of the A compartment (in a2 and a3, Figure 1A). It seems, therefore, that planar polarity is specified almost normally in the absence of Fj.

Removal of the *fj* Gene in Clones

We label the clones genetically so that each cell of the clone can be distinguished from its neighbors. Within the A compartment, fj^- clones are abnormal, but only when they are located in approximately the front half of the compartment (Figures 2A and 2B). Each cell typically produces little groups of posteriorly pointing hairs arranged in neat mediolaterally oriented rows (Figure 2B); but, within affected fj^- clones, the rows of hairs are jumbled (Figure 2A). Also, the hair orientation is disturbed, with most of those at the back of the clone, and the wild-type hairs behind it, being reversed (Figure 2A). Our impression is that, the further anterior the clone, the more disturbance within and the more reversal of hair polarity behind. In the posterior part of the A compartment, removing *fj* from clones has no effect on the orientation and arrangement of hairs; all such clones are exactly like controls in which only the marker gene, *pwn*, is missing (Figure 2B).

The effects of fj^- clones on polarity in the P compartment can only be assayed in the p3 territory — because only p3 cells make hairs, while p2 and p1 cells are bald (Figure 1A). Some clones in the p3 territory form disheveled and incorrectly polarized hairs; however, most appear normal. We found no clear cut cases of non-autonomous effects outside these P clones. Note that the p3 region is remote from the presumed peak of Fj near the front of the A compartment.

Thus, endogenous Fj activity production appears to be required in the front half of the A compartment. In this region, clones of *fj* mutant cells cause surrounding cells to make hairs that point inward, and this suggests that the hairs point down the gradient of Fj protein (see Figure 3).

Clones that Ectopically Express Fj Protein

We have used various *Gal4/UAS* and *G80* techniques to make marked clones of cells that produce ectopic Fj protein. Different levels of Fj expression were achieved with two Gal4 drivers of different strengths.

In the A compartment, clones of cells in which *UAS.fj* is expressed under the control of the weaker driver (*abx/ubx.Gal4*) cause a polarity phenotype when they are located in the back of A, but not when they are elsewhere. Within the clone, there are whorls, and these can extend outside the clone in the anterior, but not the posterior, direction. Note that this is opposite to the phenotype of the fj^- clones in two ways: first, *UAS.fj* clones cause polarity changes if they are at the back of the A compartment, while fj^- clones cause changes only at the front. Second, fj^- clones cause a reversal of polarity behind the clone, while *UAS.fj* clones alter polarity in front. Clones within the P compartment appear normal.

With the stronger driver (*tub.Gal4*), the phenotype is more definite; there are few whorls, and, instead, the hairs are reversed within the anterior part of the clone, and this reversal extends anterior to the clone itself (Figures 2C and 2D) [23]. These non-autonomous effects can spread as much as 6 or 7 cell diameters. This effect is found over most of the A compartment and includes clones in the anterior region of the bristled cuticle (a3) that cause extensive reversal anterior to them (in a2)

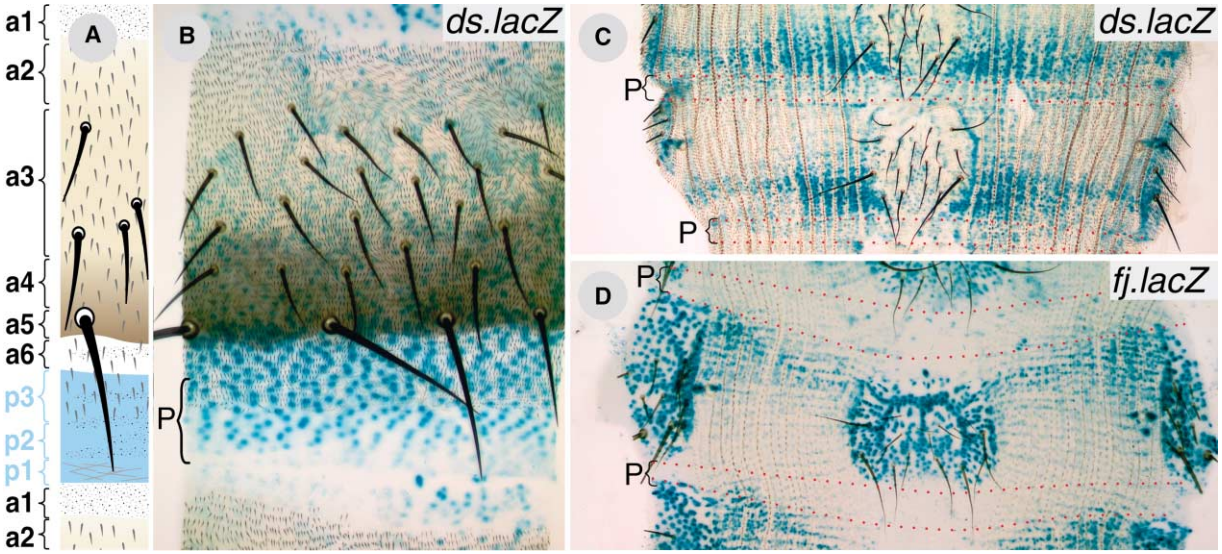

Figure 1. The Pattern of *ds* and *fj* Expression, as Indicated by Enhancer Traps

(A) A diagram of the anatomy of a segment of the dorsal epidermis showing the disposition of types of cuticle. The P compartment is shown in blue [17]. In all figures, anterior is toward the top of the page, the outlines of the clones are indicated, and red arrows indicate the zones of reversed polarity.

(B) *ds.lacZ*. One segment of the tergites. There is strong expression in a single band that includes most of the P compartment and the back of the A compartment; it is weaker elsewhere.

(C) *ds.lacZ*. Ventral view. We see strong bands centered at the back of each A compartment and grading away forward and backward. The approximate boundaries of the P compartments are indicated by red dots.

(D) *fj.lacZ*. Ventral view. In the pleura, we see strong bands centered near the front of the A compartments.

(Figure 2C). However, some clones within the extreme anterior portion of the a2 region cause little or no changes in polarity anterior to the clone (Figure 2D). This difference could be because cells at the extreme anterior of A normally make a large amount of Fj protein, so overexpressing the gene there might have little impact on the landscape of concentration. Thus, the behavior of A clones that lack or overexpress *fj* (Figure 2F) suggests that changes of polarity are induced wherever there is a difference in levels of Fj between the clone and its immediate neighborhood. All the results fit nicely with this idea and argue that there is normally a gradient of Fj that is high at the front of A and low at the back (Figure 3).

In the P compartments, these *UAS.fj* clones are also associated with polarity reversals. But here, consistently, hairs within the back half of the clone as well as hairs *behind* are reversed and now point anteriorly (Figure 2E). This contrasts with effects of *UAS.fj* clones located at the other side of the A/P boundary in the posterior of the A compartment (in the a6, a5, a4, and a3 territories). In this case, hairs in the anterior half of the clone, as well as in *front* of the clone, point anteriorly (Figures 2C and 2D). Thus, in both cases, polarity reversals are observed in territories farthest from the apparent source of Fj activity; however, in the A compartment, hairs point *away* from the ectopic *UAS.fj* source, whereas, in the P compartment, hairs point *toward* the source (Figure 2F). These results support a model in which the A and P compartments have opposing gradients of Fj activity, and cells within each compartment are programmed to respond to the vector of Fj activity by secreting hairs that point down the gradient in A but up the gradient in P (Figure 3).

UAS.fj Clones near the Compartment Boundaries

Clones situated near the boundaries between the A and P compartments raise new problems. Consider first *UAS.fj* clones at the back of the A compartment — the interface between posterior A and anterior P cells. These clones reverse the hairs in front of the clone, which would be normal for clones in the back half of A. The back of the clone is itself made of A cells; however, they abut P cells behind them, and these P cells are also reversed (Figures 4A and 4D). Apparently, polarity effects (and maybe the ectopic Fj protein) can cross over the parasegment boundary from A to P.

Now consider clones at the front of P; these clones would be expected to reverse the P cells behind them, and they do. However, they might also be expected to reverse the cells in the A compartment in front of them, but they do not (Figures 4B and 4D). Perhaps polarity effects crossing over from P to A are blocked [13]. Some rare clones may give some insight (Figure 4C): the clone illustrated is very thin and is mainly confined to the most posterior cell or two of the A compartment and extends laterally for several cells. Behind it, the P cells are extensively reversed, and yet, in front of it, the A cells are little affected — just as if the clone were a P clone. Both of these examples might be explained if, normally, Hh induces cells in posterior A (say, the a6 region) to sequester and/or destroy ambient Fj. This would create a local sink for Fj at the point farthest from its source and help build the gradient of Fj (compare with [24], for

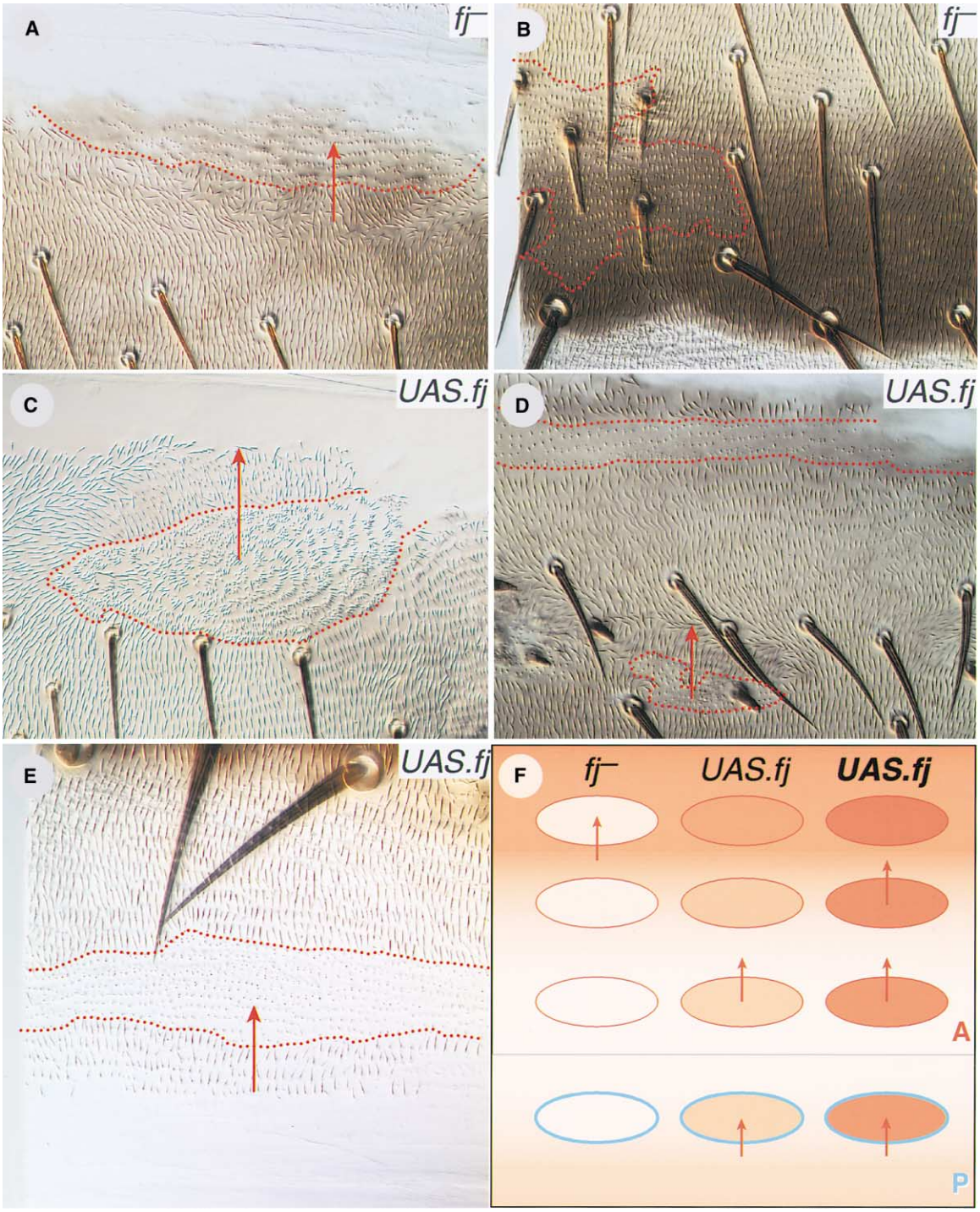

Figure 2. Clones Lacking and Overexpressing *fj*

(A) A fj^- clone situated far anterior in the A compartment (it is marked with *pawn*, which makes finer hairs and depauperate bristles). It reverses the polarity of cells behind it.

(B) A fj^- clone, as in (A), but situated posterior within the A compartment. It makes cuticle of normal polarity.

(C–E) Clones overexpressing *fj*. (C) A clone in the anterior part of A: it reverses hairs in front. The clone is marked with *tricornered* (*trc*), which makes multiple hairs and scraggy bristles. Note that the *trc* pattern of hairs indicates cell shapes, and, in the clone, these appear to form a concentric pattern. (D) A large clone marked with *pawn* at the extreme front of A that has no phenotype, and posterior to it, a small clone that reverses in front. (E) A clone in the P compartment, marked with *pawn*, that reverses polarity behind it.

(F) A summary of the polarity effects of *fj* clones. Clones in the P compartment are edged in blue. *fj* is expressed at the front of A and may also be expressed at the back of P.

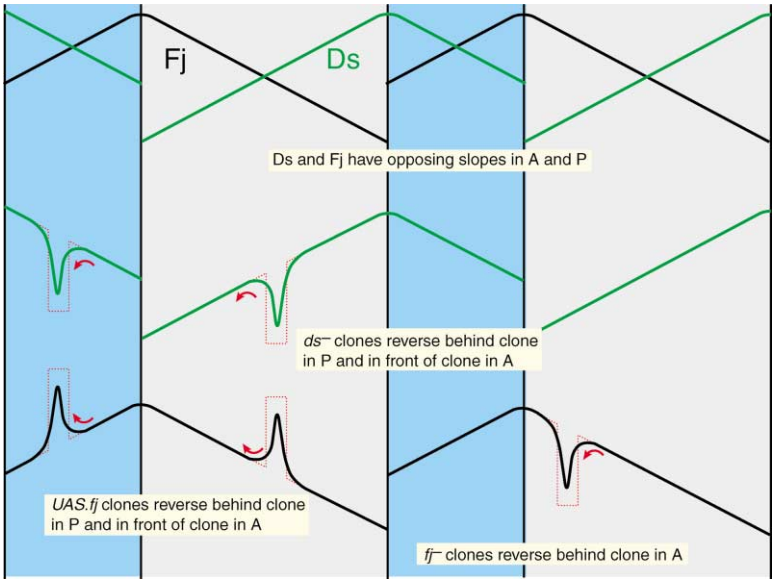

Figure 3. Gradients and Polarity

This figure models the gradients and illustrates how different directions of slope, as shown for Fj and Ds, can give the observed results. For simplicity, we do not show the activity gradient of Ft or the consequences of removing Ft activity, but both should be similar to that of Fj. We imagine that the clones either remove Fj, Ds, or Ft activity (mutant clones) or augment Fj (*UAS.fj*). Spreading or diffusion of either the protein itself, or some consequence of it, will set up new slopes as indicated. If the vector of each slope or some derivative of it is read to give the polarity, then the reversals of polarity (in front or behind the clone) should be as we observe. Red arrows mark the places where the gradient vector is reversed.

Wingless [Wg]). Overexpression of *fj* at the back of A might make enough protein to overcome this sink, thus creating an ectopic peak of Fj extending both anteriorly into A and posteriorly across the A/P boundary into the P compartment, with consequent polarity reversal in both compartments. By contrast, overexpression of Fj in P (or by just one row of cells at the back of A) might not generate sufficient Fj across the boundary to overwhelm the sequestering activity of A cells, so only cells in P would see an ectopic peak and be repolarized.

Opposite Effects of *dachsous* and *fat* on Polarity in A and P

We have found two other genes that resemble *fj* with regard to compartment-specific effects: *dachsous* (*ds*) and *fat* (*ft*). In both cases, *UAS* transgenes cannot be easily made, so we have studied only the effects of removing the gene.

dachsous, ds

Ds is a giant integral membrane protein with many cadherin domains [25], and there is a vertebrate homolog [26]. We have monitored *ds* gene expression using a *ds.lacZ* transgene. In each segment of the tergites, *ds.lacZ* is expressed in one band per metamere with a peak near the A/P border that extends into both compartments (Figure 1B). This single band is more clearly apparent in the pleura and appears to be centered in a more anterior location than in the tergite or sternite (Figure 1C).

ds^- flies are lethal, but some hypomorphic mutants survive to adulthood with defective limbs — the tarsi show polarity defects [27]. In the abdomen of these flies, the anterior parts (a2, see Figure 1A) of the A compartments are fairly normal, but much of the rest of the A and P compartments is affected by whorls. Remarkably, hair orientation in the back half of the P compartments, both dorsal and ventral, is reversed (Figure 5A).

In the tergites, ds^- clones are characterized by whorling hairs within the clone (Figure 5B). They cause some swirly repolarization of the hairs in front of the clone in the A compartment, but not behind (Figure 5C). These whorls could indicate that there has been a loss of overall polarity, even though some local coordination between adjacent cells remains. In the P compartment, ds^- clones induce clear reversal of hairs behind the clone without affecting the front (Figure 5D). Just as with clones ectopically expressing *fj*, those situated at the back boundary of the A compartment reorient hairs outside the clone, both anterior to the clone (A cells) and posterior to it (P cells) — but hairs within the clone are more whorly than with *fj*-expressing clones.

Thus, apart from the whorls, ds^- clones are reminiscent of *UAS.fj* clones; both cause non-autonomous reversals in opposite ways in the A and the P compartment. Accordingly, Ds, like Fj, may form opposing gradients in A and P, each being interpreted with opposite signs. Because loss of Ds activity mimics gain of Fj activity, we deduce that the gradients of Fj and Ds activity are reciprocal to each other (Figure 3), a conclusion that fits with the expression pattern of both genes in the pleura (Figures 1C and 1D).

fat, ft

Like *ds*, *ft* encodes a huge molecule with many cadherin repeats [28], and as with *ds*, null mutant flies do not develop. A vertebrate homolog has been defined [29, 30]. The mutant imaginal discs grow excessively, and there are some effects on the polarity of bristles [31]. Clones of ft^- cells in otherwise wild-type discs are abnormally large [28]; in the abdomen, these clones tend to be creased, as if they were trying to grow beyond their normal compass.

In the A compartments of the tergites, ft^- clones tend to disturb and reverse polarity behind the clone (Figure 6A), while, in the P compartments, they tend to reverse in front (Figure 6B). Thus, ft^- clones, like ds^- and fj^- clones, have opposite effects on polarity in A and P. When the ft^- clones are near the A/P boundary, they behave as would be expected from the provenance of the cells neighboring the clone: clones at the back of the A compartment fail to reverse the P cells behind (P

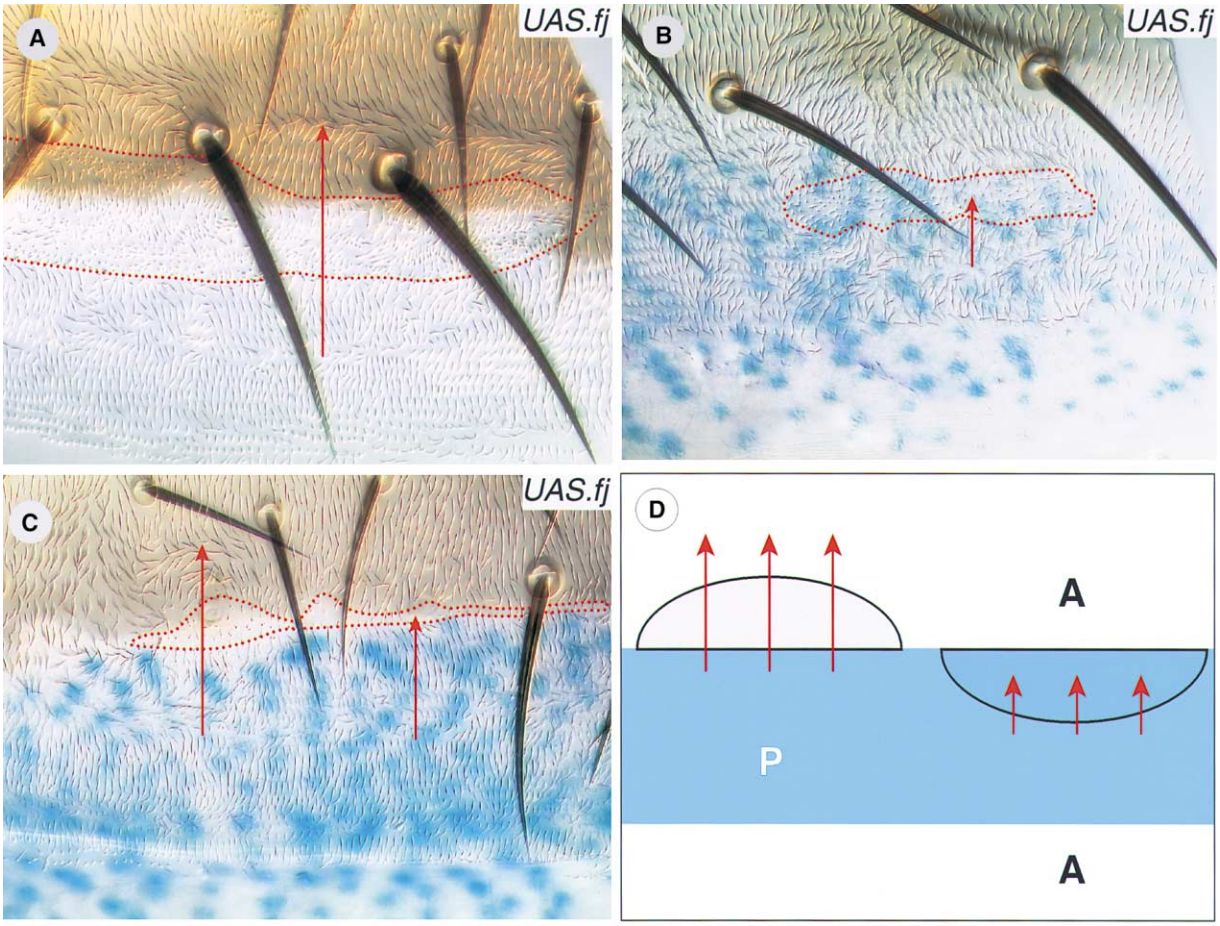

Figure 4. Clones Overexpressing *fj* that Are near the A/P Border

(A) This clone belongs to the A compartment and is marked with *trc*; it abuts the A/P border. Behind it, the cells in P are reversed, as are the cells in A in front and the cells of the clone itself.

(B) A clone located at the front of the P compartment. Note that it reverses behind but has no effect on polarity of the A cells in front. The nuclei of P cells are marked with *hh.lacZ*.

(C) Another A compartment clone, but this one is narrow and runs along the compartment boundary. Note that the polarity of the P cells (marked with *hh.lacZ*) behind is extensively reversed, but there is only slight reversal anteriorly — at a point where the clone is a little broader (compare with 5A). The a6 region (see Figure 1A) is unusually thin in this segment, both near and far from the clone.

(D) A summary of the polarity effects of clones near the A/P border. On the left, a clone in A resembles 4A. On the right, the clone in P resembles 4B.

cells normally reverse in front of a ft^- clone), and P clones fail to reverse A cells in front of them (A cells normally reverse behind a ft^- clone).

Thus, ft^- clones, like ds^- and fj^- clones, have opposite effects on polarity in A and P. Further, the effects of ft^- clones are similar to those of fj^- clones but are opposite to those of *UAS-fj* and ds^- clones. For example, in the A compartment, hairs point toward ft^- clones but away from *UAS-fj* clones, whereas, in P, they point away from ft^- clones but toward *UAS-fj* clones. Using the logic deployed with *fj* and *ds* (Figure 3), we infer that Ft activity is reflected like that of Fj, forming a peak at the segment boundary and declining to a trough at the A/P boundary. But note that ft^- clones can cause polarity reversals anywhere within A, as well as in anterior P — but fj^- clones do so only in anterior A. This difference argues for a model in which Fj is produced only by cells flanking the segment boundary and acts non-autonomously on cells further away, whereas Ft activity might be required autonomously in all cells, with any differential in Ft activity between neighboring cells determining their polarity.

Comparison with the Eye

The three genes *ds*, *ft*, and *fj* are functionally linked: mutations in all three damage the tarsi in a similar way [27]; *ds* and *ft* encode similar cadherin molecules [25, 28], and they and *fj* interact genetically [25, 32].

Recently, for the *Drosophila* eye, Yang et al. [33] have proposed that the products of *ds*, *ft*, and *fj* work together in a linear pathway in the developing ommatidia. This pathway begins with a gradient of Wg and leads to the differential activation of Fz in the presumptive R3 and R4 cells. According to their model, graded Wg spreads into the eye from sources at the dorsal and ventral poles, induces Ds expression, represses Fj expression, and thereby generates reciprocal Ds and Fj gradients. Fj

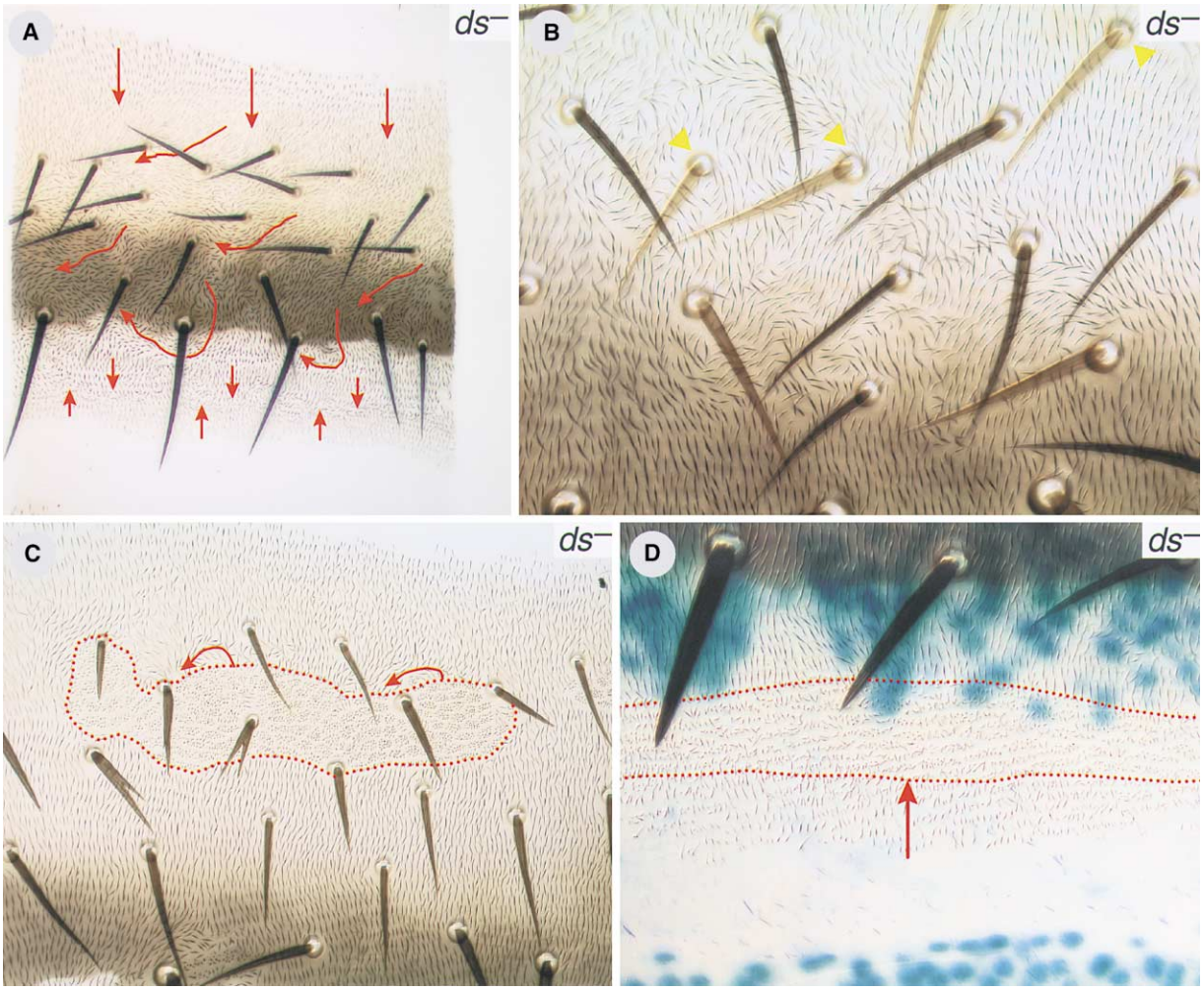

Figure 5. *dachsous* and Polarity

(A) A segment of a fly strongly hypomorphic for *ds*; note that, apart from the most anterior regions of the A compartment, the hairs show disturbed and whorly polarity. At the back of the P compartment, the hairs are reversed.

(B) A clone of *ds*$^{-}$ cells, marked with *yellow* and near some *yellow* bristles (arrowheads). The zone of whorly hair orientation indicates the epidermal extent of the clone.

(C) A *ds*$^{-}$ clone in the A compartment, marked with *crinkled* (*ck*) (making numerous hairs per cell and damaging the bristles). Note that anterior, but not posterior, to the clone, there are hairs with disturbed orientation.

(D) A *ds*$^{-}$ clone in the P compartment. Posterior to the clone, the orientation of hairs is reversed. We could not determine polarity within this clone because of the *ck* phenotype.

activity then represses Ds activity and reinforces this reciprocity. In turn, the Ds gradient then patterns the activity of Ft, which is ubiquitously expressed. Finally, the gradient of Ft activity promotes the activation of Fz in the more equatorial cell and directs it to become the R3 cell, while the more polar cell becomes the R4 cell [34–37].

Our present results point to parallels between the action of Fj, Ds, and Ft in the eye and abdomen. In both cases, a morphogen (Wg in the eye, and Hh in the abdomen) appears to govern polarity through the induction of reciprocal gradients of Fj and Ds expression. Further, in the abdomen, Hh organizes polarity at least in part through the induction of Wg [13]. Hence, as in the eye, peak Wg activity occurs where *fj* is repressed and where *ds* is expressed. Finally, our results suggest that the gradient of Ds in the abdomen is reciprocal to that of Ft activity, consistent with the model proposed for the eye. These parallels suggest that the three genes are part of a mechanism common to the eye and abdomen and presumably elsewhere.

Developmental Compartments and Polarizing Signals

Our results argue that, in the abdomen, the compartmental provenance of responding cells is crucial. This is particularly clear for clones that either lack or overexpress *fj*. We find that, in the A compartment, hairs point down gradients of Fj activity, while, in the P compartment, they point up. This discovery can help explain how all cells in the abdominal epidermis make hairs that have the same polarity, even though, in both compart-

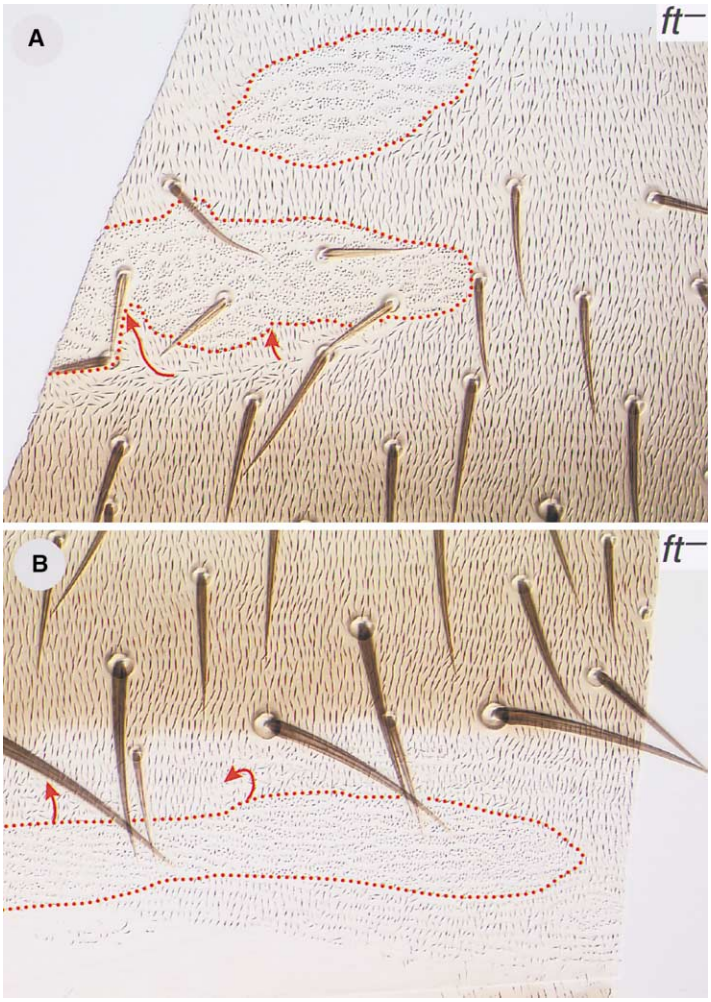

Figure 6. *ft*⁻ Clones and Polarity

(A) The clones are marked with *stubby chaete* (*stc*), a mutation with a similar phenotype to *trc* and *ck*. Two clones in the A compartment; the lower one has reversed hairs behind it. Variation in the amount of reversal is typical with *ft*⁻ clones; the likelihood of reversal does not appear to depend on the position of the clone.

(B) A clone in the P compartment; anterior to the clone there are disturbed hairs, and some are reversed.

ments, the gradients of Fj and Ds decline in opposite directions (Figure 3). However, it presents other problems.

One problem is that we previously proposed that Hh drives polarity by inducing a gradient morphogen, X, whose slope specifies polarity [13, 17]. The model is that Hh enters the A compartment from the P compartment behind it and acts through *wg* and *optomotor blind* (*omb*) to induce X. For simplicity, we conjectured that X might form a monotonic gradient, spreading forward from its peak at the back of the A compartment all the way to the front of the P compartment of the next segment [13]. According to this conjecture, all cells in both A and P make structures that point posteriorly because all respond to the common vector of a monotonic gradient of X.

However, our present results argue for reflected gradients centered around the A/P compartment boundary and against a monotonic gradient for X. Thus, we now surmise that Hh induces reflected gradients of Fj, Ds, and Ft activity. It is instructive to compare the imaginal discs with the abdomen. In the *discs*, unidirectional Hh signaling across the A/P boundary induces the morphogens Decapentaplegic (Dpp) and Wg, and these then spread both anteriorly and posteriorly and create reflected gradients that pattern both compartments [38, 39]. In the *abdomen*, Hh also induces Wg (in the tergites and sternites) and Dpp (in the pleura). At least in the tergites, Wg then spreads posteriorly from its source at the back of the A compartment to induce *omb* and specify cell pattern in the P compartment. Thus, the combined activities of Hh in A cells, and of Hh-induced Wg moving back into P cells, generates a zone of Omb expression spanning the A/P boundary [13]. We now suggest that this band of Omb organizes the reflected gradients of Ds and Fj, which in turn, through Ft, help polarize the cells. Thus, the combined actions of Fj, Ds, and Ft might constitute what we have previously called X.

Another problem is raised by our finding that cells in the A and P compartments interpret the polarizing activities of Fj, Ds, and Ft with opposite sign. In the wing, gene products such as Fz and Dsh accumulate transiently along the distal edge of each cell and forecast both the site and distal direction of hair outgrowth [6, 8]. Further, wing hairs invariably point away from *UAS.fz* clones and toward *fz*⁻ clones, and this suggests that these subcellular localizations could be a readout of differential Fz activity [6–9]. We find that *UAS.fz* and *fz*⁻ clones in the abdomen behave like their counterparts

in the wing, whether in the A or P compartment — in all cases, hairs point away from *UAS.fz* clones and toward *fz*$^{-}$ clones (unpublished data). Thus, we infer, that in the normal abdomen, Fz and Dsh accumulate along the posterior edge of both A and P cells, even though the controlling gradients of Fj, Ds, and Fat in the A compartment have the opposite slopes of those in the P compartment.

How might A and P cells be programmed so that bidirectional activity gradients of Fj, Ds, or Ft lead to a unidirectional slope of Fz activity? We suggest that a transcription factor, Engrailed, encoded by the selector gene that distinguishes P from A cells [40], also alters the response of P cells relative to A cells — so that, in A cells, Fz might accumulate at the cell edge where Fj is lowest, while, in the P cells, it might accumulate where it is highest (cf Figure 3). The result would be a localized accumulation of Fz along the posterior edge in all cells, whether in A or P. A precedent comes from yeast, where haploid (a or α) cells bud axially near prior budding sites, while diploid (a/α) cells bud in a bipolar fashion at the site farthest from the previous bud. In yeast, this switch in polarity is also governed by transcription factors encoded by the mating-type locus [41].

But this new model raises yet another challenge: consider the pleura, which is formed by a sheet of cells spanning several segments, all of which secrete hairs that point posteriorly. If, for example, the localization of Fz in each cell were controlled by the graded activity of Ft, then these Ft gradients would need to be precisely coextensive with the compartments. Otherwise, some cells would read gradients with the wrong sign and make hairs that point in the wrong direction. This could be most critical at the boundaries between the A and P compartments, where the gradient landscape of Ft should be forming peaks or troughs and hence might be relatively flat. This challenge could be resolved if, in a later and/or independent process, cell polarity were locally coordinated, for which there is some evidence. For example, clones of cells that lack or overexpress Fz can cause local reversals in hair polarities that propagate a few cell diameters beyond the clone borders [8, 10, 11].

Genetic Pathways and Redundancy

In the abdomen, there are observations that do not fit with a simple linear pathway as proposed for the eye [33]. For example, hair polarities are not randomized in *fj*$^{-}$, *ds*$^{-}$, or *ft*$^{-}$ mutant tissues, and even entirely *fz*$^{-}$ flies show relatively normal polarity in most regions. Nevertheless, consistent changes in polarity are generated by disparities in the activity of each of these polarity genes, usually across clone borders. Hence, cell polarity may depend on multiple signals of which the mutually reinforcing effects of Fj and Ds are but one example.

Polarity and Growth

Mutations that cause a reduction in cell division are common, but those, such as *ft*, that cause increased growth are rare. The *ft* gene may be a link between planar polarity and growth — it has been suggested that a morphogen gradient may control both. If the slope or vector of a morphogen is used to specify planar polarity, the local steepness of that same gradient might provide a measure of dimension. This measure would then help determine the probability of cell division and apoptosis, regulate the rate of net growth, and limit the final size [42].

Experimental Procedures

The mutant alleles and transgenes used in this work are as follows [16, 27]: ***ft***$^{-}$: *ft*^{15}, a truncated form at the 32 cadherin domain of Fat; ***ds***$^{-}$: *ds*^{UAO71}, a strong *dachsous* allele; ***ds.lacZ***: *ds*^{2D60b}, an enhancer trap insertion at *dachsous*; ***fj***$^{-}$: *fj*^{d1}, a deletion of *four-jointed*; ***UAS.fj***: *fj*$^{Scer\UAS.cZa}$; ***ptc.lacZ***: *EcoI\lacZ*$^{ptc\text{-}AT96}$; ***en.Gal4***: *Scer\GAL4*$^{en-e16E}$; ***ptc.Gal4***: *Scer\GAL4*$^{ptc\text{-}559.1}$; ***hh.lacZ***: *hh*^{P30}; ***hh.Gal4***: *Scer\GAL4*$^{hh-MAT29}$; ***hs.FLP***: *Scer\FLP1*$^{hs.PS}$; ***abx/ubx>f***$^{+}$***>Gal4-lacZ***: *Scer\GAL4*$^{Scer\FRT.Ubx}$; ***tub.Gal4***: *Scer\GAL4*$^{Tub84B.PL}$; ***tub.Gal80***: *Scer\GAL80*$^{Tub84B.PL}$; ***CD2y***$^{+}$: *Rnor\CD2*$^{hs.PJ}$; ***FRT***: *P{FRT(w*^{hs}*)}39*, *P{neoFRT}40A*, *P{neoFRT}42D*, *P{FRT(w*^{hs}*)}2A*.

Clonal Analysis

Unless stated otherwise, clones were induced by heat shocking either embryos or larvae of the following genotypes at 34°C or 37°C: ***ft***$^{-}$ **clones:** *y hs.FLP; ft*$^{-}$ *stc FRT39/CD2y*$^{+}$ *FRT39; ptc.lacZ/+*; ***ds***$^{-}$ **clones:** *y w hs.FLP; Dp(1;2)sc*^{19} *FRT40A ptc.lacZ/ds*^{UA071} *ck FRT40A*; ***fj***$^{-}$ **clones:** *y hs.FLP; FRT42D pwn fj*$^{-}$*/FRT42D CD2y*$^{+}$; ***UAS.fj* clones:** i) *y w hs.FLP; FRT42D pwn UAS.fj/; FRT42D tub.Gal80 CD2y*$^{+}$*; hh.lacZ/tub.Gal4*; ii) *y w hs.FLP tub.Gal4/y w hs.FLP; UAS.fj fj*$^{-}$*/CyO; tub.Gal80 FRT2A/CD2y*$^{+}$ *trc ri FRT2A*; iii) *w/hs.FLP*; *abx/ubx>f*$^{+}$*> Gal4-lacZ/UAS.fj*.

Abdominal cuticles containing marked clones were dissected and mounted in Hoyer's medium. Detection of β-Gal activity was carried out as in [16]. Images were captured with Auto-Montage (Syncroscopy).

Acknowledgments

We thank Paul Adler, Peter Bryant, David Strutt, and the Bloomington Stock Center for helpfully sending us stocks. G.S. is an Investigator of the Howard Hughes Medical Institute. J.C. and P.L. are supported by the Medical Research Council, UK.

Received: April 11, 2002
Revised: May 21, 2002
Accepted: June 11, 2002
Published: July 23, 2002

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