Epithelial planar polarity in the developing *Drosophila* eye

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SUMMARY

Experiments with the insect ectoderm have suggested that planar polarity in epithelia results from the local orientation of cells to the slope of a gradient of positional information. Here we show that planar polarity in the *Drosophila* eye is inverted when the morphogenetic wave that sweeps through the presumptive retinal epithelium is induced to move in the reverse direction. We suggest that the movement of the morphogenetic wave may be causal in establishing the planar polarity of this epithelium.

Key words: planar polarity, epithelium, chirality, pattern formation, *Drosophila*, eye

INTRODUCTION

A key problem in developmental biology is how the patterning of cells can be coordinately organized over long distances. A good example of this long-range coordination is the planar polarity seen in insect epithelia (Nübler-Jung, 1987; see Lawrence, 1992) and manifested by the differentiation of cuticular structures such as hairs, bristles or ridges, all oriented in the same direction (usually 'pointing' posterior) (Fig. 1A, Wigglesworth, 1940). This phenomenon has been investigated extensively in the ectoderm of many different insects using surgical manipulations (for example; Piepho, 1955; Lawrence, 1966; Stumpf, 1967; Nübler-Jung, 1987).

In *Drosophila,* molecular and genetic analyses of planar polarity have focused largely on the ectoderm and have examined the orientation of hairs and bristles (Gubb and Garcia-Bellido, 1982; Vinson et al., 1989; Theisen et al., 1994) but have examined only cursorily the planar polarity in the developing retina (Theisen et al., 1994; see Gubb, 1993). The developing eye is an attractive system with which to study epithelial polarity since the establishment of the cellular patterns has been described in fine detail and the cellular arrangements and orientation of ommatidia are faithful indicators of the epithelial polarity (see below).

The *Drosophila* compound eye is an assembly of hundreds of sub-unit ommatidia, each of which is an asymmetrical cellular cluster (Fig. 1B) that can occur in one of two chiral forms (Fig. 1C,D). In the dorsal region of the eye the ommatidia are the mirror image of those in the ventral region and the two types meet at a line of mirror reflection termed the equator (Dietrich, 1909) (Fig. 1C,D). The right eye is the mirror reflection of the left (Fig. 1E) and thus the chiral form found in the dorsal right eye is found ventrally in the left eye.

The ommatidia in a particular half of the eye collectively manifest two planar polarities – the anterior/posterior (A/P) and equatorial/polar (Eq/Pl) axes. Ommatidia orient with photoreceptors R1,2,3 lying to the anterior and photoreceptors R3 and R4 lying away from the equator towards the pole (Fig. 1D). Reversal of either axis will switch the chiral form of an ommatidium (Fig. 1F), and analysis of the chiral forms of the ommatidia can therefore be used to indicate the success of experimental interventions designed to alter planar polarities. In this paper we describe experiments performed to determine the mechanism that establishes the retinal A/P polarity.

During the development of the eye, a wave of morphogenesis sweeps across the presumptive retinal epithelium from posterior to anterior, leaving in its wake a smoothly graded series of developing ommatidia (Ready et al., 1976). An indentation in the tissue called the morphogenetic furrow is an overt feature of this moving front. Cells behind it cluster into proto-ommatidia and begin to differentiate as photoreceptors (Ready et al., 1976). A self-propagating signaling mechanism has been proposed to account for the movement of the morphogenetic front. The differentiating cells behind the furrow secrete hedgehog (Hh), a protein thought to diffuse from these cells. The unpatterned tissue directly anterior responds to hh by expressing decapentaplegic protein (Dpp – a protein of the TGFβ family) (Heberlein et al., 1993; Ma et al., 1993). Cells expressing *dpp* subsequently organize into ommatidia, begin to differentiate as photoreceptors, secrete Hh and signal more anterior expression of *dpp*. This cycle of cells receiving the hh signal and subsequently sending this signal could therefore account for how the wave of morphogenesis sweeps across the epithelium (Heberlein et al., 1993; Ma et al., 1993).

The movement of the morphogentic wave is a striking feature of the retina's development and raises the question of whether the sweep of the wave could be involved in establishing the anterior/posterior (A/P) polarity of the epithelium. Previous studies of A/P epithelial polarity in the insect retina have suggested that ommatidia orient by referring to the slope of an underlying gradient of positional information (Lawrence and Shelton, 1975). To distinguish between these possibilities we have reversed the direction of the morphogenetic wave and examined the chirality of the emerging ommatidia. The A/P
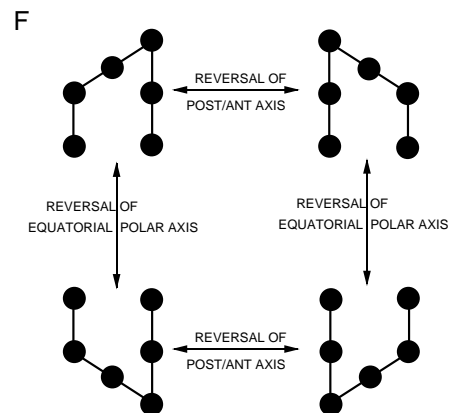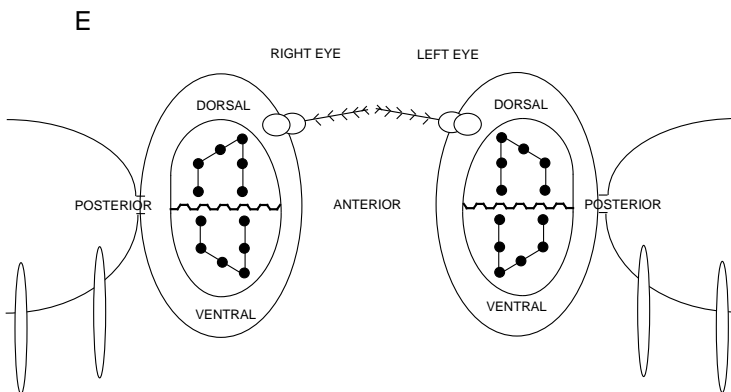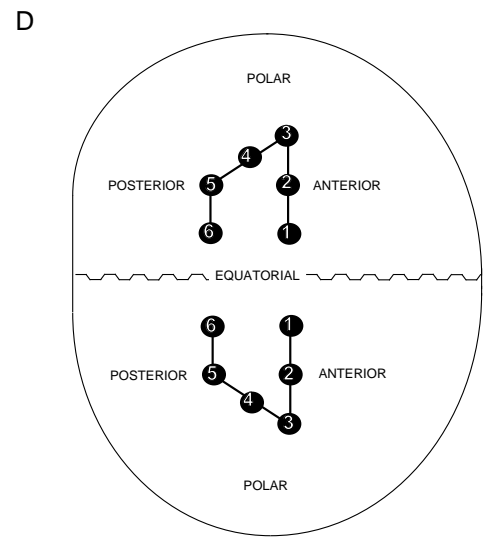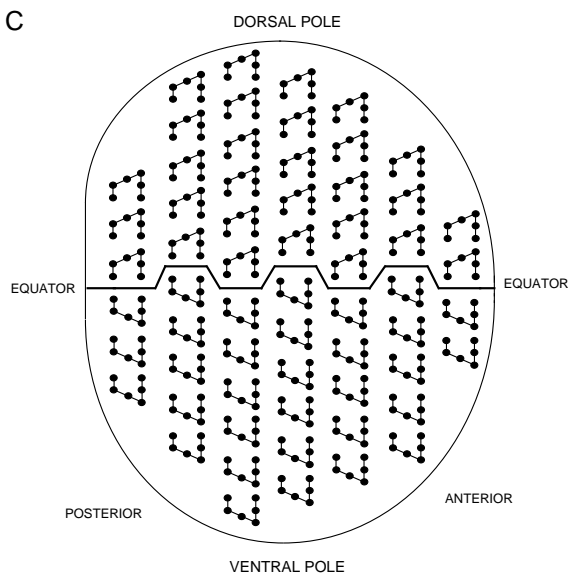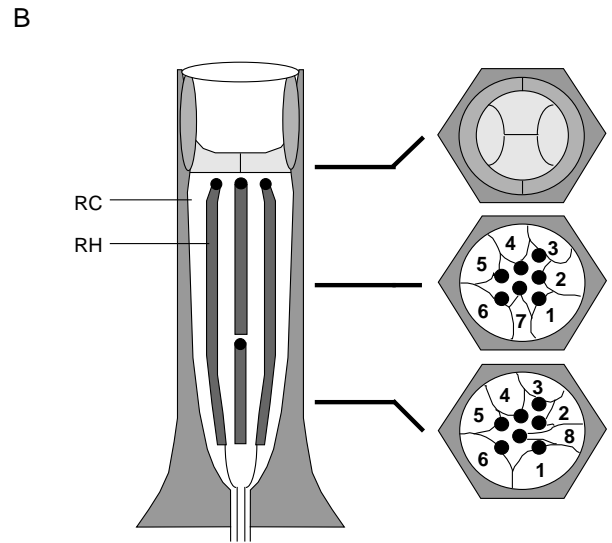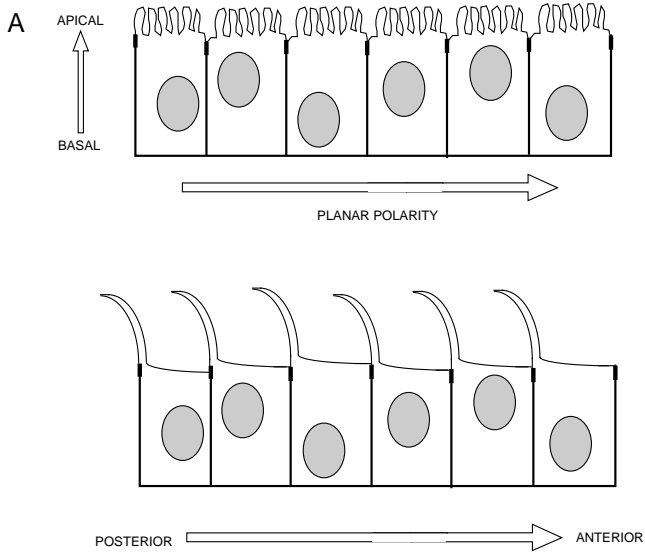
polarity of the epithelium inverts coincidentally with the reversal of the morphogenetic wave, suggesting that the movement of the wave and the establishment of epithelial A/P polarity are intimately linked. Further to this we argue that the movement of the wave may be causal in establishing the A/P epithelial polarity.

MATERIAL AND METHODS

Generating clones of marked cells

Clones of mutant cells were generated by *flp*-mediated recombination as described by Xu and Rubin (1993). The presence of *ptc^S2* and *DCO* clones was verified using the cell marker transgene, hsp70-CD2, (Dunin-Borkowski and Brown (1995) and used as described by Jiang and Struhl (1995).

ptc^S2 and *DCO* clones were induced by incubating larvae for 2 hours at 37°C at 24-48 and 48-72 hours of development. The eye imaginal discs were dissected from late third instar larvae.

Double staining of eye imaginal discs

Dissected eye imaginal discs were double stained using di-amino-benzidine and mounted as described in Campbell et al. (1993). Double immunofluorescence was monitored as described by Basler and Struhl (1994).

Sectioning of adult eyes

Eyes with clones lacking pigment were dissected, fixed, embedded and sectioned according to the method of Tomlinson and Ready (1987b).

Fly stocks

Stocks for the *flp*-mediated recombination were 40w⁺F, 42w⁺F (Xu and Rubin, 1993), *ptc^S2* (Philipps et al., 1990), CD2L.1 y⁺, CD2R.1

y⁺, (Jiang and Struhl, 1995), *dpp^10638* and dpp^BS3.0 (Blackman et al., 1991).
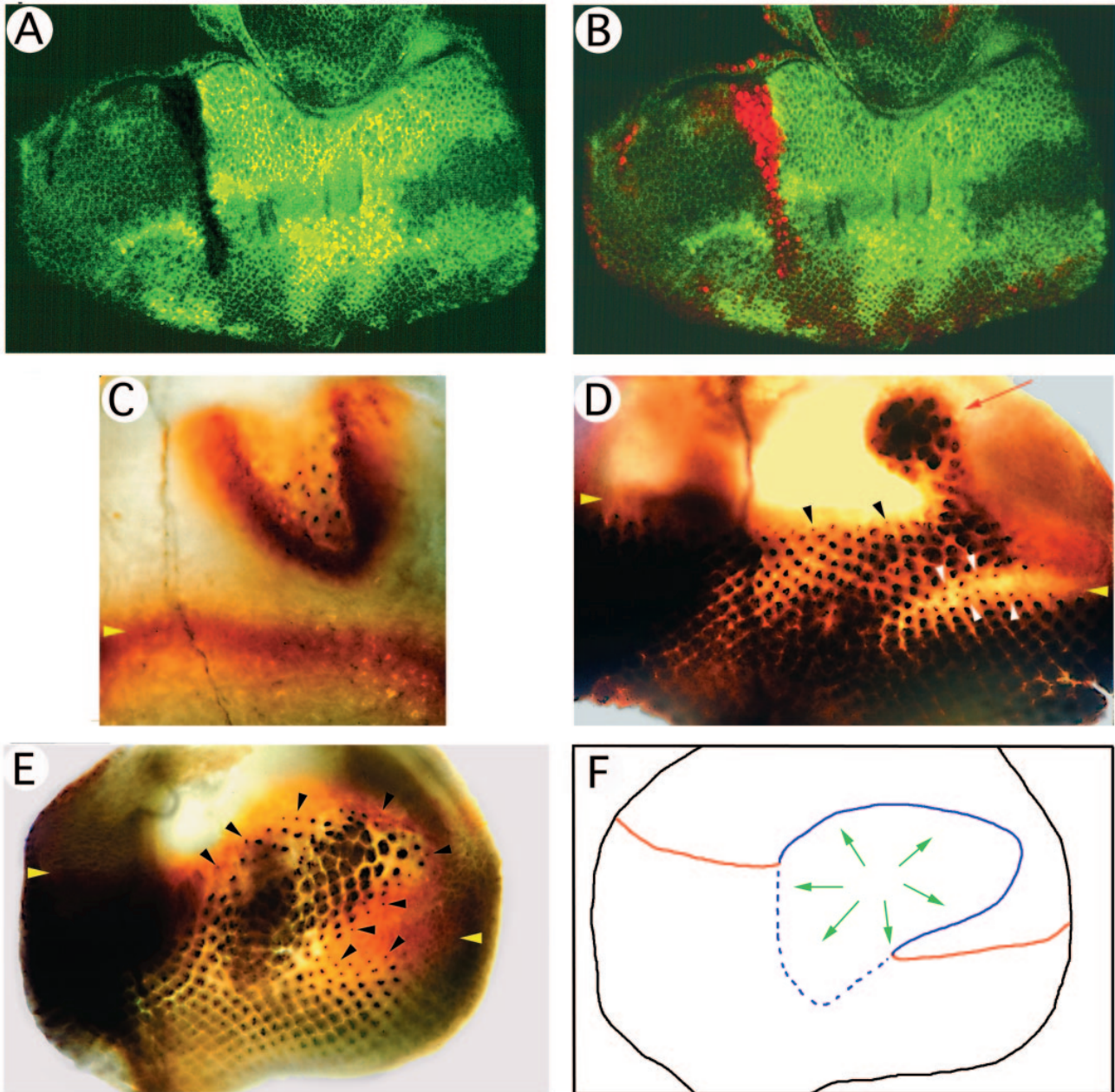
RESULTS

Initiation of radial expansion of the morphogenetic wave

Hedgehog (Hh) is thought to be the primary motive signal driving the advancing wave front across the presumptive retina (Heberlein et al., 1993; Ma et al., 1993). Differentiating cells expressing *hh* elicit *dpp* expression in undifferentiated cells lying a short distance anteriorly. The cells expressing *dpp* subsequently begin to differentiate as photoreceptors and express *hh* and then signal further anterior expression of *dpp*. Thus the *dpp*-expressing cells represent the front of the Hh signaling from the differentiating cells posteriorly. Ectopic expression of *hh* would be a possible mechanism for initiating eye morphogenesis ahead of the normal wave front. In the appendages of the fly, the consequences of ectopic expression of *hh* can be phenocopied by the loss of *patched* (*ptc*) or *Protein kinase A (DCO)* gene functions (Capdevila et al., 1994; Jiang and Struhl, 1995; Li et al., 1995). In an attempt to trigger an ectopic morphogenetic wave ahead of (anterior to) the endogenous wave front, we induced clones of *ptc⁻* or *DCO⁻* in the developing eye and assayed their consequences. The initiation and consequences of ectopic morphogenetic waves have been independently documented by Strutt et al. (1995) and Heberlein et al. (1995).

Clones of *ptc⁻* and *DCO⁻* were induced by flipase induced somatic recombination (Xu and Rubin, 1993) and the clones were marked in the disc by the absence of the CD2 marker protein (Dunin-Borkowski and Brown, 1995), and in the adult by the loss of the pigmentation gene *white*. Clones of *ptc⁻* and *DCO⁻* had similar effects with the exceptions that *ptc⁻* clones appeared to have more extensive effects. In particular we frequently observed *ptc⁻* but not *DCO⁻* clones to extend outside the normal limits of the retina (Fig. 2D) and in adults we observed ommatidia to invade the head capsule as far as the midline placed ocelli (data not shown).

Fig. 2 shows the consequences of *ptc⁻* clones in the developing retina. Ahead of the furrow, loss of *ptc* gene function led to ectopic *dpp* expression, initially within the clone itself (Fig. 2A,B). Subsequently however, cells within the anteriorly positioned clone began photoreceptor differentiation and the *dpp* expression was then found surrounding the differentiating patch (Fig. 2C). Hence it appeared that cells surrounding the clone were being signalled by the differentiating cells to express *dpp* and thus a radial expansion of the morphogenetic wave was beginning in the wild-type tissue surrounding the clone.

The radial expansion of an ectopic morphogenetic wave is most clearly detected by the graded series of developing ommatidia left in its wake. mAb 22C10 recognizes the developing photoreceptors (Fujita et al., 1982) and in an incipient ommatidium only the R8 cell is labeled. As maturation proceeds more cells become labeled and progressively larger cell clusters stain (Tomlinson and Ready, 1987a). Fig. 2E shows a precociously differentiating field of ommatidia ahead of the morphogenetic furrow and in its periphery the

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Fig. 1. (A) Epithelia can be polarized in at least two axes. The (non-planar) apical basal polarity is maintained by tight junctions that separate the apical plasma membrane (usually thrown into conspicuous microvilli) from the basolateral plasma membrane. The planar polarity is the polarity that runs in the plane of the epithelium. One of the most obvious manifestations of the underlying planar polarity is the secretion of hairs, which occurs at the same position within each cell and the shaft is projected in a specific direction. (B) A schematic ommatidium showing the spatial arrangements of the cells. The photoreceptors (RC) bear rhabdomeres (RH) which in cross sectional profile appear as dark circles. The rhabdomeres of the outer photoreceptors (R1-R6) run the depth of the retina and are organized in an asymmetrical trapezoidal shape. Central to the trapezoids lie the rhabdomeres of the central cells R7 and R8 - these cells are ignored in subsequent descriptions of the chiral shapes. (C) A schematic eye showing the orientation of the ommatidia in the two halves of a right eye. The ommatidia in one half of the eye are the mirror reflection of those in the other. The two types meet about the conceptual line called the equator. The distant points from the equator are the dorsal and ventral poles. (D) Two axes polarize each ommatidium. The trapezoidal shape of each ommatidium reflects the two orthogonally positioned polarizing axes. The open end of the trapezoid (R1,6) lies towards the equator and away from the pole indicating the Eq/Pl axis. The long side of the shape (R1,2,3) lies to the anterior, indicating the A/P axis. (E) The right eye is the mirror image of the left. There are not dorsal and ventral specific ommatidial forms, rather there is the dorsal right/ventral left type and the ventral right/dorsal left form. (F) Reversing either of the two polarizing axes of an ommatidium will change it into the other chiral form.

Fig. 2. Loss of function *ptc^S2* clones lead to the ectopic expression of *dpp*, the subsequent maturation of developing photoreceptors and the initiation of a morphogenetic wave which sweeps out radially from the clone. Posterior is down in all cases. (A,B) Clones of *ptc^S2*, detected by the absence of the CD2 marker protein (green) induce *dpp*, as evidenced by enhancer trap *dpp^10638* (red). (C-E) Eye discs stained for the *dpp-lacZ* reporter construct dpp^3.0, which visualizes *dpp* expression in the morphogenetic furrow (Blackman et al., 1991; Ma et al., 1993) (brown), and the neuronal marker mAb 22C10 (black), which labels developing photoreceptors. (C) The advancing furrow is highlighted by strong *dpp* expression (arrowhead). Ahead of it (above) is an ectopic patch of eye differentiation. The black dots are the developing photoreceptors and the horseshoe-shaped band of *dpp* indicates an expanding front. (D) Ectopic retinal differentiation can sometimes spread outside the normal region of the retinal field and extend well into the region of the head capsule (red arrow). This observation raises the unresolved question of whether the retina expands by recruitment of cells otherwise destined to differentiate as head capsule, or by proliferation of committed retinal cells (Lawrence and Shelton, 1975; Green and Lawrence, 1975; Nowel and Shelton, 1980). The white and black arrowheads point to very young ommatidia with only the R8 cell staining and indicating the front of differentiation. On the right, two fronts moving towards each other are indicated by the white arrowheads. Note that the endogenous fronts of differentiation (indicated by the yellow arrowheads) are at different levels on the left and right of the picture. We infer a retardation effect upon the endogenous wave by the ectopic one (and vice-versa). The meeting of these ectopic and endogenous fronts will appear in the adult as in Fig. 3A. Because the advancing front leaves behind it a smoothly graded series of developing ommatidia, by reading the developmental states of the ommatidia we can observe the time axis and infer the expansion of the wave. (E) Radial expansion of the ectopic waves can be frequently observed. The time axes indicated by the state of development of the ommatidia run along the radii of the patch with the youngest ommatidia (black arrowheads) to the periphery. Note the *dpp* staining (brown) outside (temporally ahead of) the region of differentiation. (F) Summary diagram of (E) to indicate the radial expansion of this ectopic eye patch.
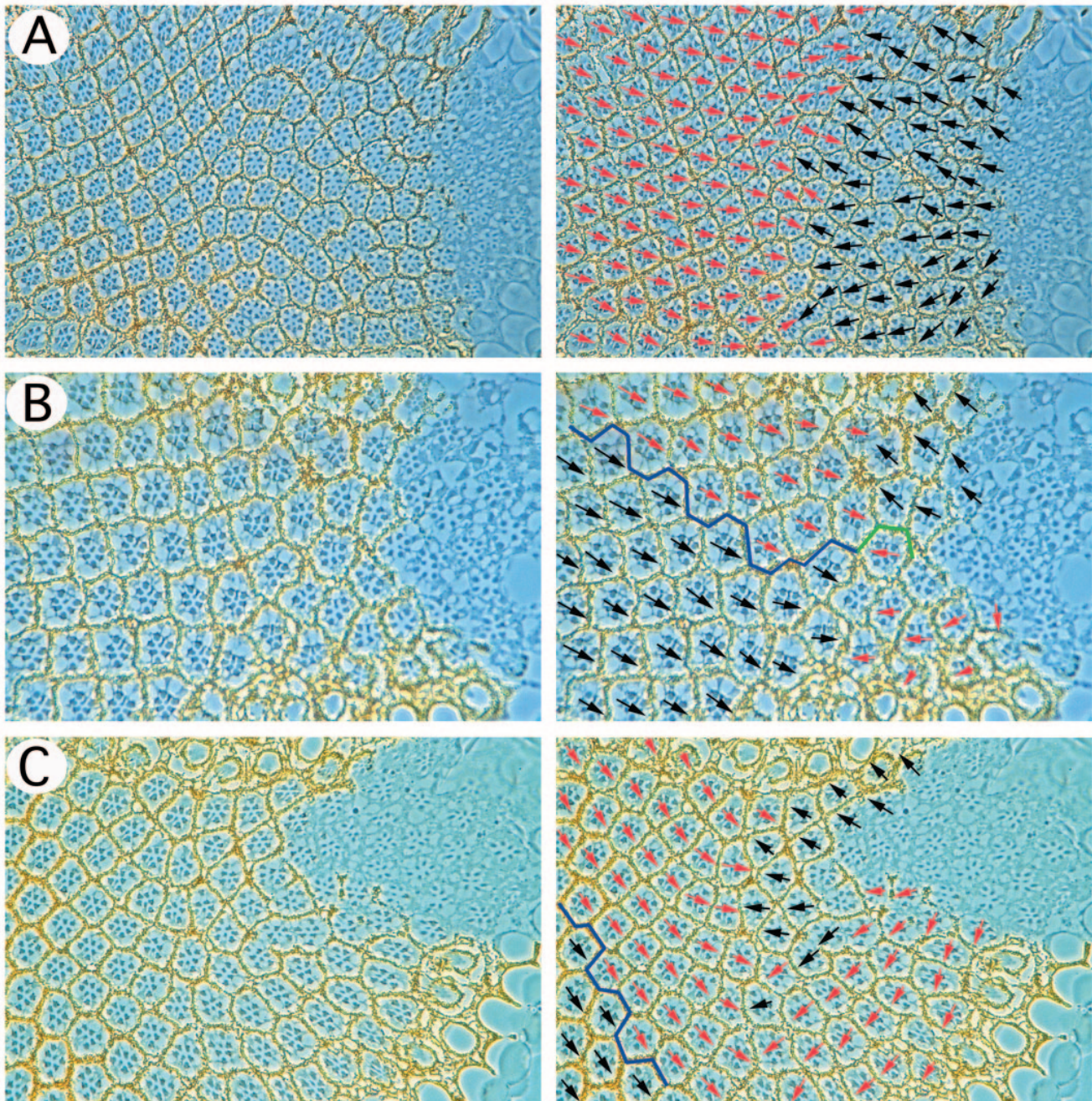
Fig. 3. The effects on epithelial polarity of *ptc*^S2 and *DCO*^E95 clones. Each figure has an adjacent color-coded explanatory duplicate. Ommatidial orientations and chirality are indicated by colored arrows pointing from the posterior side to the anterior side of an ommatidium. Red arrows indicate the dorsal right/ventral left type and black arrows are their chiral opposites, the dorsal left/ventral right form. The clone in each picture is the unpigmented region to the right, but the effects of the clones can be seen within the pigmented regions. Data shown is schematically summarized in Fig. 4. (A) A dorsal right eye, anterior is to the right. In a normal eye all arrows would be red and pointing to the right. The effect of an anteriorly positioned *ptc*^S2 clone is to invert the chiral form in the tissue posterior to it (now black) and the orientation of the ommatidia (now pointing posteriorly). Note that the inverted ommatidia still have their R3,4 side upwards, lying towards the dorsal pole. The equator is off the picture to the bottom. (B) The effect of an equatorially and anteriorly positioned *DCO*^E95 clone. The equator runs from top left towards bottom right and the opposite chiral forms can be seen to invert either side of it. The ommatidia directly posterior to the patch are of the mirror image (color change) and point in the opposite direction to those in the more posterior (left) wild-type region of the tissue. Note that the ommatidia in the inverted region are respecting the equator; normally there should be red ommatidia above the equator and black ones below, here there are black ones above and red ones below. (C) The effects of a *ptc*^S2 clone on chiral shapes in positions equatorial to the clone. Note the equator to the bottom left. The arrows pointing either side of the equator show the anterior direction of the eye. In the region influenced by the clone ommatidia now reorient respecting the center of the clone as posterior (the arrows are pointing away from the center). Anterior to the clone (below it) ommatidia are of the correct chiral form (red) and posterior to it (to its left and up a little) ommatidia are changed into the opposite (black) chiral form. The two types meet to the left (equatorial) of the clone at the centre of the picture where a small amount of chiral confusion may occur.

ommatidia containing only the R8 cell staining can be seen. Internal to this peripheral region lie ommatidia containing three labeled cells (R8,2,5) and in the central region more mature ommatidia are found. Hence there is clear evidence for the radial expansion of the ectopic morphogenetic wave (Fig. 2F).

The presence of ectopic retinal differentiation can have a pronounced retardation effect upon the movement of the endogenous morphogenetic wave (Fig. 2D). This suggests that the front projects an inhibitory effect upon the retinal differentiation programme, well ahead of itself, which is only slowly relieved as the Hh signaling front approaches.

A/P polarity inversion following the reverse flow of the morphogenetic wave

To determine whether changing the direction of the morphogenetic waves alters the epithelial polarity, we examined the chiral shapes of ommatidia in and around *ptc⁻* and *DCO⁻* clones in adult eyes. In tissue lying anterior to a clone the wave flowed in the appropriate posterior to anterior direction; posterior to the clone the wave flowed directly backwards in an anterior to posterior direction, and in positions dorsal and ventral to a clone the wave flowed perpendicular to the normal posterior to anterior direction.

(i) Ommatidial chirality and orientation within *ptc⁻* or *DCO⁻* clones

Clones are marked by the absence of pigment in the cells and appear on the right hand side of each picture in Fig. 3. Within the clones the ommatidia are variably mutant, some having extra photoreceptors and others less. Those ommatidia that are normally constructed appear to be of either chiral form and to orient randomly, indicating a disorganization of the planar polarity.

(ii) Ommatidial chirality and orientation in tissue posterior to *ptc⁻* or *DCO⁻* clones

In tissue posterior to clones where the wave had earlier flowed backwards in the anterior to posterior direction, ommatidia were of the inappropriate chiral form (Fig. 3A; see Fig. 4 for a summary). This region of chiral inversion can extend for many ommatidial columns and presumably reflects the backward flow of the ectopic wave before it met with the anteriorly advancing endogenous wave. Although the ommatidia are of the wrong chiral form they still orient with their polar side towards the pole and the chiral inversion appears to result from their inversion in the A/P rather than the Eq/Pl axis. Further demonstration of this A/P polarity inversion comes from clones lying in the equatorial region. Fig. 3B shows the reorganization of ommatidial chirality in tissue posterior to an equatorially positioned *DCO⁻* clone. Posterior to the clone the ommatidia are of the inappropriate chiral form, but they still show mirror reflection either side of the equator. This observation suggests that in the region of inappropriate chiral forms the equatorial/polar planar polarity is in place and functioning.

(iii) Ommatidial chirality and orientation anterior to *ptc⁻* or *DCO⁻* clones

Anterior to the clones, the morphogenetic wave would have flowed in the correct posterior to anterior direction and ommatidial chiralities and orientations in these positions are correct (data not shown, summarized in Fig. 4).

(iv) Ommatidial chirality and orientation dorsal and ventral to *ptc⁻* or *DCO⁻* clones

Normally, the two polarizing axes of the retina lie orthogonal to each other. Since the radially expanding morphogenetic front moves out from the clones in all planar directions of the epithelium, then at specific positions (right angles to the normal A/P axis) it is superimposed on the Eq/Pl axis. These positions are indicated by the question marks in Fig. 4. Since ommatidial chirality monitors the two (normally orthogonally positioned) axes, then in positions where the two axes are superimposed we might anticipate confusion in ommatidial forms, and symmetrical or randomly chosen chiralities. In the region equatorial to a *ptc* clone, the ommatidia to the anterior of the clone are of the normal chiral form (color-coded with red arrows), those to the posterior are the opposite, inappropriate type (color-coded with black arrows) (Fig. 3C). The two meet in the equatorial region in an apparently smooth interface, suggesting an absence of chiral confusion in this position. However, there are two limitations to this interpretation. First a small amount of random chirality in this region of interface would be difficult to identify and second, although some clones appear as shown in Fig. 3C, others show a significant amount of chiral randomization with an accompanying low frequency of symmetrical ommatidia. Accordingly it remains unclear whether ommatidial chiralities are perturbed in regions where the morphogenetic wave flows in the Eq/Pl axis.

DISCUSSION

We show here that when the morphogenetic wave that sweeps the presumptive retina is reversed, the ommatidia adopt the inappropriate chiral form. We will argue below two major points. First, that the chiral inversions occur because the A/P rather than the Eq/Pl polarity of the epithelium has been reversed, and second, that the reverse sweep of the wave may be causal in changing the epithelial polarity.

Chiral inversions following A/P polarity reversal

The chiral shape of an ommatidium reflects the two orthogonally positioned planar axes of the epithelium (Fig. 1D) and reversal of either axis alone will cause the chiral shape of an ommatidium to change (Fig. 1F). The chiral inversions that occur when the morphogenetic wave flows in the opposite direction appear to result from a reversal of the A/P rather than the Eq/Pl axis. Firstly, the chirality inversions respect the equator with (inappropriately) the 'black-type' on one side and the 'red-type' on the other (Figs 3B, 4B). Secondly, ommatidia that exhibit chiral inversion orient with their 'anterior' sides (R1,2,3) to the posterior but still maintain their Eq/Pl orientation with R3,4 lying toward the pole (Fig. 4A). These features are characteristic of the reversals of the A/P and not the Eq/Pl axis (Fig. 1F). Fig. 4 schematically summarizes the pattern reorganization which accompanies the radial expansion of the morphogenetic wave. Ommatidia normally orient in a regular array to the uniform A/P axis. The effect of the clone is to 'bend' (a gravitational allusion) the ommatidia around the clone, with the centre of the clone functioning as the supernumerary posterior direction.

Normally, ommatidia of the two chiral forms only meet at
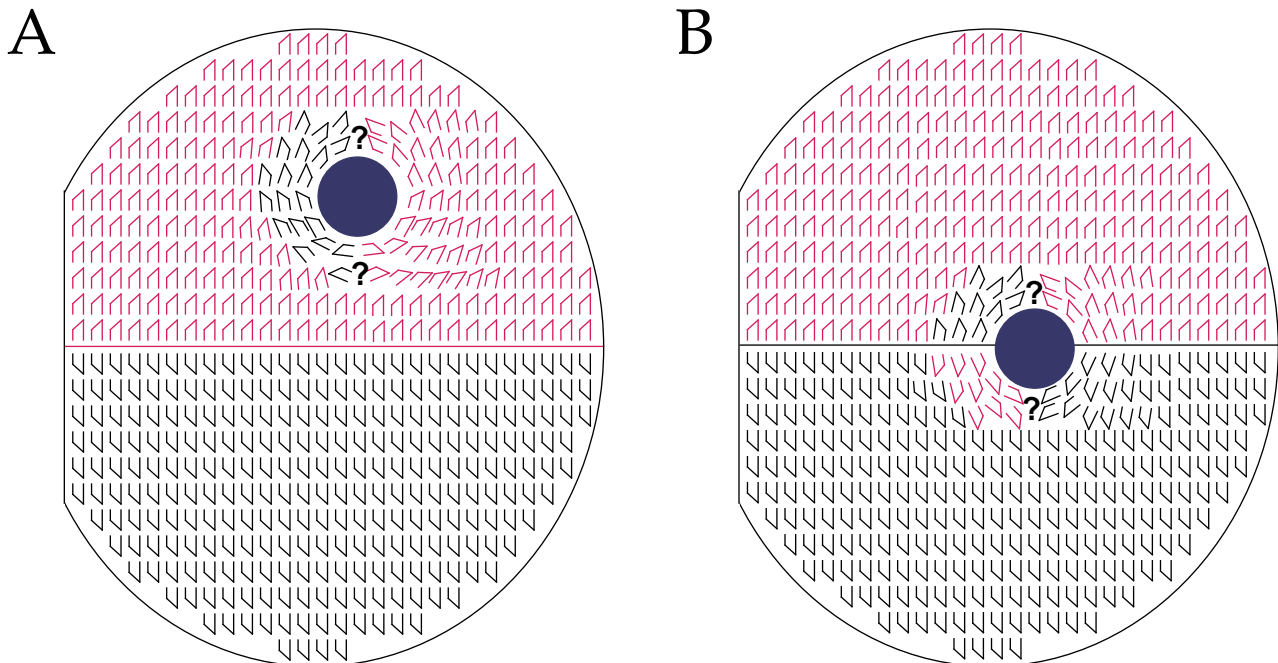
Fig. 4. A schematic representation of the reorganization that occurs surrounding the *ptc^{S2}* or *DCO^{E95}* clones. The clones are depicted by the blue circles and the surrounding effects are indicated by the changes in orientation and chirality (colour) of the ommatidia. (A) A dorsally positioned clone. Anterior to the clone (to the right) the ommatidia are of the correct chiral form. In this position the ectopic wave front moved through the tissue in the appropriate direction. To the posterior of the clone the wave front moves in the wrong direction and ommatidia are of the wrong chiral form (black). (B) An equatorially positioned clone. The details are the same as for A except that the inappropriate chiral forms posterior to the clone now show a mirror reflection either side of the equator. Note that all ommatidia in the region of influence of a clone are now respecting the centre of the clone as posterior rather than to the left which is the typical orientation of ommatidia. The clone has a pseudo-gravitational effect 'bending' the ommatidia around it. Above and below the clones the radiating wave front has run in the Eq/Pl axis and the two polarizing influences necessary for chiral identity are now superimposed. The question marks indicate that sometimes extensive chiral confusion is seen in these regions and in others not. Note that above and below the clones, 'red' and 'black' ommatidia can meet at pseudo-equators (positions of the question marks). These pseudo-equators result from reflection of the chiral forms in the A/P axis, and not the Eq/Pl axis, which is responsible for the normal equator.

the equator, but as a consequence of the induced inversions, ectopic interfaces of the two occur. Although these interfaces resemble equators, it is of interest to note that they result from the reflection of ommatidia in the A/P axis (which is the equivalent of the reflection seen between right and left eyes - Fig. 1E) rather than the mirror image inversion that occurs in the Eq/Pl axis about the equator.

**Wave reversals and the inversion of planar polarity**

Two different mechanisms could account for the observed correlation between the direction of the morphogenetic wave and the epithelial A/P polarity. First we will examine a model in which cells orient in response to the direction of a static gradient of positional information lying inherent in the epithelium and in which the movement of the wave is not instructive in establishing the polarity. We will then describe models in which the movement of the wave is causal in laying down the polarity.

(i) Long-range gradient models of epithelial polarization

Long-range gradients of positional information have been proposed to account for the epithelial polarity in the insect segment (Locke, 1959; Lawrence, 1966; Stumpf, 1967). These models envisage a high point of a gradient at one extreme of the segment and a low at the other. Cells at any position would

establish appropriate orientation by reference to the local slope of the gradient. Such a mechanism has been proposed by Lawrence and Shelton (1975) to be operating in the insect eye, with ommatidia establishing their A/P polarity by reference to a gradient lying in the retinal epithelium (Fig. 5A). In this kind of model, the loss of *ptc* or *DCO* gene function in a clone in the anterior part of the retina would establish a supernumerary high point of the gradient (Fig. 5A, II'). To the anterior of the new high point the gradient slopes in the normal posterior to anterior manner, but to the posterior the gradient slopes the wrong way. Since, in this model, the ommatidia read the slope of the gradient, their polarities will be correct anterior to the clones but reversed posteriorly.

The above model accurately predicts the polarity reversals we see around the *ptc^-* and *DCO^-* clones but does not account for why there is a corresponding reversal of the morphogenetic wave when the polarity is inverted. One explanation could be that the gradient within the epithelium directs both the polarity of the ommatidia and the movement of the morphogenetic wave. However, the wave-propagation models of Heberlein et al. (1993) and Ma et al. (1993) argue that Hh-signaling drives the morphogentic wave across the retina independently of any static gradient: differentiating cells expressing *hh* elicit the differentiation of undifferentiated neighbours and their subsequent expression of *hh*, and by this mechanism the hh signal
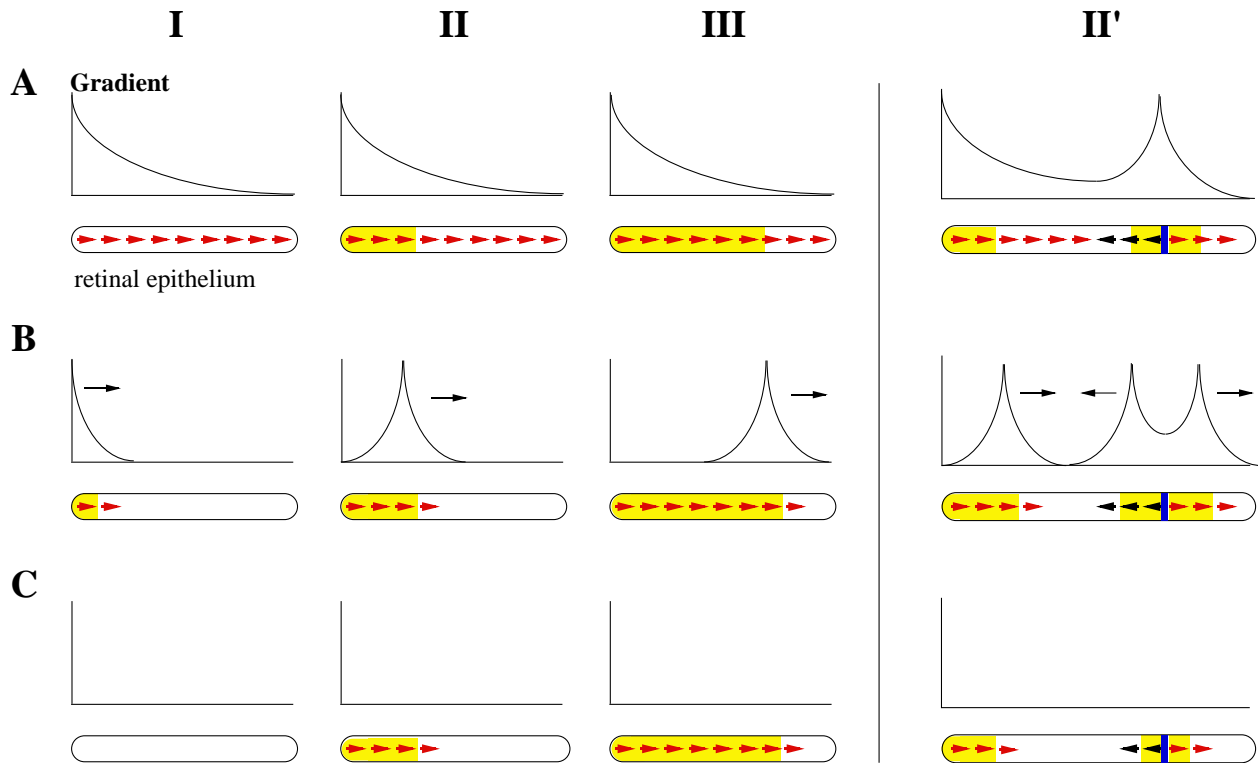
Fig. 5. Models for the establishment of epithelial planar polarity. (A,B,C) Three different models depicting the wild-type situation at progressively later time points (I, II, III) and that with a *ptc* or *DCO* mutant clone (II′) at the stage corresponding to (II). Underneath the gradient profiles the retinal epithelium and its polarization state are schematically indicated. Red arrows denote cells with one polarity, black the other. Yellow indicates differentiating tissue. The relative positions of gradient peak, polarized front and the extent of the yellow area are arbitrarily chosen. In (II′) the mutant clone is indicated in blue. (A) Long-range gradient model. The entire epithelium is polarized through a static gradient and the wave of development subsequently runs through this polarized tissue. In II′ the clone (blue) has created a new gradient high point which organizes polarity either side of it. (B) The moving gradient model. A high point of a signaling molecule sweeps as a wave through the epithelium and polarizes the cells ahead of it, possibly near the peak where the slope is steep. The gradient profile has been chosen arbitrarily and would probably be asymmetrical, being higher than shown behind the peak. In (II′) the gradient peak moves out as a circular wave (here shown in cross-section) and only the part moving posteriorly is inverting the polarity (black arrows underneath the profile). (C) The short-range interaction model. The moving front of differentiation is able to polarize the cells directly ahead of it. These cells subsequently differentiate and then polarize their immediate neighbours.

can propagate in an epithelium. All that is required for the posterior to anterior flow of the wave is for the initial *hh* expression to occur posteriorly. A second explanation for the correlation of polarity and direction of wave flow is that when the *ptc⁻* or *DCO⁻* clones are made, they initiate two independent mechanisms – a new high point of the gradient, and the radial expansion of the wave. However, Heberlein et al. (1995) have shown that ectopic *hh* expression alone produces the same phenomena described for *ptc⁻* and *DCO⁻* clones. Accordingly, ectopic *hh* appears to both elicit the polarity organizing mechanism and drive the propagation of the wave. This is inconsistent with a static long range gradient model because *hh* expression itself moves across the epithelium with the wave front and would be repeatedly triggering new high points of the polarizing gradient. This concept of the wave moving the polarizing high point corresponds to one of the wave-dependent mechanisms described below.

(ii) Wave-dependent models of epithelial polarization

Two models may describe polarization of the retinal epithelium by the action of the morphogenetic wave sweeping across it. The wave may progressively move the high point of a polar-

izing gradient across the retina (Fig. 5B). First the posterior regions of the epithelium would experience the gradient and then as the wave moves, the polarity will be imposed on progressively more anterior regions. In this scenario, the mutant clones would trigger the radial expansion of the wave carrying the high point of the gradient. Anterior to the clones the wave would move the gradient high-point in the normal posterior to anterior direction, appropriately polarizing the epithelium, but the posterior-moving part of the wave would cause the polarity reversal (Fig. 5BII′). In another conceptually different form of wave model, local cell-cell communication can progressively polarize the epithelium as the wave moves anteriorly (Fig. 5C). An example of this would be where an undifferentiated cell would lie directly anterior to a differentiating and polarized cell. Neighbour/neighbour signaling would now orient the cell relative to the differentiating cell. As the wave moves, this cell would then begin to differentiate and impart the same polarity onto its anterior neighbour.

Some long-range gradient models depict the diffusion and resulting graded distribution of a signaling molecule from a fixed source (Lawrence, 1966; Stumpf, 1967). Conceptual difficulties with these ideas are how the gradient can be established

and maintained over long distances, and how cells in positions progressively distant from a source are able to read the slope of a progressively flattening gradient. In contrast to this model, the idea of a morphogenetic wave laying down the polarity as it moves is attractive, since it can, depending on the distance it moves, establish the coordinately organized orientation of cells over long distances. Waves of differentiation traversing epithelia occur elsewhere in development (e.g. expansion of the primitive streak (reviewed by Bellairs, 1986), or mediolateral expansion of the field of feather primordia in the avian dermis (see Senegel, 1973), raising the possibility of wave-dependent planar polarity mechanisms in these tissue and elsewhere.

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