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A polarity field is established early in the development of the *Drosophila* compound eye

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Abstract

The photoreceptors within the ommatidia of the *Drosophila* compound eye form a trapezoid. This occurs in two chiral forms in the dorsal and ventral half of the eye. We have used two manipulations to induce ectopic ommatidia, in combination with molecular markers for specific positions in the retinal field. We find that ectopic morphogenetic furrows induced on the eye field margin (or midline) and those induced in the body of the field have different consequences for the establishment of retinal polarity. Furthermore, the dorsal/ventral vector field is established early in development, prior to and independent of the initiation of the morphogenetic furrow. An 'early equator' model is presented to account for these and previously published data. © 1997 Elsevier Science Ireland Ltd.

Keywords: Drosophila; *patched*; *wingless*; Polarity; Compound eye

1. Introduction

The *Drosophila* compound eye develops from a monolayer epithelium. Such epithelia may be considered as two-dimensional fields which can be patterned in both dimensions and this patterning may be a consequence of a system of positional information. Several possible molecular mechanisms may specify coordinates of positional information, including morphogen gradients and systems of cell polarity based on local signals (Wigglesworth, 1940; Crick, 1970; Wolpert, 1971; Lawrence et al., 1972; Meinhardt, 1977; Meinhardt, 1978; Bonhoeffer and Huf, 1982; Adler, 1992; Bryant, 1993; Doe, 1996).

In the adult, ommatidia in the dorsal half of the compound eye are oriented with the R3 photoreceptor cell dorsal and anterior and the R7 ventral and ommatidia in the ventral half of the eye are inverted. The boundary across which this pattern inverts is known as the equator and the dorsal and ventral margins are known as the poles (Fig. 1A) (Dietrich, 1909; Ready et al., 1976). Early in life the presumptive eye fields are unpatterned columnar epithelia in the eye-antennal imaginal discs (Weismann, 1864). Beginning in the third larval instar a progressive wave of development, known as the morphogenetic furrow, moves across this epithelium from posterior to anterior (Fig. 1B) (Ready et al., 1976). Furrow progression is induced by Hedgehog expressed on its posterior side (Heberlein et al., 1993; Ma et al., 1993). In the furrow subsets of cells form first 'rosettes' and then five-cell 'preclusters' in a process that does not depend on cell lineage (Ready et al., 1976; Tomlinson, 1985; Wolff and Ready, 1991). After the passage of the furrow the clusters recruit additional cells by means of local signaling (Ready et al., 1976; Banerjee and Zipursky, 1990; Simon, 1994). As the furrow moves it lays down a new column of ommatidial clusters roughly every 2 h (Basler and Hafen, 1989). However, the ommatidial clusters in one column are not initiated at the same moment, i.e. the first cluster is formed at the center of the furrow (the midline or future equator) and then subsequent clusters are formed dorsal and ventral to this at about 10-min intervals (Wolff and Ready, 1991). This point at the center of the furrow is known as the 'firing center'. The firing center has been described as an inductive node which transmits information in two directions, i.e. induction of new ommatidial columns towards the anterior (Fig. 1B,

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white arrow) and induction of new ommatidial clusters towards the dorsal and ventral poles (Fig. 1B, black arrows) (Gubb, 1993).

The posterior cell of the precluster is the future R8 photoreceptor and anterior to it are two pairs of cells (R2/5 and R3/4) (Fig. 1C). The preclusters are initially bilaterally symmetrical with their axes orthogonal to the furrow. Within a few hours clusters in the dorsal half of the right eye rotate counter-clockwise (first 45° and then 90°) and those in the ventral half rotate clockwise, so that after about 15 columns the epithelium is further polarized and the equator is established (Fig. 1D) (Ready et al., 1976). The completion of this rotation requires the *nemo* and *roulette* genes (Choi and Benzer, 1994b). Some mutations at loci which act autonomously in cuticular polarity also show retinal phenotypes (such as *frizzled*) (Gubb, 1993; Zheng et al., 1995; Heslip et al., 1997) and these genes are likely to encode components involved in the receipt or interpretation of positional information.

There are two sub-fields in the dorsal/ventral dimension, i.e. the equatorial/polar fields. We and others have suggested that two orthogonal vectors of positional information may act on each cluster to confer orientation and chirality, i.e. direction to the equator and direction to (or of) the furrow ('two vector models', see white and black arrows in Fig. 1C) (Gubb, 1993; Ma and Moses, 1995). The focus of our study is the nature of the equatorial/polar vector. Several conditions have been used to reorient the direction of furrow progression and these affect ommatidial polarity in the retina. When *wingless* function is removed early in larval development the furrow initiates all along the posterior and dorsal margins of the eye field (and to a lesser extent on the ventral side) (Ma and Moses, 1995; Treisman and Rubin, 1995). After 48 h under these conditions the furrow is shaped like a capital Greek letter gamma (Γ). Clusters formed by the endogenous (vertical) part of the furrow rotate to adopt their correct orientations, dorsal and ventral to the equator. This alone demonstrates that *wingless* is not directly required for normal rotation at this time. However, clusters formed by the horizontal leg of the furrow fail to rotate. Mosaic clones which ectopically express *wingless* in the body of the retinal field can

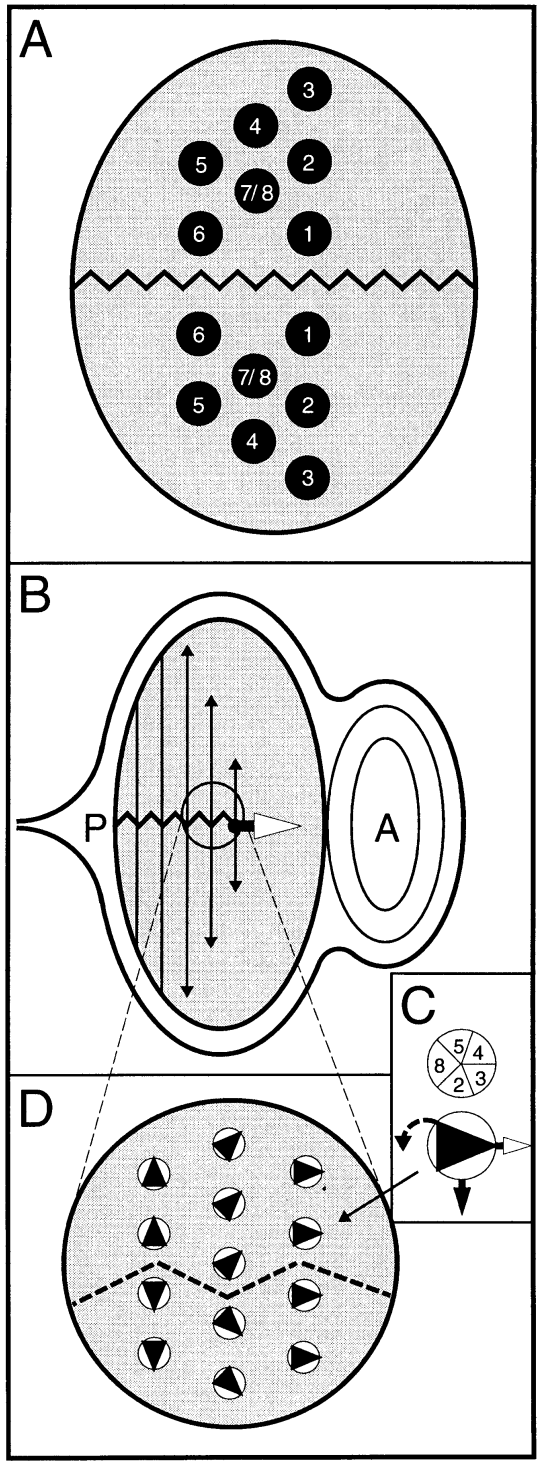

Fig. 1. The development of polarity and chirality in the *Drosophila* retina. In all panels anterior is to the right and dorsal is up. (A) Diagram of the right hand eye of an adult *Drosophila*. The photoreceptor cell rhabdomeres are shown for one ommatidium above the equator and one below it. Note that these two forms are mirror images reflected in the equator. The R7 rhabdomere lies apical to that of R8. (B) Diagram of a late third instar eye imaginal disc. A, antennal disc; P, posterior edge of the eye field where the furrow initiated. The jagged, horizontal line shows the equator and the dot at its right end shows the firing center which is moving in the direction of the white arrow. Every 2 h the firing center initiates a new ommatidium adjacent to the midline and then new ommatidia are induced dorsal and ventral to it at 10-min intervals shown by vertical black arrows. (C) Detail of a single five cell precluster from the dorsal domain of the right eye. The future R8 cell is the posterior central cell. Anterior to the future R8 lie the precursors to R2/5 and R3/4. The five cell precluster is initially bilaterally symmetrical and can be represented by the black arrow-head in a ring symbol shown below. The initial orientation is at right angles to the furrow. The direction to the furrow and equator are shown as open and filled arrowheads, respectively. This vector combination results in a counter clockwise rotation of clusters in the dorsal half of the eye field. (D) Three stages by which clusters in the dorsal and ventral half of the right eye rotate (first 45° and then 90°) to lie eventually with their R3 cells the most dorsal or ventral. Ommatidia in the ventral half of the right eye are inverted relative to the dorsal half. Thus, the ommatidia come to have the R7 cells facing each other across the equator as shown in (A).

cause polarity inversions in adjacent wild type tissue (Treisman and Rubin, 1995). Thus, it may be that while *wingless* has no late function in transmitting polarity information, it does have an early function in establishing the equatorial/polar field and a later role in inhibiting furrow initiation on the margins. Therefore. it is particularly important to distinguish early events and signals (long before furrow initiation) from late events and signals (during furrow progression).

Clones which express ectopic Hh anterior to the endogenous furrow (and loss of function *patched* or *Pka* clones) induce ectopic furrows which can propagate through the surrounding wild type (or heterozygous tissue) away from the site of induction (Chanut and Heberlein, 1995; Heberlein et al., 1995; Li et al., 1995; Ma and Moses, 1995; Pan and Rubin, 1995; Strutt and Mlodzik, 1995; Wehrli and Tomlinson, 1995). *patched* clones can induce morphogenetic furrows which fall into classes. In class I, the *patched* clone is small, approximates to a single point of induction and does not intersect with the eye field midline or margin. Class I clones produce a circular furrow with four zones of cluster orientation surrounding the clone in the adult retina and with no true equator between any of them (Ma and Moses, 1995). Class II *patched* clones do intersect the eye field midline or margin and produce a different result; two polarity fields are seen (inverted in the anterior/posterior axis), with an ectopic equator between them in the adult retina (Chanut and Heberlein, 1995; Strutt and Mlodzik, 1995). The mechanistic difference between these two classes of *patched*-induced furrows is the central focus of this study.

In all of these experiments, the clones are induced early in development, about 2 days prior to furrow initiation. Thus, it is possible for clones to have early and late effects (before and after furrow initiation). We have set out to resolve this by repeating these experiments, but analyzing the results in the developing larval disc rather than in the adult. This was done by marking the clones to determine their size and position relative to the disc margin and by employing visible markers of the polarity fields. We find that *patched* clones in the body of the eye field do not cause ectopic expression of field markers, but that clones on the margin (or midline) do. We propose that early in development (before furrow initiation) a signal emanates from the margin and propagates inwards (likely to be Wingless itself). This first signal establishes the position of the midline/equator. Later, a second signal emanates from the midline/equator and propagates out towards the poles. This second signal is interpreted by the nascent clusters and confers their dorsal/ventral orientation. Reception of this second signal requires the product of the *frizzled* gene and thus the second signal may also be conveyed by a member of the Wnt family of proteins. *patched* clones made early on the margin or midline can disturb and reorient these signals and perturb the field. Clones made early but off the midline or margin cannot.

2. Results

2.1. PD and Eq-1 are early markers of retinal field polarity

Sun et al. (1995) reported the isolation of a number of P-element insertions expressing both *white* and β-galactosidase in the compound eye, under the influence of genomic transcriptional regulatory elements. Two of these provide persistent markers for aspects of positional information in the retina. The 'PD' element expresses *white* and β-galactosidase in a 'posterior dot' at the posterior margin of the eye, at the point where the optic stalk joins the eye field (Fig. 2A–D) (Sun et al., 1995). This position is of particular interest as it marks the point of origin of the morphogenetic furrow, the equator and the firing center. This expression is present in the second instar, before furrow initiation (Fig. 2A) and persists after the furrow has initiated and as it progresses (Fig. 2B,C,D). The 'Eq-1' element expresses *white* and β-galactosidase in a broad domain along the midline of the retinal field (Fig. 2E–H) (Sun et al., 1995). Like PD, the Eq-1 expression is present before furrow initiation (Fig. 2E) and persists into the adult. As the disc grows, the Eq-1 expression domain broadens and eventually also encompasses the anterior/dorsal margin of the retinal field (Fig. 2G,H). Interestingly the Eq-1 expression is clearly present in the midline, anterior to the furrow and prior to its initiation. Therefore, there is some molecular correlate of the field midline even before the firing center passes through and thus the firing center does not itself create the midline. We used these two P-element insertion lines to mark the posterior margin and midline in experiments in which we manipulate the orientation of the furrow (see below).

2.2. Ectopic furrows can affect polarity markers

We induced ectopic morphogenetic furrows by shift of $wg^{l\text{-}12}$ homozygotes to the non-permissive temperature for 48 h. This treatment has been shown to cause ectopic initiation of the furrow in the late third instar (i.e. not immediately at the time of the temperature shift in the second instar) (Ma and Moses, 1995; Treisman and Rubin, 1995). The ectopic initiation is mostly on the dorsal and anterior margins and by the late third instar results in a Γ-shaped furrow. We examined the expression of β-galactosidase driven by both the PD end Eq-1 elements under these conditions (Fig. 3) and we observed ectopic expression on the dorsal and anterior side in all cases and occasionally also on the ventral side (data not shown). These differ from furrows induced by anterior margin *patched* clones (see below), in that the ectopic neuronal field induced by wg^{ts} is never separated from the endogenous field and thus does not form a separate polarity field. Therefore, clusters born behind these ectopic furrows fail to rotate as described previously (Ma and Moses, 1995).

We also used loss of function *patched* mosaic clones to induce ectopic furrows as, in the retina, *patched* functions as

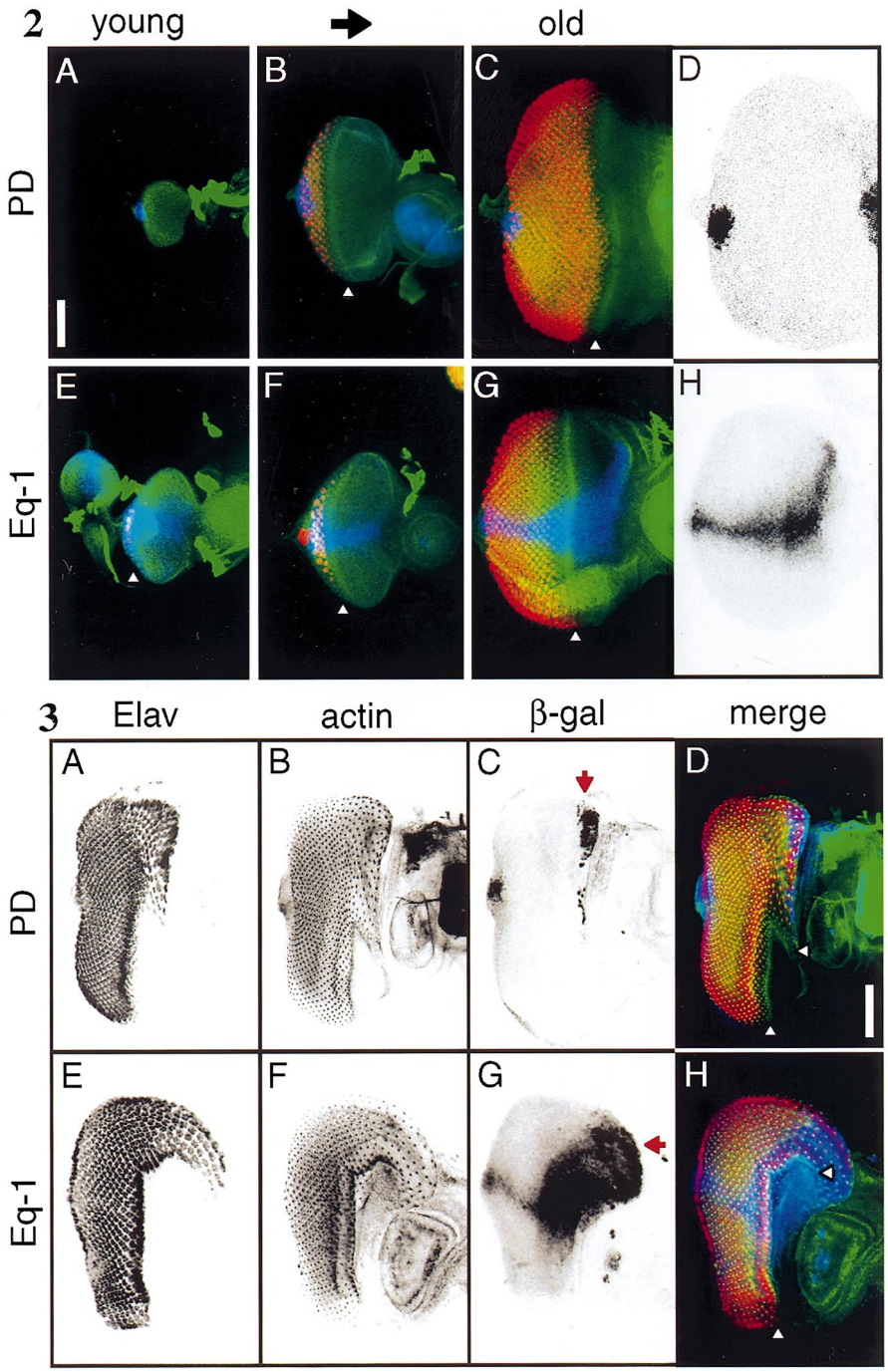

Fig. 2. PD and Eq-1 are markers of the point of furrow initiation and of the midline. All panels show larval eye-antennal imaginal discs, stained for the expression of three proteins, i.e. β-galactosidase (blue), cytoplasmic actin (by phalloidin, green) and a nuclear neural antigen (Elav, red). (A–D) PD; (E–H) Eq-1. (A) A late second instar disc. (E) Two discs; one (vertically oriented on the left) is from the late second instar and the other (on the right) is from the early third. (B,F) Early third instar discs. (C,G) Late third instar discs. (D,H) Only the blue (β-galactosidase) signal from (C) and (G) are shown for clarity. Note that PD is expressed at the point of furrow initiation and Eq-1 is expressed on the midline. Note also that PD and Eq-1 express β-galactosidase before furrow initiation (A,E) and that Eq-1 expresses β-galactosidase anterior to the moving furrow (F–H). We also observed expression of both PD and Eq-1 in the antennal primordia as was seen by Sun et al. (1995) for Eq-1 but not for PD. White arrowheads in (D) and (H) show the furrow. Anterior is shown to the right, the scale bar in (A) represents 50 μm and all panels are to the same scale.

Fig. 3. Ectopic furrows induced by loss of *wingless* affect polarity markers. All panels show late third instar eye discs dissected from $wg^{l\text{-}12}$ homozygotes after 48 h at the non-permissive temperature. (A–D) A disc carrying the PD element. (E–H) A disc carrying Eq-1. (A,E) The expression of Elav, a nuclear neural antigen. (B,F) Cytoplasmic actin (visualized with phalloidin). (C,G) β-Galactosidase expression. (D,H) The three images merged (Elav in red, phalloidin in green and β-galactosidase in blue). The red arrows in (C,G) show ectopic β-galactosidase expression and the white arrowheads in (D,H) mark the ends of the Γ-shaped furrow. Note the ectopic β-galactosidase expression on the anterior/dorsal margin (red arrows). Anterior is shown to the right, dorsal is up, the scale bar in (D) represents 50 μm and all panels are to the same scale.

a negative regulator of furrow initiation (see Fig. 4) (Chanut and Heberlein, 1995; Ma and Moses, 1995; Strutt and Mlodzik, 1995; Wehrli and Tomlinson, 1995). The precise shape, size and position of the resulting furrow depends in part on the shape, size and position of the clone. We used an Myc epitope to negatively mark the clones (Xu and Rubin, 1993) and simultaneously visualized the position of developing neural clusters (with Elav) and the expression of β-galactosidase driven by the PD or Eq-1 elements. We examined 28 discs with *patched* clones carrying the PD element and 32 with Eq-1 (some discs have multiple clones). In most, but not all cases (see below), these result in furrows with associated ectopic ommatidial clusters. In cases with clones that happen to lie neither on the margin nor on the midline, the ectopic site of furrow initiation is not associated with ectopic β-galactosidase expression from either PD (27 cases, Fig. 4A–D) or Eq-1 (35 cases, Fig. 4E–H). However, in those cases where the clones do lie on the midline or margin, the ectopic site of furrow initiation is associated with ectopic β-galactosidase expression from either PD (seven cases, Fig. 4I–L) or Eq-1 (11 cases, Fig. 4M–P). Note that clones show ectopic PD expression even before furrow initiation (green arrows in Fig. 4J,K). Thus, *patched* clones induced early in development can induce ectopic furrow initiation days later (in the third instar), but the position of the clone is critical in determining whether the retinal tissue polarity field is affected. These changes observed in the two β-galactosidase markers (PD and Eq-1) do indeed reflect actual changes in tissue polarity, as corresponding changes are found in *patched* clones observed in the adult retina (Chanut and Heberlein, 1995; Ma and Moses, 1995; Strutt and Mlodzik, 1995; Wehrli and Tomlinson, 1995).

While most retinal *patched* clones induce ectopic furrows, not all do. We examined a set of 50 such clones and found that 42 of them did produce ectopic clusters and eight of them did not (we call these clones 'sterile'). There are possible artifacts that could account for this sporadic occurrence of this negative result. One artifact could be genetic heterogeneity in the *Drosophila* stocks, such that about 16% of the larvae were not of the correct genotype to produce *patched* clones. However, in all eight of these cases, the same disc contained other *patched* clones that were neurogenic, demonstrating that all eight individual larvae were of the correct genotype. Another possibility is that in some cases the FLP recombinase is able to induce somatic recombination at ectopic sites on the chromosome arm which do not carry an FRT. We eliminated this second possibility by both positive and negative controls. To test for possible non-FRT mediated FLPase-induced mitotic recombination two control experiments were conducted. The FRT lies in polytene region 43D, *patched* lies in 44D3 and the two Myc expression elements lie in 45F and 47F. Any ectopic recombination event (or cryptic endogenous FRT-like sequence) between 44D3 and 45F could yield a negatively Myc-marked clone that is actually wild type for *patched* and thus not able to induce ectopic furrows. As a positive control we constructed flies with the FRT-43D chromosome and both Myc elements (as above) in trans to an FRT-43D chromosome and induced FLPase (as described above). We scored for adult retinal mosaic clones (by virtue of the *white* genes in the Myc elements). Over 80% of such flies had retinal clones (most had multiple clones). Thus, FLPase is capable of producing retinal mosaics at a very high rate. As the negative control we constructed flies with the FRT-43D chromosome and both Myc elements (as above) in trans to a chromosome with no FRT element at all and induced FLPase (as above). We found no retinal mosaic clone in over 400 such flies scored. Thus, ectopic FLP-mediated somatic recombination is more than two orders of magnitude more rare than FRT-mediated recombination (for this chromosome arm). As we found sterile clones at a 16% frequency (eight out of 50), one cannot account for them by invoking ectopic recombination.

Thus, the sterile clones reveal an actual failure to induce ectopic furrows. Even such sterile clones can re-specify polarity field markers if they lie on the margin or midline (green arrow in Fig. 4J). This also demonstrates that the establishment of the polarity field does not require the morphogenetic furrow. The eight sterile clones cluster in the anterior dorsal quadrant of the eye (Fig. 5) but do not define a cleanly separable domain. Nor does it appear that there is any clear correlation with clone size. We can only conclude that the anterior dorsal quadrant has a lower susceptibility to *patched*-mediated furrow induction than the remainder of the field. The anterior dorsal quadrant is the primary domain in which *wingless* acts to repress furrow initiation and thus *wingless* may be acting in this domain to counteract (in part) the effects of the *patched* clones.

2.3. Retinal axon pathfinding is cooperative and is correlated with the presence of basal glia

We noticed that the photoreceptor cells in the ectopic ommatidia formed by *patched* clones produce axons that do not always follow the normal polarity field towards the posterior and the optic stalk. Normally the efferent retinal axons follow their predecessors to form a fan-like array leading to the optic stalk and the brain. This acts through a system of molecules that includes the product of the *dreadlocks* gene (Garrity et al., 1996). We found that in those cases in which a field of ectopic ommatidial clusters had merged with the endogenous furrow, that the axons of the ectopic clusters do follow the others to the optic stalk (Fig. 6A). However, in those cases in which a field of ectopic ommatidial clusters is still disconnected from those formed by the endogenous furrow, the axons of the ectopic clusters do not find a path to the optic stalk, but converge on the center of their local field (Fig. 6D). This may be consistent with a homotypic affinity of the photoreceptor growth cones for the axon tracts formed by their predecessors which also discriminates the polarity of the axons

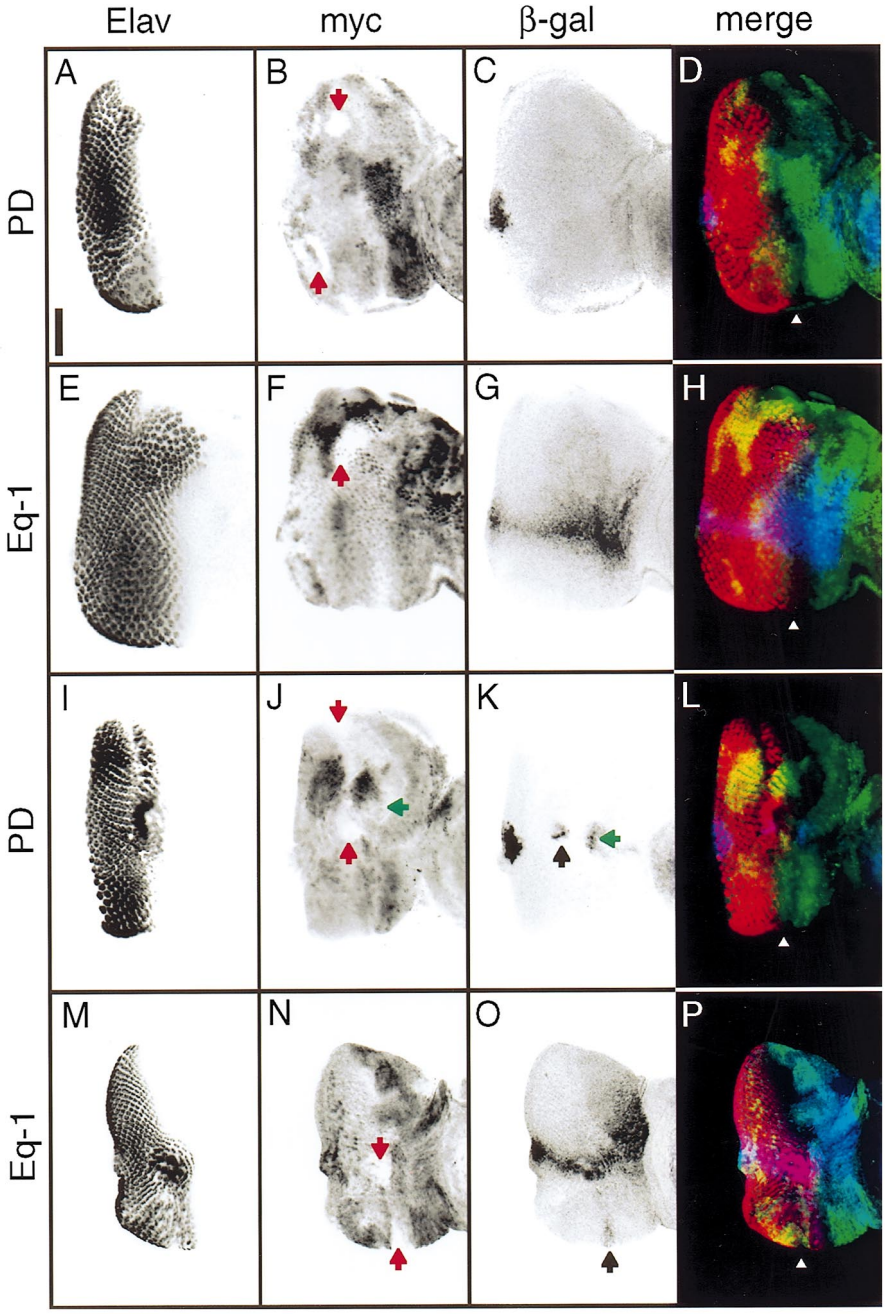

Fig. 4. Ectopic furrows induced by *patched* clones may or may not affect polarity markers. All panels show third instar eye discs containing *patched* mosaic clones. (A,E,I,M) The expression of Elav, a nuclear neural antigen. (B,F,J,N) The Myc epitope which negatively marks the mosaic clones; clones are shown by arrows. (C,G,K,O) β-Galactosidase expression. (D,H,L,P) A merged image of the preceding three panels; the white arrowheads indicate the endogenous furrow (Elav in red, Myc in green and β-galactosidase in blue). (A–H) Discs with clones that do not lie on the margin or midline; β-galactosidase expression is unaffected. (I–P) Discs with clones on the margin or midline; β-galactosidase expression is affected. Note that the dorsal Myc-clone shown in (J) (red arrow) is not intersecting the margin and therefore does not show ectopic PD expression (K). Arrows in (B,F,J,N) show clones (green arrows in (J,K) show a sterile clone that leads to ectopic PD expression prior to furrow initiation). Arrows in (K,O) show ectopic β-galactosidase expression. (A–D,I–L) The PD element. (E–H,M–P) The Eq-1 element. Anterior is shown to the right, dorsal is up, the scale bar in (D) represents 50 μm and all panels are to the same scale.

(seeking the posterior direction). In isolated ectopic fields the growth cones can only grow towards their neighbors and this results in the inward valency of the axons in these field. Once the endogenous furrow merges with the ectopic field, then stalled growth cones in the ectopic field can restart and follow the path to the optic stalk. This phenomenon may be similar to the development of axon tracts in insects' central nervous systems (reviewed by Tessier-Lavigne and Goodman, 1996).

The field of retinal axons is associated with basal glial cells that migrate in from the optic stalk in the opposite direction (Choi and Benzer, 1994a). We have visualized these glia with an anti-Repo antibody (Campbell et al., 1994; Xiong et al., 1994; Halter et al., 1995). These glia

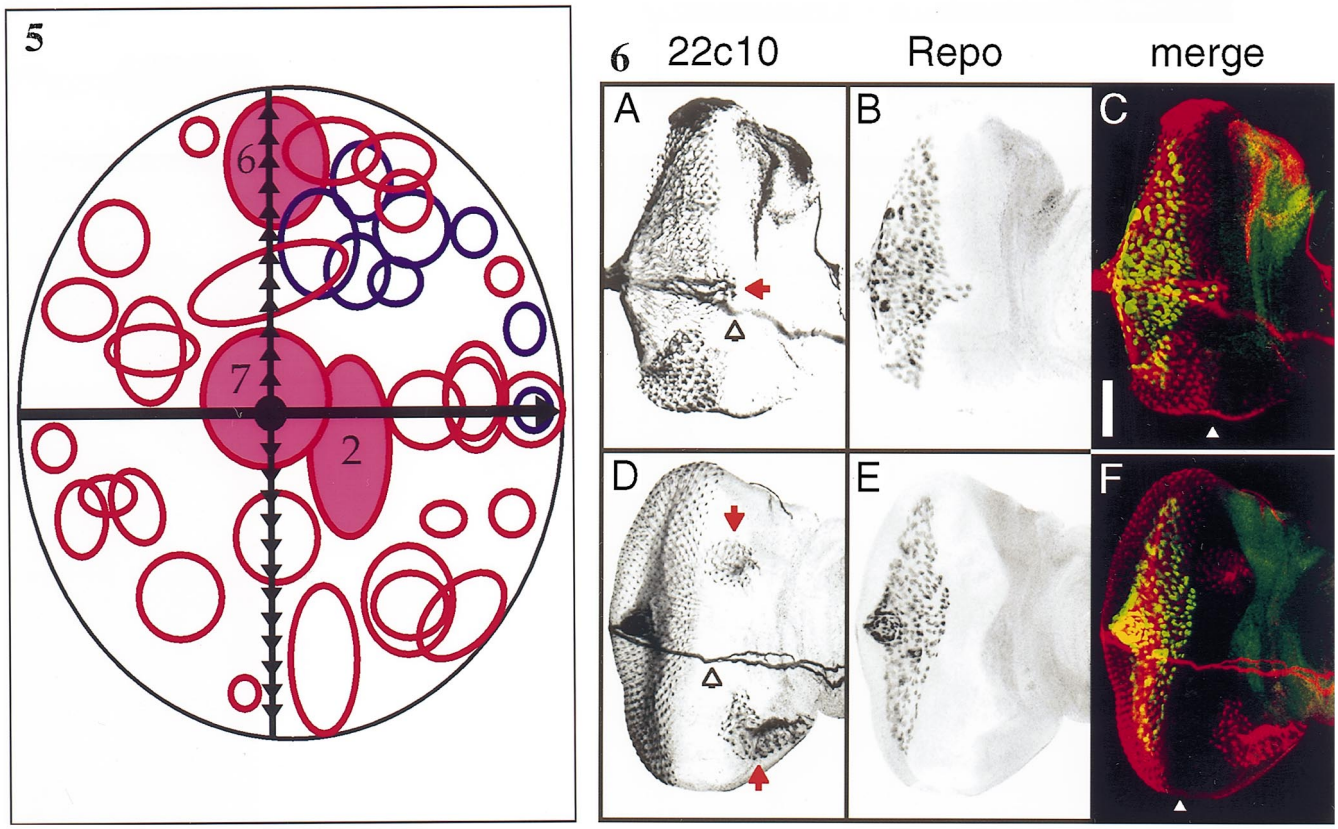

Fig. 5. Map of the location of 42 neurogenic and eight sterile *patched* mosaic clones. In this diagram anterior is to the right and dorsal is up. The black horizontal arrow represents the midline/equator and the vertical black line with arrowheads represents the furrow. The black dot at the intersection of the furrow and midline shows the firing center. The colored shapes represent the approximate size and position of 50 clones. The red shapes represent clones that produced ectopic ommatidia. The three red shapes filled with pink represent multiple cases of very similar clones (the numbers 6, 7 and 2 indicate how many of each). The blue shapes represent sterile clones without ectopic neural clusters. Note that the sterile clones cluster in the dorsal/anterior quadrant (see text).

Fig. 6. Ectopic ommatidial axons follow the endogenous field, when they are in contact with it. All panels show third instar eye imaginal discs with fields of ectopic ommatidia induced by *patched* clones. (A–C) An example in which the ectopic and endogenous fields have merged. (D–F) An example in which the ectopic and endogenous fields have not yet merged. Red arrows in (A,D) indicate the ectopic fields. Arrowheads in (A,D) are pointing to the larval photoreceptor (Bolwig's nerve). (A,D) A stain for a neural cell-surface antigen with mAb 22C10. (B,E) The basal glial cells stained with an anti-Repo antibody (see Section 4). (C) A merged image of (A,B); (F) a merged image of (D,E), in which mAb 22C10 is shown in red and anti-Repo is shown in green. Red arrows in (A,D) show ectopic photoreceptor fields. Note that the axons from ectopic fields only find the optic stalk when they have merged with the endogenous field and that this is correlated with the entry of basal glia. Anterior is shown to the right, dorsal is up, the scale bar in (C) represents 50 μm and all panels are to the same scale.</fig

are excluded from isolated ectopic fields (Fig. 6E), but soon after the endogenous field merges with the ectopic field, these glia enter (Fig. 6B). This is consistent with the homophilic axon guidance model described above, except that the glial cells may have philopodia with an affinity for retinal axons, but in the opposite polarity to those of the photoreceptor growth cones.

3. Discussion

Polarity and chirality in the *Drosophila* compound eye develop during larval life. As the morphogenetic furrow passes across the eye field preclusters are formed and subsequently these rotate in opposite directions in the dorsal and ventral domains of the eye (Ready et al., 1976). Later the R3 and R4 photoreceptor cells break the bilateral symmetry of the ommatidium, such that R3 lies polar and anterior to R4 (Tomlinson, 1985). These rotations and cell movements must follow the receipt of positional information by cells in the developing cluster. We and others have proposed models for this information based on two vectors, one being the direction from the cluster to the furrow and the other being the direction from the cluster to the equator (or to the pole, which are formally equivalent) (Gubb, 1993; Ma and Moses, 1995). The first vector (direction to the furrow) determines the anterior/posterior axis of a cluster and may be inherent in the pattern of cell movements in the furrow itself. The second vector (direction to the equator) determines the dorsal/ventral axis and direction of the cluster and together with the first vector determines handedness (chirality). This second vector implies the ability of the clusters to sense a distant landmark (the midline/equator) and how this could occur has been the subject of some

controversy. Gubb (1993) suggested that the direction of the propagation of cluster induction away from the firing center is itself informative and that the equator is a direct product of the movement of the firing center as a node. However we did not find this firing center model consistent with our data from ectopic furrows generated by loss of *wingless* or *patched* function (which do not produce ectopic equators) (Ma and Moses, 1995). Thus, we proposed that the midline/equator has an existence independent of the firing center, that the equator vector remains unaltered by the orientation of the furrow and this results in the production of four lobes of ommatidial polarity with no new equator formed (Fig. 7A). In contrast, others have found that *patched* clones that occur on the margin or midline produce two lobes of ommatidial chirality with a novel equator between them (Fig. 7B) (Chanut and Heberlein, 1995; Strutt and Mlodzik, 1995).

How can these apparently contradictory data be integrated into one model for retinal polarity? We now propose an early equator model: early events establish the dorsal/ventral polarity of the retinal field and establish the midline/equator and only later does the furrow initiate and then the firing center follows the midline, but does not form it. Initial support for this idea comes from the discovery of markers such as Eq-1 and PD that are expressed in specific parts of the field, even before furrow initiation (see above) (Sun et al., 1995). Indeed, Eq-1 expresses β-galactosidase in the midline, ahead of the firing center. However, it could be that the firing center follows the midline, but that inductive propagation from it carries the polarity information to the clusters. To test this we examined the expression of these field markers in cases in which the direction of furrow movement had been manipulated (by loss of *wingless* or *patched* function). Consistent with the early equator model we find that events which initiate furrow movement on the margin or the midline re-specify the field markers, while those that lie off the margin or midline do not. Thus, these treatments can alter the geometry of the retinal tissue polarity field early in development and later furrow induction and the firing center follows the altered field that is already established. Transplantation studies in the Hemipteran *Oncopeltus* in which anterior tissue was rotated resulted in changes in retinal polarity beyond the bounds of the transplant, consistent with the existence of a polarity field anterior to the front edge of retinal morphogenesis (Lawrence and Shelton, 1975). In the Lepidopteran *Ephestia*, cuticle from the presumptive eye field was transplanted into varying dorsal/ventral and anterior/posterior positions ahead of that insect's morphogenetic furrow. The transplanted cells migrated back to their original positions, consistent with the existence of a system of retinal positional information anterior to the furrow (Nardi, 1977). Further evidence for a pre-existing field of positional information

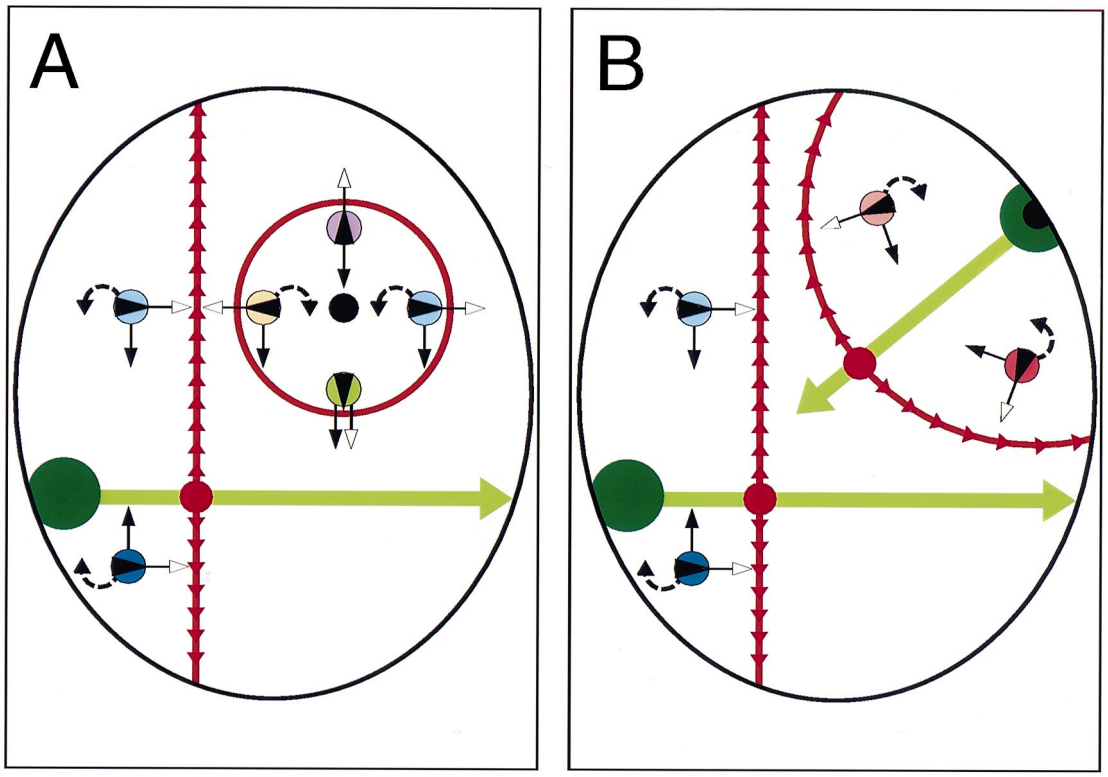

Fig. 7. *patched* clones which are on the margin (and midline) act differently from those which are not. (A,B) Diagrams of third instar eye discs. The furrow is shown as a red line or arc, the firing center(s) as red dots, the expression of PD as a dark green circle, the midline/equator (and expression of Eq-1) as a light green arrow and *patched* clones as black dots. Clusters are shown by the same symbol as used in Fig. 1. Note that the clone in (A) which does not lie on the margin or midline produces a furrow without a firing center, does not alter expression of PD or Eq-1 and does not form an ectopic equator. The clone in (B) lies on the margin and does re-specify PD, Eq-1 and the equator.

comes from the cloning and characterization of a novel PBX-class homeoprotein, *mirror*, which is expressed in the dorsal half of the eye in *Drosophila*. *mirror* expression has been shown in second instar eye imaginal discs prior to furrow initiation and anterior to the morphogenetic furrow in later stages. Loss of function *mirror* clones can alter the direction of the endogenous equator or lead to the establishment of ectopic equators along the clone boundaries (McNeill et al., 1997).

What molecular signals underlie the equatorial signal? Recently, Brodsky and Steller (1996) showed that the *four-jointed* (*fj*) gene shows a graded expression in equatorial-polar direction along the equator in third instar eye imaginal discs. The *four-jointed* gene encodes a putative cell surface or secreted protein. The function of the gene product in the establishment of dorsal-ventral identity of photoreceptor cell clusters remains to be investigated. Another candidate is Wingless itself. Wingless is expressed on the dorsal (and less on the ventral) margin and clones that express ectopic Wingless can reorient adjacent ommatidia (Treisman and Rubin, 1995). Wingless is not likely to act late (i.e. while the furrow is moving) as the late removal of *wingless* function does not affect rotation, except where the furrow is reoriented. However, Wingless could act early to signal from the margins inwards. There could also be a second signal from the midline (perhaps as revealed by Eq-1) and this could be induced by early Wingless. Mosaic clones for *frizzled* affect retinal polarity (Zheng et al., 1995) and these have a 'domineering non-autonomy' on adjacent wild type tissue, as do *frizzled* clones in the wing blade (Adler, 1992). Proteins similar to Frizzled have been shown to act as Wnt receptors (reviewed by Orsulic and Peifer, 1996) and the action of *frizzled* clones might imply that the signal is another Wnt protein. It is perhaps significant that the 'shadow' cast by the *frizzled* clones is away from the equator and this could imply that the source of this signal is the midline. In summary it may be that an early Wingless signal from the disc margin inwards induces a second Wnt signal from the midline outwards (received by Frizzled). These signals on the margin and midline may account for the special nature of *patched* mosaic clones that fall on these lines. Indeed, *patched* may normally function in the regulation of both of these signaling molecules.

4. Experimental procedures

4.1. Drosophila stocks, mosaic clones and temperature-shift experiments

The *ptc* allele was ptc^{7M59} (=tuf^{5} in Lindsley and Zimm, 1992). PD and Eq-1 are two *P*[lacW] insertion lines showing spatially restricted expression patterns (Sun et al., 1995). The *wg* temperature sensitive mutation was $wg^{l\text{-}12}$ (=wg^{IL} in Lindsley and Zimm, 1992). The FLP/FRT system was used for mosaic experiments (Golic and Lindquist, 1989; Xu and Rubin, 1993): males of the genotype w^{1118} P[ry^{+}hsp70:FLP]1; P[ry^{+}hsp70:neoFRT]43D ptc^{7M59}/+ were crossed to virgin females of the genotype *w* PD; P[ry^{+}hsp70:neoFRT]43D P[mini-w^{+}; hs-ΠM]45F, 47F or w^{1118}; P[ry^{+}hsp70:neoFRT]43D P[mini-w^{+}; hs-ΠM]45F, 47F; Eq-1. To induce clones the progeny were heated once at late embryogenesis and twice in the first instar (37°C in narrow glass vials in a water bath for 1 h). Myc expression was induced by heat (as above) followed by 1 h recovery at room temperature prior to dissection. Flies of the genotypes *w* PD; $wg^{l\text{-}12}$/TSTL/+ or w^{1118}; $wg^{l\text{-}12}$/TSTL/Eq-1 were raised at 16.5°C until the second instar and then shifted to 29°C for 48 h. TSTL is a T(2:3) translocation double balancer between SM5 *Cy cn* and TM6B *Tb Hu e*. Eye discs were prepared from Tb^{+} third instar larvae.

4.2. Histology

Eye discs were prepared as described by Tomlinson and Ready (1987), as modified by Tio and Moses (1997), mounted in Vectashield (Vector Labs, H-1000) and examined by laser-scanning confocal microscopy. Primary antibodies were rabbit anti-β-galactosidase (Cortex Biochem, CR7001RP2, 1:6250), mouse mAb 22C10 (gift of Larry Zipursky and Seymour Benzer, 1:50) (Fujita et al., 1982), rat anti-Elav (Iowa, Developmental Studies Hybridoma Bank, 1:150) (Bier et al., 1988; Robinow and White, 1991), mouse anti-Myc (Wisconsin Hybridoma Facility, 1:25) and rat anti-Repo/RK2 (rat anti-RK2; gift of Larry Zipursky, generated in Tomlinson's lab, 1:2000). Secondary antibodies were Cy5 conjugated goat-anti-rabbit (Jackson Labs, 111-176-003, 1:2500), FITC conjugated donkey-anti-mouse (Jackson Labs, 715-095-151, 1:500) or goat-anti-rat (Jackson Labs, 112-096-003, 1:200) and LRSC conjugated donkey-anti-rat (Jackson Labs, 712-085-153, 1:100). Cytoplasmic actin was detected with rhodamine or FITC conjugated phalloidin (Molecular Probes, R-415 or F-432).

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