INTRODUCTION

Proper epithelial morphogenesis requires that cells act according to their position to coordinately execute particular differentiation programs. In the absence of continual inductive interactions from neighboring tissues, epithelial polarity and pattern formation could be achieved by two different but nonexclusive mechanisms. First, patterning could be driven by a pre-established global morphogen gradient such that cells respond differently to different concentrations of the morphogen. Alternatively, once patterning is initiated, it could be propagated by more local gradients or direct cellular interactions (Gubb, 1993; Lawrence, 1992). The developing compound eye of *Drosophila* provides an attractive system in which to study the relationships between epithelial polarity and tissue patterning.

The *Drosophila* retina consists of approximately 800 nearly identical unit eyes or ommatidia, each of which contains a core of eight photoreceptor neurons (R1-R8) that are surrounded by their accessory cone and pigment cells. Within each ommatidium, the photoreceptor cells are arranged in a trapezoidal pattern (Fig. 1). Owing to the asymmetry of the trapezoid, the exact orientation of each ommatidium relative to both the anterior/posterior (A/P) and dorsal/ventral (D/V) axes of the eye is obvious (Fig. 1B). The long side of the trapezoid, formed by R1, R2 and R3, faces the anterior border of the eye, while the short side, formed by R5 and R6, faces posteriorly. In addition, ommatidia in each eye exist in two chiral forms, which reflect along a line of mirror-image symmetry, the equator, that bisects the eye horizontally (Dietrich, 1909). While in the dorsal half of the eye (above the equator) the side of the trapezoid formed by R3, R4 and R5 faces the dorsal margin, this side faces ventrally in the ventral half of the eye (below the equator) (Fig. 1B). R3, R4 and R5 therefore define the polar side of the ommatidium.

The adult retina develops from the eye imaginal disc, which is a proliferating monolayer epithelium until the third larval instar, when patterning and differentiation of retinal cells begins. Differentiation starts at the posterior margin and progresses across the epithelium reaching the anterior margin approximately two days later. The front edge of the differentiating field is marked by a dorsoventral indentation in the epithelium, the result of a transient change in cell shape, called the morphogenetic furrow (MF) (Ready et al., 1976; Tomlinson, 1985). As the furrow progresses, it leaves in its wake parallel rows of gradually assembling ommatidial clusters. The various developmental stages are thus laid out in a smooth spatial gradient, with older clusters positioned furthest from the MF.

The expression of neural antigens by developing R-cells follows a fixed sequence (Tomlinson and Ready, 1987a). Expression is first observed in R8 and is followed sequentially
by the pairs R2/R5, R3/R4, R1/R6, and finally, by R7 (Fig. 1C). Although the stepwise neural differentiation of R-cells highlights the initial asymmetry of developing clusters along the A/P axis (the R3/R4 pair lies closest to the MF, Fig. 1C), this asymmetry already exists prior to neural differentiation as evidenced by histochemical (Wolff and Ready, 1991) or ultrastructural (Tomlinson, 1985) methods. As ommatidial clusters assemble, they rotate as a unit by exactly 90°; in a left eye disc, clusters in the dorsal half rotate clockwise, while clusters in the ventral half rotate counterclockwise, leading to the formation of the equator (Fig. 1C). Consequently, the side of the ommatidium formed by the R3/R4 pair, which initially faced anteriorly, ends up facing away from the equator towards the dorsal or ventral poles of the disc (Fig. 1D). The initial bilateral symmetry of developing ommatidia is broken as the R4 cell (the posterior cell of the R3/R4 pair) loses contact with R8, and clusters become chiral (Tomlinson, 1985). Therefore, ommatidial assembly in the correct orientation with respect to both the anterior/posterior (A/P) and equatorial/polar (Eq/Pl) axes of the eye.

Little is known about how A/P and Eq/Pl asymmetries arise in the retinal epithelium. However, the analysis of mutations that alter ommatidial orientation is starting to provide some clues (Gubb, 1993; Theisen et al., 1994). For instance, frizzled (fz) disrupts primarily the direction of ommatidial rotation (Zheng et al., 1995), while nemo and roulette appear to affect the execution of the rotation program (Choi and Benzer, 1994). Recently, it has been proposed that ommatidial rotation is controlled by a bi-directional signal emanating from the equator (Zheng et al., 1995). The nature of this signal or how its source might become restricted to the D/V midline is unknown. The equator does not represent a border, as evidenced by the observation that clones of cells can contribute to both dorsal and ventral ommatidia (Becker, 1957; Ready et al., 1976); it must therefore be established through cell-cell interactions.

The posterior-to-anterior progression of the MF across the eye disc is believed to occur by successive cycles of induction (for a review see Heberlein and Moses, 1995). Differentiating cells posterior to the MF express and secrete hedgehog (hh), which is thought to diffuse anteriorly into the MF where it induces the expression of the TGF-β family member decapentaplegic (dpp). Cells in the furrow then begin to differentiate as photoreceptors, express hh and induce more anteriorly located cells to express dpp (Heberlein et al., 1993; Ma et al., 1993). The reiteration of this process ensures the posterior-to-anterior progression of morphogenesis across the eye disc. Ectopic expression of hh in clones of cells located anterior to the MF leads to ectopic expression of dpp, precocious neural differentiation and the propagation of ectopic MFs (Heberlein et al., 1995). The direction in which ectopic furrows move across the eye disc appears to be determined solely by the position of hh-expressing cells. As in other imaginal discs (for recent reviews see Kalderon, 1995; Perrimon, 1995), dpp expression in the eye is negatively regulated by patched (ptc) (Ma and Moses, 1995; Wehrli and Tomlinson, 1995) and the gene encoding the major catalytic subunit of protein kinase A (DCO) (Pan and Rubin, 1995; Strutt et al., 1995). Loss of ptc and DCO function anterior to the MF phenocopies the effects of ectopic expression of hh.

In this study, we have assessed the role of the MF in establishing the axes of symmetry in the eye. For this purpose, we manipulated the direction of MF progression by either ectopic expression of hh or by local loss of ptc function and assayed its effects on both A/P and Eq/Pl asymmetry in developing and adult eyes. We find that an ectopic furrow, in addition to perturbing the disc in the A/P direction, can establish an ectopic axis of mirror-image symmetry (equator) that parallels the ectopic A/P axis. We propose a model for coordinate regulation of A/P and Eq/Pl polarity in the developing eye.

### MATERIALS AND METHODS

#### Fly stocks

Stocks carrying hsFLP1 and the FRT at 42D are as previously described (Xu and Rubin, 1993). The y, FL122 stock was obtained from G. Struhl. The w; FRT, ptcS2 and the y, FL122; Tub>y+>hh stocks were obtained from D. Kalderon. The F7 and B347 enhancer trap insertions were kindly provided by M. Brodsky and H. Steller. The FRT43D, ptcS0/CyO and the FRT43D, arm-lacZ stocks were obtained from K. Moses and M. Mlodzik, respectively. Line WR122 maps to the same cytological position as B347 and their expression patterns are indistinguishable. The AE127 enhancer-trap insertion in svp was obtained from the Rubin laboratory collection.

#### Generation of mosaics

**Adult ptcS2 clones**

w, hsFLP1; FRT42D, P[w+] females were crossed to w; FRT42D, ptcS2/CyO males. Progeny were raised at 23°C and subjected to two 1 hour heat shocks at 38.5°C after 48 and 72 hours (or 72 and 96 hours) of development. Clones were identified in adult progeny by the local loss of the w+ marker. The frequency of clones was ~5%.

**Adult Tub>hh clones in F7 background**

w, hsFLP1; Tub>y+>hh/CyO females were crossed to y, w; F7-P[w+], lacZ/TM6B males. Larval progeny was treated as ptcS2 clones. Clones were recognized by roughening of the eye and were obtained at a frequency of ~2%.

**Disc ptc clones**

All disc clones were induced using the FL122 transposase source, which is ~10-fold more active than hsFLP1. y, FL122; FRT43D, ptcS0/CyO males were crossed to y, w; FRT43D, arm-lacZ/CyO females. FRT42D, ptcS2; dpp-lacZ/SM6-TM6B males were crossed to y, FL122; FRT42D, arm-myc females. Larvae were subjected to one 30 minute heat shock (38.5°C) after 30-48 hours of development at 23°C. Frequency of clones was ~20%. Both ptc alleles used are loss-of-function and gave identical results.

**Disc Tub>hh clones**

y, FL122; Tub>y+>hh/CyO females were crossed to males (or vice-versa) carrying one of the following markers: AE127 (an enhancer-trap insertion in svp), F7 (an enhancer-trap insertion with dorsal white and lacZ expression), B347 or WR122 (enhancer trap insertions with equatorial lacZ expression), or a stock carrying a dpp-lacZ reporter (construct BS3.0, Blackman et al., 1991) on the second chromosome. Larvae were treated as described for ptcS2 clones. Frequency of clones was ~40%. Control discs (carrying CyO rather than Tub>y+>hh) were identified by their attached y- mouth hooks. With few exceptions, mosaic larvae died as pupae.

#### Histology

Adult Drosophila heads were fixed and embedded in Durcupan resin according to previously described methods (Tomlinson and Ready, 1987b). 2 µm sections were mounted in DPX (Fluka) and viewed under phase microscopy. Multiple photographs of serial sections of each mosaic eye were assembled to produce the composites shown in Fig. 2.
**Immunohistochemistry**

Third instar eye imaginal discs were immunostained with the following antibodies: rat-anti-Elav (a gift from G. M. Rubin), mAbBP104 (a gift from C. S. Goodman), or rabbit anti-β-galactosidase essentially as described before (Kimmel et al., 1990). Secondary antibodies: goat anti-mouse-HRP was obtained from Bio-Rad, donkey anti-rat-HRP and donkey anti-rabbit-HRP were from Jackson Laboratories. Staining for β-galactosidase activity with X-GAL (Simon et al., 1985) was usually performed after antibody staining. Discs were mounted in 80% glycerol/PBS; due to the abnormal structure of the discs analyzed distortion sometimes occurred during the mounting procedure. Discs were viewed and photographed with a Leica DMR microscope.

**RESULTS**

**Loss of ptc function has non-autonomous effects on A/P and Eq/Pl polarity in the adult eye**

In the course of previous studies, we had observed that ectopic expression of hh in the developing eye could lead to alterations in the polarity of adult ommatidia. Expression of hh under the control of the Tubulin-α1 promoter in clones of cells (Tub>hh clones) generated by the flp-out method (Basler and Struhl, 1994) caused disruptions in the normal ommatidial array, an effect that spread into genetically wild-type regions of the eye (Heberlein et al., 1995). Sections through such eyes revealed that ommatidia apparently located posterior to Tub>hh clones displayed reversed A/P polarity. However, because the exact position of Tub>hh clones could not be established in retinal sections, the location of the affected ommatidia relative to the clone’s boundaries could not be determined.

The effects of ectopic expression of hh in imaginal discs can be mimicked by loss of ptc function (Ma and Moses, 1995; Wehrli and Tomlinson, 1995). We therefore analyzed the effects of local removal of ptc function on ommatidial polarity. Marked homozygous mutant ptc clones were generated by the FLP/FRT recombination system (Golic, 1991; Xu and Rubin, 1993); ptc-78b-galactosidase activity was recognized by the absence of the cell-autonomous marker white+ (Fig. 2) (see Materials and Methods). Although clones that alter the organization of surrounding wild-type tissue were observed throughout mosaic eyes, we concentrated our analysis on clones located near the edges to minimize potential interference between normal and ptc-78-induced patterning (see below).

The structure and orientation of ommatidia within ptc-78 clones is abnormal regardless of their position in the eye. In addition, ptc-78 clones altered the orientation of surrounding wild-type clusters. ptc-78 clones positioned near the anterior border often induced reversal in the A/P polarity of wild-type ommatidia located immediately posterior to them (Fig. 2C-F). The long side of the trapezoid formed by R1, R2 and R3, which normally faces anteriorly, now points towards the posterior of the eye. This effect spreads for several ommatidial columns. Normal and polarity-reversed ommatidia meet along a relatively smooth dorsoventral line of mirror-image symmetry, which reflects across the anterior side of the trapezoid (Fig. 2C-F). When anterior clones are positioned near the dorsoventral midline of the eye, as in the clone depicted in Fig. 2C,D, the field of A/P-reversed ommatidia is traversed by an ‘equator’ that aligns closely with the normal equator.

**Fig. 1.** Organization and sequential assembly of ommatidia. (A) Schematic representation of a left eye with a dorsal and a ventral field of regularly arrayed ommatidia (trapezoidal shapes) reflecting each other along a median line of mirror-image symmetry, the equator (Eq). (B) Close up of two ommatidia facing along their equatorial side. In each cluster, the rhabdomeres of the outer photoreceptors R1-R6 are distributed along the sides of an asymmetric trapezoid; the smaller rhabdomeres of R7 and R8 are positioned centrally (the R7 rhabdome occupies the distal retina, while the R8 rhabdome lies proximally). The two ommatidia are chiral forms that cannot be transformed into each other by simple rotation within the plane of the retina. The orientation of each ommatidium can be schematized by a one-sided arrow; the long side is aligned with the anterior side of the cluster (formed by the rhabdomeres of R1, R2 and R3), and its short side with the polar side (formed by the rhabdomeres of R3, R4 and R5). (C) Schematic representation of the sequential assembly of ommatidial clusters, as visualized with neuronal specific markers (Tomlinson and Ready, 1987), and (D) close up of two mature clusters at the disc’s equator. Clusters can be represented by a vector starting in R7 and ending between R3 and R4. This vector defines the equatorial/polar (Eq/Pi) axis of a cluster. This axis is originally aligned with the A/P axis of the disc, but eventually aligns with the disc D/V axis as clusters rotate by 90° during maturation. This rotation is clockwise in the dorsal portion of a left disc and counterclockwise in its ventral portion.

This observation suggests two possibilities. Either the Eq/Pi orientations of both normal and A/P-reversed ommatidia are determined by the same spatial cues, or the ptc-78 clone generates an equator that happens to coincide with the normal equator. To distinguish between these possibilities, we analyzed more off-centered ptc-78 clones. Fig. 2E,F shows the patterning of ommatidia near an anterior and dorsally located clone. The A/P-reversed ommatidia seem to belong to two distinct fields of opposite Eq/Pi chirality reflecting along a line of mirror-
image symmetry that runs ventrally and posteriorly from the clone. Thus, an ectopic equator, albeit a short one, appears to have formed near this clone.

As was previously observed with Tub>hh clones, ptc- clones can cause considerable overgrowth and deformation of the eye. Therefore, tangential sections of such eyes normally yielded useful information over only small areas. Reconstruction of two examples where serial sections were legible over exceptionally large portions of retina are shown in Fig. 2G,H.

Although both eyes contained two ptc- clones, normal patterning appears to have been modified by the posterior clones only as alterations of A/P polarity are not observed near the smaller anterior clones. Two more or less parallel lines of equatorial mirror-image symmetry coexist in the eye reconstructed in Fig. 2G; the upper one is likely to correspond to the normal equator, whereas the lower one initiates near the ptc- clone. Although both equators are quite obvious, and extend for many ommatidial columns, some distortions occur where the two

Fig. 2. Ommatidial orientation in the vicinity of ptc-S2 clones in adult eyes. Tangential sections through wild-type (B) and mosaic eyes carrying ptcS2/ptcS2 clones (D,F) and the corresponding interpretations of ommatidial orientation (A,C,E,G,H), obtained by reconstruction of serial sections, are shown. The position of ptcS2/ptcS2 clones is recognized by the lack of pigment. In all panels, posterior is to the right and dorsal to the top. Ommatidia are represented by one-sided arrows, as defined in Fig. 1B. Ommatidial polarity is color-coded as follows: blue and lavender identify chiral forms that reflect across the ‘normal’ equator; red and orange identify chiral forms reflecting across ectopic equators. Equators are defined as lines of mirror-image symmetry generated by reflection across the equatorial side of ommatidia. Circles mark ommatidia with abnormal number or arrangement of photoreceptors whose orientation could not be ascertained. Insets show the approximate position of clones (white patches) as recorded prior to sectioning; the approximate positions of normal (purple arrow) and ectopic (green arrow) equators are also indicated. (A,B) Wild-type eye. (C,D) Eye containing an anterior-medial ptc-S2 clone. A field of ommatidia with reversed A/P polarity (located posterior to the clone) is traversed by an equator that coincides with the normal equator. The area shown in D corresponds to the green rectangle in C. (E,F) Eye containing an anterior-dorsal ptc-S2 clone. A field of ommatidia with reversed A/P polarity (located posterior to the clone) is traversed by an equator that is completely contained within the dorsal half of the eye. (G) Reconstruction of an eye containing a posterior-ventral (and an anterior-medial) ptc-S2 clone. Two long equators (encompassing approximately 17 ommatidial columns each) run more or less parallel through this eye defining two distinct ommatidial fields. Some distortion occurs where the two fields meet, perhaps due to interferences between rotational signals emanating from each field. No reversal of A/P polarity or major distortion is observed in the vicinity of the small anterior clone. (H) Reconstruction of an eye containing a posterior-medial (and an anterior-medial) clone. Two equators intersect near the posterior clone. No major distortion of ommatidial orientation is seen near the anterior clone. Because the reconstructions shown in G and H are based on multiple sections, individual sections fail to be informative and are therefore not shown.
ommatidial fields meet. For example, ommatidia located ventrally to the anterior portion of the normal equator have abnormal polarity (as if they had over-rotated). The eye depicted in Fig. 2H also contains two lines of equatorial mirror-image symmetry, positioned at approximately a 90° angle. In this case, it is unclear which, if any, corresponds to the normal equator, as both appear to originate from the posterior-medial ptc clone. These data (and that from several additional clones not shown) suggest that ectopic equators can be induced in the wild-type tissue near ptc clones.

Our data show that multiple equators can coexist in adult eyes. There does not seem to exist a preferential position or direction for an equator, as we can find them in all regions of the eye, and they can run in any direction across the eye. Multiple equators can also occur upon injury to the disc (Campos-Ortega, 1980). In this case, scar tissue is found between the two eye fields containing separate equators. We do not observe scarring in eyes containing ptc or Tub>hh clones, suggesting that our ectopic equators are generated by a different mechanism. Ommatidia in the vicinity of ptc or Tub>hh clones do not always reorient in a coordinate manner or form ectopic equators. However, in ommatidial fields that form new equators, the individual ommatidia are always positioned such that their anterior side (formed by R1, R2 and R3) faces away from the clones. This suggested that patterning was mediated by a wave that originated in the clone and progressed outwards into wild-type tissue. As previously shown for Tub>hh and DCO clones (Heberlein et al., 1995; Pan and Rubin, 1995; Strutt et al., 1995), ptc clones give rise to ectopic morphogenetic furrows that move away from the clone (Wehrli and Tomlinson, 1995; Ma and Moses, 1995; see below). We hypothesized that the position and orientation of ectopic equators might be dictated by the direction of ectopic furrow progression. To test this, we analyzed the orientation of ommatidia developing in the wake of ectopic furrows in third instar imaginal discs.

**Ommatidial assembly can occur in all directions in the developing eye disc**

The effect of loss of ptc function in the developing eye was analyzed in mosaic discs from third instar larvae. Mutant ptc tissue was recognized by the loss of the cell-autonomous marker arm-lacZ (Vincent et al., 1994; see Materials and Methods) (Fig. 3A-E). Developing ommatidia were detected with an antibody that recognizes the neural-specific Elav protein (Robinow and White, 1988). Elav is expressed in all differentiating photoreceptors as they are progressively recruited into ommatidia (Fig. 3A,F). ptc clones located ahead of the MF are associated with fields of precociously differentiating ommatidia (Fig. 3B-E). In these fields, differentiation is graded; more mature ommatidia are found near the center of clones, while less mature clusters lie near the border or in the surrounding wild-type tissue. To visualize the MF, ptc clones were induced in larvae carrying a dpp-lacZ

![Image](image-url)
The direction of ommatidial assembly in relation to the direction of MF propagation was analyzed in these discs. Normally, Elav is first detected in a characteristic triplet, formed by R8, R2 and R5, that lies parallel to the furrow. As clusters mature, Elav appears in R3 and R4, which are added to the anterior side of the R2-R8-R5 triplet (Fig. 1C). Therefore, the A/P axis of individual ommatidia can be deduced from the configuration of Elav-expressing cells. Close examination of ectopic ommatidial fields shows that the R2-R8-R5 triplets emerge from an ectopic furrow parallel to it (Fig. 3H), and that the R3/R4 pair is added to the side of the triplet closest to the ectopic furrow. This holds true whether differentiation spreads radially from a central clone (Fig. 3H) or in one direction from a clone near the disc’s margin (Fig. 3I). As a result, the axes of ectopic five-cell clusters are not necessarily aligned with the A/P and D/V axes of the disc.

Two main conclusions can be drawn from these data. First, ommatidial assembly occurs in a coordinate and polarized fashion behind ectopic furrows in a manner that is indistinguishable from normal ommatidial assembly. Second, the eye disc can accommodate simultaneously several directions of ommatidial assembly. This suggests that the normal posterior-to-anterior gradient of ommatidial differentiation is not due to a prepattern laid out across the disc prior to MF initiation, but rather, that ommatidial assembly coincides with, and may be a consequence of, the direction of MF progression. Thus, the furrow not only establishes a zone of competence for neuronal differentiation, but it also imparts polarity to the assembly process.

**Ectopically induced ommatidia rotate independently of the normal equator**

Ectopic ommatidial fields created by local removal of *ptc* function or by ectopic *hh* expression are indistinguishable (Heberlein et al., 1995; Wehrli and Tomlinson, 1995; Ma and Moses, 1995), and all of the experiments described below were performed with larvae carrying *Tub>hh* clones for two reasons. First, *Tub>hh* clones were obtained at a higher frequency than *B347-lacZ*, forming, as rotation nears completion, the distance between the R1 and R6 nuclei increases as R7 squeezes between them. In addition to the asymmetry generated by R7, the boundaries of individual clusters can be readily visualized using Nomarski optics, allowing the unambiguous assessment of polarity in most ommatidia. In a wild-type left eye disc (Fig. 4A-C), clusters rotate clockwise (CW) in the dorsal hemisphere and counter-clockwise (CCW) in the ventral hemisphere. The equator is roughly aligned with the optic stalk.

To assess ommatidial rotation in ectopic neural fields, we generated *Tub>hh* clones in larvae carrying the *svp-lacZ* marker (see Materials and Methods). Fig. 4D shows an ectopic ommatidial field far ahead of the normal furrow, on the ventral side of the disc. As is always observed near *Tub>hh* or *ptc* clones, substantial overgrowth of the epithelium accompanies this ectopic ommatidial field. Analysis of ommatidial orientation in this field (Fig. 4E and F) revealed that clusters appear to have rotated coordinately, defining an ectopic equator that runs orthogonally to the normal equator. Fig. 4G shows a disc in which ectopic ommatidial differentiation apparently initiated posteriorly and somewhat ventrally, resulting in an asymmetric disc with a large expansion ventral to the optic stalk. Careful assessment of the orientation of ommatidia in this disc revealed the presence of two parallel equators. The normal equator, although somewhat distorted, is roughly aligned with the optic stalk. An additional equator bisects the ventral region of the disc: a close-up view of this region is shown in Fig. 4H I. Therefore, this disc contains four domains of coordinately rotating ommatidia, which alternate their direction of rotation (from dorsal to ventral) as follows: CW-CCW-CW-CCW. It is likely that this disc would have developed into an adult eye similar to that depicted in Fig. 2G.

These results show that fields of ectopic ommatidia rotate coordinately creating a local equator that is separate and, apparently independent from the normal equator. This suggests that ommatidial rotation can occur independently from polarity cues affecting the disc as a whole, and appears to be controlled locally, within each ommatidial field.

**An equatorial marker is induced in ectopic ommatidial fields**

While there are physical manifestations of the equator (rotation of the ommatidia in the disc, mirror-image symmetry in the adult eye), its chemical nature remains elusive. Recently, several enhancer-trap insertions have been generated, in which *lacZ* or white expression coincides with the equator. For instance, in eye discs from larvae homozygous for insertion *B347* (M. Brodsky and H. Steller, personal communication), β-galactosidase expression (*B347-lacZ*) is restricted to a narrow triangular region between the optic stalk and the furrow and centered about the equator (Fig. 5A). There is no expression ahead of the furrow, which suggests that *B347-lacZ* expression is a consequence of furrow progression. We therefore thought that this enhancer-trap might be a good probe for an equator-like activity in ectopic ommatidial fields.

Ectopic ommatidial differentiation was induced in *Tub>hh* larvae bringing the *B347* insert. Double staining with the anti-Elav antibody (to determine ommatidial rotation) was not possible in these experiments, due to weak β-galactosidase expression in heterozygous *B347* larvae. Therefore, we doubly stained discs with mAbBP104 (Hortsch et al., 1990), which,
due to more sparse staining, allowed the visualization of B347 expression. Fig. 5C,D shows discs with two domains of B347-lacZ expression. In each case, one B347-lacZ domain is aligned with the optic stalk and must therefore correspond to the position of the normal equator. The additional B347-lacZ domains always occur within fields of ectopically differentiating ommatidia induced by Tub>hh clones, whether these fields are completely separate from (Fig. 5C) or adjacent to (Fig. 5D) the normal field. In the absence of markers for ommatidial rotation, we cannot determine whether ectopic B347 expression coincides with ectopic equators. Nevertheless, B347-lacZ expression is always restricted to a central subset of cells in ectopically differentiating ommatidial fields. We conclude that, in ectopic ommatidial fields, an environment similar to that found about the normal equator can be created. Curiously, the normal B347-lacZ expression domain was somewhat modified by an ectopic domain in the near vicinity (Fig. 5D). This is reminiscent of interference between normal and ectopic equators observed in sections of mosaic adult eyes and suggests long-range interactions between ommatidial fields.

Identical results to those described for B347 were obtained with another enhancer-trap insertion in the same locus, WR122 (see Materials and Methods). Equatorial WR122-lacZ expression is inhibited in frizzled mutants, which are equatorless (Zheng et al., 1995). Therefore, the expression of this marker is not only induced ectopically in places where equators can form, but it depends, directly or indirectly, upon the presence of an equator.

Expression of a dorsal eye marker is altered by ectopic hedgehog

Other enhancer-trap insertions that direct expression of white

![Fig. 4. Ommatidial rotation in ectopic fields induced by Tub>hh clones. Tub>hh clones were induced in first instar larvae carrying an enhancer-trap insertion in svp and late third instar eye discs were stained with an antibody against β-galactosidase. In all panels, posterior is to the right and dorsal to the top. The position of the MF (black arrowheads) is inferred from the location of the least mature clusters (where only R3 and R4 express svp-lacZ) and by observation with Nomarski optics. (A-C) Wild-type eye disc. The equator (black arrow) is roughly aligned with the optic stalk (blue arrowhead). (B) Close-up of the equatorial region of the disc shown in A. Note that the anterior-most R3/R4 doublets are parallel to the furrow. Expression in R1/R6 begins 2-3 columns more posteriorly as ommatidia begin to rotate. (C) Interpretation of the staining pattern shown in B, highlighting the orientation of each cluster (green arrows, defined in Fig. 1D). Rotation is clockwise in the dorsal half, counterclockwise in the ventral half. (D-F) Eye disc carrying an ectopic ommatidial field induced by Tub>hh near the ventral margin. (E,F) Close-up and interpretation of ommatidial orientation of the ectopic ommatidial field, respectively. The black arrow points to the position of the normal equator, while red arrows point to the position of the ectopic equator. (G-I) Eye disc carrying a Tub>hh clone. The ventral domain of this disc is enlarged by a Tub>hh clone presumably located near the posterior-ventral margin. The normal equator was somewhat distorted during the mounting procedure, probably due to the unusual shape of the disc. However, reconstruction of this area (not shown) confirmed the presence of an equator (black arrow). (H) Close-up of the ventral portion of the disc shown in G and (I) the corresponding interpretation of ommatidial orientation. Green bars indicate clusters whose Eq and Pl sides could not be distinguished. Red arrows point to the position of the ectopic equator.
or lacZ in either the dorsal or ventral eye have been isolated (M. Brodsky and H. Steller, personal communication). Flies carrying the F7 insertion express white (F7-white) in the dorsal half of the eye (Fig. 6A); the ventral border of expression coincides closely with the equator (not shown). We induced Tub>hh clones in larvae carrying the F7 insertion and analyzed the resulting adult eyes (see Materials and Methods). Because mutant cells cannot be marked in this experiment, the presence of clones was inferred from roughening or distortion of the eye surface. Multiple eyes were obtained, in which Tub>hh clones were associated with ectopic F7-white expression (or derepression) in the ventral portion of the eye (Fig. 6B). Conversely, in several cases, F7-white expression was turned-off in the dorsal eye near dorsal-marginal clones (Fig. 6C). Therefore, Tub>hh clones, which we have shown can alter the polarity of ommatidia, induce the expression of a dorsal marker in the ventral eye, while inhibiting its expression in the dorsal eye. While we envisioned that expression of white would be correlated with ommatidial orientation in these eyes, sections through some of these eyes failed to show a precise correlation (although aberrantly oriented ommatidia were observed in the vicinity of such clones, data not shown). We therefore

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**Fig. 5.** Expression of an equatorial marker in ectopic ommatidial fields induced by Tub>hh. Tub>hh clones were induced in first instar larvae carrying the enhancer-trap insertion B347 and late third instar eye discs were doubly labeled for β-galactosidase activity (blue) and with mAbBP104 (brown). The position of the MF is marked with white arrowheads, black arrowheads point to the optic stalk and black arrows point to ectopic domains of B347-lacZ expression. Posterior is to the right, dorsal to the top. (A,B) Eye discs from wild-type larvae homozygous (A) or heterozygous (B) for B347. Note the fan-like pattern of B347-lacZ expression from the posterior region near the optic stalk to the MF in panel A. Expression is noticeably weaker in heterozygous larvae (B) and is confined to a posterior region near the optic stalk. (C,D) Eye discs from larvae carrying Tub>hh clones. Ectopic B347-lacZ expression is associated with ectopic ommatidial fields that are completely separate from (C) or adjacent to (D) the normal fields. Staining in these larvae, heterozygous for B347, is weak.

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**Fig. 6.** Change in expression of a dorsal marker by Tub>hh clones. Tub>hh clones were induced in first instar larvae carrying the F7 enhancer-trap insertion and analyzed in either adult eyes (A-C) or third instar larval eye discs doubly stained for β-galactosidase activity (blue) and with mAbBP104 (brown) (D-F). In all panels but F, posterior is to the right and dorsal to the top. In F, the two discs face each other on their dorsal side. (A) Structurally wild-type eye. F7-white expression is confined to the dorsal half of the eye, above the D/V midline (black arrow). (B,C) The approximate position of clones is inferred from roughening of the eye surface. In the eye shown in B, ectopic F7-white expression (green arrowhead) is observed near a ventral-anterior Tub>hh clone. The eye shown in C contains an anterior-dorsal Tub>hh clone that is associated with inhibition of F7-white expression (green arrowhead). (D) Wild-type eye disc. Expression of F7-lacZ is restricted to the dorsal-anterior region of the disc ahead of the MF (black arrow). (E) Ectopic ventral F7-lacZ expression (green arrowhead) is associated with ectopic ommatidial differentiation (black arrow) near the ventral anterior margin. Note that expression spans areas fated to become cuticle/ocelli as well as retina (yellow arrowhead). Staining in these larvae, heterozygous for B347, is weak.
conclude that F7-white expression is affected by ectopic hh, although not directly via its effect on ommatidial rotation.

In third instar larvae, the F7 insertion directs expression of β-galactosidase (F7-lacZ) in the dorsal-anterior portion of eye discs, anterior to the furrow (Fig. 6D). In Tub>hh discs, we observed ectopic F7-lacZ expression in regions of unpatterned epithelium (Fig. 6E,F). In the vast majority of mosaic discs examined, ectopic F7-lacZ expression was associated with ectopic ommatidial fields, leading us to believe that both phenomena are the result of a single event. Therefore, Tub>hh can, directly or indirectly, induce (or derepress) a normally dorsal disc marker ahead of ectopic furrows. Based on these results, we envision two possible mechanisms for asymmetric expression of F7. First, the furrow (or hh) might send a dorsoventrally asymmetric signal(s) to unpatterned anterior eye tissue resulting in asymmetric induction of gene expression. Alternatively, the furrow (or hh) might send a symmetric signal that is interpreted differently in the dorsal and ventral halves of the disc. We favor the latter possibility for the following reasons. The head cuticle that surrounds the eye (and which develops from tissue that borders the eye field proper (Haynie and Bryant, 1986)) has obvious D/V and A/P polarity, as evidenced by the position of numerous bristles and the ocelli. This asymmetry must be independent of asymmetry in the eye field as it can still form in the absence of an eye. It is possible that dorsal expression of F7-lacZ in the eye reflects a long-range interaction between the furrow (or hh) and determinants that pattern the head cuticle. Consistent with this proposal, the F7 reporter is expressed, in addition to the eye field, in regions fated to become head cuticle and ocelli (Fig. 6D-F). Moreover, ectopic expression of F7-lacZ induced by Tub>hh always encompasses the margin of the eye field (Fig. 6E,F). The relevance of these observations to normal development is difficult to ascertain at the moment, but they may hint at a role of the furrow (or hh) in coordinating growth and patterning in the eye and the surrounding head. Curiously, we often observe ectopic ocelli and bristles in the head cuticle located near Tub>hh or ptc- clones.

**DISCUSSION**

The developing retinal epithelium exhibits clear signs of cellular polarity as ommatidial assembly and rotation occur asymmetrically with respect to the A/P and D/V axes of the disc. This asymmetry extends to the structures that develop from surrounding tissue, such as bristles and ocelli. The degree to which polarity of retinal and cuticle fields is coordinated is at present unknown. We have manipulated the site of initiation of the morphogenetic furrow, and hence its direction of propagation, and assessed the consequences on retinal patterning. The mechanisms underlying normal development are inferred from this analysis. We find that the polarity of ommatidial assembly as well as the direction of ommatidial rotation can be a consequence of the direction of furrow propagation. We therefore propose that the establishment of retinal polarity can occur independently of global patterning determinants and need not predate the morphogenetic furrow.

**How is A/P polarity established in ommatidia?**

It has recently been shown that ectopic furrows, induced by ectopic hh expression (Heberlein et al., 1995) or loss of either DCO (Pan and Rubin, 1995; Strutt et al., 1995) or ptc (Ma and Moses, 1995; Wehrli and Tomlinson, 1995) function, can progress in all directions across the eye disc. Therefore, the furrow’s normal posterior-to-anterior propagation may not be a reflection of epithelial polarity, but rather, a consequence of the posterior site of initiation. In addition, the direction of assembly of individual ommatidia behind ectopic furrows strictly parallels the direction of furrow propagation. This suggests that the polarity of ommatidial assembly is determined by the direction of furrow propagation and is also, ultimately, a consequence of the posterior site of furrow initiation. The mechanisms by which the polarity of ommatidial assembly and the direction of furrow progression are coupled are unknown.

**How is ommatidial rotation controlled?**

During normal eye development, retinal differentiation begins in a few ommatidial clusters located at the posterior edge of the eye disc. This may act as a ‘point source’ of signals, such as hh, that begin pushing the furrow anteriorly across the disc. As the disc widens in the D/V axis, ommatidial assembly and neural differentiation occur, in addition to the posterior-to-anterior gradient, in an center-outward temporal gradient (Wolf and Ready, 1991). Thus, each ommatidial column starts at the equator (the ‘firing center’, Ma and Moses, 1995) and progresses gradually towards the poles. It has been proposed that this center-outward asymmetry could generate an equator by a propagative mechanism involving bidirectional polarized communication between cells (Gubb, 1993). Indeed, Carthew and collaborators (Zheng et al., 1995) have recently provided evidence that a rotational signal (directly or indirectly mediated by fcz) emanates from the equator. Interestingly, fcz mutations disrupt both the center-outward progression of ommatidial assembly and the coordinate rotation of ommatidia, suggesting that both phenomena are linked. Our data do not address the nature of the rotational signal, but they suggest that ectopic furrows can generate their own source of the putative signal and that an eye disc can accommodate more than one such source.

The central position of the ‘firing center’, and thus the equator, may reflect a special property of the retinal epithelium at the D/V midline, established prior to furrow progression. Alternatively, it may reflect the propagation, during furrow progression, of a property specific to the site of initiation. We find that ectopic ommatidia can undergo coordinate rotation leading to the formation of ectopic equators, regardless of their position in the disc. We believe that these equators are genuine because they appear during ommatidial maturation (as does the normal equator), they are accompanied by the expression of an equatorial marker and they persist to adulthood. Occasionally, ptc- or Tub>hh clones create what appears to be a complete ectopic eye field (Figs 3I, 4D, 5C and 6E). It can be argued that, in these cases, a global D/V polarity system has been generated within the ectopic field, which would in turn induce a novel equator. We cannot at this time eliminate this possibility. However, in the majority of our experiments, multiple equators are observed in eye discs or adult eyes displaying only minor structural abnormalities. We therefore suggest that ommatidial rotation and equator formation are not controlled by a single global system of D/V polarity, but rather, reflect a self-organizing property of ommatidial fields. This does not
exclude the possibility that, during normal eye development, the position of the equator is dictated by global D/V determinants, not through direct effects on the retinal epithelium, but rather, by determining the site of furrow initiation.

How do ectopic equators form?
It is likely that Tub\(>hh\) or pte\(^{-}\) clones near the disc’s margin artificially recreate a ‘point source’ similar to that found at the normal site of furrow initiation. We speculate that this could generate a rudimentary ‘firing center’ that then progresses across the disc in association with the furrow. This hypothesis may explain the discrepancy between our results and those obtained by loss of wingless (\(wg\)) function (Ma and Moses, 1995). \(wg\) is normally expressed at the margins of the eye field, where it prevents furrow initiation: removal of \(wg\) function (by means of a temperature sensitive allele) results in an ectopic furrow propagating ventrally from the dorsal edge (Ma and Moses, 1995; Treisman and Rubin, 1995). Ommatidia developing behind this furrow do not rotate. Ma and Moses interpreted this observation in the context of a model whereby ommatidia only rotate when the furrow that creates them is not parallel to the disc’s equator. Implicit in their model is the proposal that the equator predates retinal differentiation and governs the rotation of all ommatidia in a disc. Our data do not support this proposal. We suggest instead that removal of \(wg\) function fails to generate a ‘point source’ of signals (and therefore a ‘firing center’) due to simultaneous furrow initiation along the length of the margin.

In summary, we propose that once the site of furrow initiation is established, the developing ommatidial field can generate signals that mediate coordinate ommatidial rotation. This does not preclude the existence of long-range asymmetric patterning determinants that establish the site of furrow initiation and coordinate proper development of the retina and surrounding structures.

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