frizzled regulates mirror-symmetric pattern formation in the Drosophila eye

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SUMMARY

Coordinated morphogenesis of ommatidia during Drosophila eye development establishes a mirror-image symmetric pattern across the entire eye bisected by an anteroposterior equator. We have investigated the mechanisms by which this pattern formation occurs and our results suggest that morphogenesis is coordinated by a graded signal transmitted bidirectionally from the presumptive equator to the dorsal and ventral poles. This signal is mediated by frizzled, which encodes a cell surface transmembrane protein. Mosaic analysis indicates that frizzled acts non-autonomously in an equatorial to polar direction. It also indicates that relative levels of frizzled in photoreceptor cells R3 and R4 of each ommatidium affect their positional fate choices such that the cell with greater frizzled activity becomes an R3 cell and the cell with less frizzled activity becomes an R4 cell. Moreover, this bias affects the choice an ommatidium makes as to which direction to rotate. Equator-outwards progression of elav expression and expression of the nemo gene in the morphogenetic furrow are regulated by frizzled, which itself is dynamically expressed about the morphogenetic furrow. We propose that frizzled mediates a bidirectional signal emanating from the equator.

Key words: Drosophila, frizzled, spiny legs, polarity, photoreceptor

INTRODUCTION

Pattern formation often involves coordinated morphogenesis of differentiated cells dictated by their positions within a field of cells. This is evident in the Drosophila compound eye, which develops a mirror-image symmetric pattern of ommatidia from an unpatterned epithelium. Ommatidia in the dorsal half of the eye are mirror-image patterns of those in the ventral half and the two chiral forms meet along a precise boundary that bisects the eye called the equator (Dietrich, 1909). Pattern formation in the eye begins in the morphogenetic furrow of the larval eye imaginal disc. The furrow moves across the eye disc in a posterior-to-anterior direction, initiating the periodic formation of ommatidia. When ommatidia initially develop in the furrow, both dorsal and ventral forms are equivalent with an internal symmetry that is bilateral about the anteroposterior axis (Ready et al., 1976). As the ommatidia develop, they rotate precisely 90°; those in the dorsal half of the eye turn in the opposite direction to those in the ventral half. This specifies their initial dorsal/ventral positional identities and gives rise to the equatorial midline. During rotation, each ommatidium turns as a unit, with the photoreceptor (PR) cells maintaining cell-cell contacts with the same set of neighbors. As rotation proceeds, asymmetries become incorporated into each ommatidium by the movement of R3, R4 and R8 PR cells (Tomlinson, 1985). In both dorsal and ventral halves of the eye, the anterior cell of the symmetric R3-R4 pair displaces the posterior one and becomes an R3 cell. The posterior cell loses its contact with the central R8 cell and becomes an R4 cell. The R8 cell later migrates from the center of the cluster anteriorly to a location between the R1 and R2 cells. Together with rotation, the establishment of asymmetry within each PR cluster assigns the ommatidia their chiral identities such that the dorsal ommatidia are mirror images of the ventral ones. The mechanisms that regulate mirror-symmetry formation are not understood. However, they likely involve cell-cell interactions rather than cell lineage since a single clone of cells can contribute to ommatidia on both sides of the equator (Becker, 1957; Ready et al., 1976).

Two different mechanisms have been proposed to explain the polarity of pattern formation in adult insects. Both invoke the initial establishment of a morphogen concentration gradient. In the first model, the polarity of each cell is locally determined by the steepest slope of the morphogen gradient. Assembly of polarized cells then defines the polarity of larger scale patterns (Lawrence, 1966). In the second model, cells respond to different morphogen concentrations by differentiating to maintain graded concentrations of a local-acting secondary signal (Lawrence et al., 1972; Gierer, 1974). Thus, a stable and self-regulating gradient of differentiated states would form with respect to continuous expression of the secondary signal. Transplantation studies in Rhodnius favor the second model since sections of abdominal epidermis rotated through 180° tend to maintain their original patterns and yet morphogenetically interact with their new neighbors (Locke, 1959; Lawrence et al., 1972). Transplantation studies in Oncopeltus suggest that a similar mechanism is operating to control the polarity of ommatidia in the compound eye (Lawrence and Shelton, 1975).

We describe here a number of genes that act directly in
mirror-symmetry pattern formation in Drosophila eyes. These genes are required for correct polarity of adult cuticular structures such as hairs and bristles (Gubb and Garcia-Bellido, 1982). In particular, the frizzled (fz) gene is required for all aspects of mirror-symmetry formation. In the adult cuticle, fz mutations show directional non-autonomy along the proximal-distal axis affecting wing hair polarity (Vinson and Adler, 1987). The fz gene encodes a cell surface protein with seven transmembrane domains but not all of the sequence features characteristic of G-protein-coupled receptors (Vinson et al., 1989; Park et al., 1994; Krasnow and Adler, 1994). Here we show that fz is dynamically expressed about the morphogenetic furrow in the developing eye, and expression within and posterior to the furrow is sufficient for its function. We find that fz is required for coordinating most ommatidial morphogenetic movements posterior to the furrow, and is required for equator-outwards progression of neuronal development within the furrow. fz-mediated signaling between the presumptive R3 and R4 cells in each ommatidium influences the positional identities of these cells and rotation of the ommatidium, and fz activity is directed in a gradient fashion from the presumptive equator to each pole. The results are discussed in terms of a model for mirror-symmetry formation related to the classical model for insect epidermal polarity.

**MATERIALS AND METHODS**

**Histology, histochemistry and immunohistochemistry**

For light microscopy analysis, adult Drosophila heads were fixed and embedded, and sections were cut as described by Carthew and Rubin (1990) and Freeman et al. (1992). Samples for scanning electron microscopy were prepared as described by Kimmel et al. (1990) and Freeman et al. (1992). Samples for scanning electron microscopy were prepared as described by Carthew and Rubin (1990) and Freeman et al. (1992). Samples for scanning electron microscopy were prepared as described by Carthew and Rubin (1990) and Freeman et al. (1992). Samples for scanning electron microscopy were prepared as described by Carthew and Rubin (1990) and Freeman et al. (1992). Samples for scanning electron microscopy were prepared as described by Carthew and Rubin (1990) and Freeman et al. (1992). Samples for scanning electron microscopy were prepared as described by Carthew and Rubin (1990) and Freeman et al. (1992). Samples for scanning electron microscopy were prepared as described by Carthew and Rubin (1990) and Freeman et al. (1992).

**Mosaic analysis**

Clones of desired genotype were generated by mitotic recombination between heterozygous chromosomes and were visualized by marking a specific chromosome with a transposable element containing the cell-autonomous white (w) gene. fzKD4a clones were generated by the FLP-FRT system (Xu and Rubin, 1993). w; fz, P[FRT]80B/TM3 flies were crossed to w, P[hsFLP]; P[w+]70C; P[FRT]80B flies, and clones were induced by a 1 hour heat shock at 38°C after 42 hour development. Clones of spie1 genotype were generated by γ-irradiating (1000R) w; P[w+]47A/spie1 larvae after 54±6 hours development.

**RESULTS**

**fz mutants disrupt ommatidial polarity**

We examined the eyes of homozygous fzKD4a mutants (a transcript null allele of fz; Adler et al., 1990; P. Adler, personal communication). Like most fz alleles, homozygous fzKD4a mutants have eyes that are roughened in appearance, and the facets have lost their normal hexagonal shape and exhibit disarranged bristles (Fig. 1A-D). Tangential sections revealed a disrupted orientation of ommatidia in fz eyes. In wild type, the precise 90° rotation places the trapezoidal ommatidia in antiparallel arrays (Fig. 1E). Within each ommatidium, the R1, R2 and R3 cells are aligned such that they are anterior and parallel to the R5 and R6 cells, which are around the anterior-posterior axis. The R7 cells in the dorsal and ventral halves of the eye point towards opposite poles, forming an equatorial midline at the place where the two halve meet. Although fz ommatidia were composed of a complete set of correctly assembled PR cells and pigment cells, the mirror-image symmetry was completely abolished and no equatorial midline could be found (Fig. 1F). We analyzed the polarity phenotype in four ways. First, were the ommatidia arranged in antiparallel arrays? Second, in what directions were the R7 cells pointing? Third, were the asymmetric arrangement of R3 and R4 cells affected? and fourth, were the R8 cells positioned correctly? In fz mutants, although most ommatidia were aligned in antiparallel arrays, the R7 cells within these ommatidia were pointing to either pole randomly, regardless of their dorsal-ventral location. Approximately 27% of the ommatidia had their R1, R2 and R3 cells aligned at 45° angles from the anterior-posterior axis. A few ommatidia had their R1, R2 and R3 cells aligned parallel to the anterior-posterior axis, and almost all of their R7 cells were pointing posteriorly. When the asymmetric arrangement of R3 and R4 cells were examined in fz mutants, 19% of fz ommatidia had the R3 cell posterior to the R4 cell rather than anterior to it, and 11% had the R3-R4 pair remain symmetric. Interestingly, it was noticed that among the ommatidia that had the R1, R2 and R3 cells aligned parallel to the anterior-posterior axis, the percentage of ommatidia having symmetric R3-R4 pairs increased twofold. Another feature that normally contributes to the final chiral form of each PR cluster is that the R8 cell is positioned between the R1 and R2 cells on the anterior side of the cluster. In fz eyes, almost all R8 cells occupied a position between the R1 and R2 cells, regardless of whether they were on the anterior or posterior side of the cluster (data not shown).

To interpret better the abnormal phenotypes seen in fz adult eyes, we followed their development from the time when the morphogenetic furrow initiates pattern formation. We used a monoclonal antibody to the neural-specific elav protein to examine the morphogenesis of developing PR clusters and to assess neuronal differentiation in the eye disc. The normal sequential morphogenesis of PR clusters is summarized in Fig. 1F.
Fig. 1. Adult eye phenotypes of \( \text{fz} \) mutants. Anterior is to the left, and dorsal is up. (A-D) Scanning electron micrographs of wild-type (A,C) and \( \text{fz} \) mutant (B,D) eyes. (E-G) Tangential sections of compound eyes from wild-type (E), \( \text{fz} \) mutant (F) and \( \text{fz} \) mutant flies with one copy of the \( \text{P[w+}, \text{Glfz]} \) transgene (G). PR cells are scored by their darkly stained rhabdomeres. In wild type (E), each ommatidium consists of eight PR cells (inset). Note the characteristic trapezoidal configuration of R1-R6 cells. R7 cells are projecting to the equator illustrated by the zig-zagged line bisecting the eye. R8 cells are below the plane of section. In \( \text{fz} \) mutant eyes (F), the R7 cells are pointing to either pole randomly (arrowheads). We have labelled the cells on the R3 cell side as being R1 and R2 cells while those on the R4 cell side as being R5 and R6 cells. The arrow shows an ommatidium with its R3 and R4 cells occupying opposite anteroposterior positions. The circled ommatidium has R1, R2 and R3 cells aligned at a 45° angle to the A-P axis and the R3 and R4 cells are symmetric, as if they have both adopted the positional identity of an R3 cell. (H) Histographic summary of \( \text{fz} \) eye phenotypes. The ommatidial polarity phenotypes seen in \( \text{fz} \) eyes are categorized into three classes. A total of 657 ommatidia were scored over the entire retina. However, the distribution of phenotypes for all categories was similar in both halves of the eye. Positions of R3/R4 cells are indicated as the following: A\(^3\)-P\(^4\), the anterior cell is an R3 cell and the posterior cell is an R4 cell; A\(^3\)-P\(^1\), the anterior cell is an R4 cell and the posterior cell is an R3 cell; A\(^3\)-P\(^3\), both the anterior and posterior cells occupy the position of an R3 cell; A\(^3\)-P\(^4\), both cells occupy the position of an R4 cell.

2A. Initially, the PR clusters form as bilaterally symmetric structures. They rotate 90° either clockwise or counter-clockwise depending on which half of the eye they reside (Fig. 2D). As the rotation proceeds, the anterior cell (presumptive R3 cell) of the symmetric R3-R4 pair on both halves of the eye displaces the posterior one (presumptive R4 cell) from its contact with the R8...
cell, breaking the bilateral symmetry (Fig. 2F). The asymmetric patterning of the PR clusters is completed with the R8 cell projecting anteriorly to a position between the R1 and R2 cells (Fig. 2K). Together, the rotation and the asymmetry of each ommatidium impose the final mirror-image pattern across the entire eye. In \( f_2 \) mutant eyes, the onset of elav expression progressed sequentially in the PR cells in a manner that was almost identical to wild-type (Fig. 2B-G). Moreover, \( f_2 \) PR cells appeared to have adopted their correct PR subtype identities, as indicated by the presence of the rough protein in R2, R3, R4 and R5 cells in \( f_2 \) ommatidia (Fig. 2H,I). Rough is normally expressed only in these four cells posterior to the furrow (Kimmel et al., 1990).
Fig. 2. Abnormal morphogenesis in developing fz eyes. Anterior is to the left, dorsal is up, and areas shown are within the dorsal halves of eyes. (A) A schematic drawing of the sequential morphogenesis in wild-type eye imaginal discs that leads to the final mirror-image symmetry. The PR clusters initially form as bilaterally symmetric structures behind the morphogenetic furrow (MF). Displacement of R4 cells by R3 cells begins at two-cone cell (cc) stage, and is apparent by the four-cone cell stage. (B-G) Anti-elav antibody staining of wild-type (B,D,F) and fz (C,E,G) eye discs. elav protein is present in all neuronal cells (Robinow and White, 1991). (B,C) Region within the morphogenetic furrows (arrows) showing the center-outwards progression of neuronal differentiation. Arrowheads point to the midline of the discs where differentiation of PR cells normally initiates. (D,E) Symmetric eight-cell cluster stage. Arrowheads are placed between R3 and R4 cells indicating the orientations of PR clusters by pointing along their axes of bilateral symmetry. This symmetry is also seen in R2, R5 and R8 cells which are below the focal plane. Identities of R3 and R4 cells in E are ambiguous and are noted by asterisks. Note one ommatidium with an accelerated rate of rotation. (F,G) Four-cone cell stage. R3, R4 and R7 cells are shown at this focal plane. In wild-type (F), the anterior R3 cell has displaced the posterior R4 cell from the center of the cluster. In fz mutants (G), R3 and R4 cells (asterisks) remain symmetric. (H,I) Anti-rough antibody staining of wild-type (H) and fz (I) eye discs. Only R3 and R4 cells are shown at this focal plane (indicated by asterisks in I). Arrowheads indicate the orientations of PR clusters. (K-M) Anti-elav antibody staining of wild-type (K) and fz (L,M) 50 hour pupal retinas. Most fz PR clusters have rotated 90° by this stage to point to either pole (arrowheads, L). Some PR clusters exhibit retarded rotation (arrowhead, M). Most fz PR clusters have broken the symmetry between R3 and R4 cells, but the anterior cell of the R3-R4 pair does not always become an R3 cell (M). Symmetric R3 and R4 cells are indicated by asterisks in (M). (J,N) Cobalt sulfide staining of 60 hour wild-type (J) and fz (N) pupal retinas showing cell boundaries of cone and primary pigment cells. The equatorial (eq) and polar (pl) cone cells contact each other, and are symmetric about the A-P axis. The anterior (a) and posterior (p) cone cells are symmetric about the D-V axis. The cone cells are surrounded by two primary pigment cells which are also symmetric about the D-V axis. In fz mutants (N), the four cone cells fail to adopt their normal polarities along the A-P and D-V axes. Arrowhead shows a case in which one cell of the anterior-posterior cone cell pair is missing, and the other wraps around the remaining equatorial-polar cone cell pair.

The spatial arrangement of PR cells in most fz clusters was also normal. However, a few clusters had their R2 and R5 cells positioned slightly posterior to the R8 cell rather than slightly anterior as seen in wild-type (data not shown). The arrangement of the other PR cells in these clusters was otherwise normal. Thus, it is unlikely that the ommatidial orientation phenotype seen in adult eyes is due to reversed spatial arrangement of PR cells within individual ommatidia.

In contrast, we observed multiple morphogenetic abnormalities in fz eye discs. The PR clusters were captured at positions as if they had not rotated according to their dorsoventral position in the eye disc, but had randomly turned either clockwise or counterclockwise (Fig. 2E,G,I). As a result, the equatorial midline that is apparent posterior to the furrow of wild-type eye discs was not seen in fz eye discs. Some PR clusters did not rotate at all and many were under-rotated. A very small number of PR clusters were over-rotated, but there was no evidence of rotation of more than 180°. Thus, it seems that it is the randomized direction of rotation, rather than rotating an additional 180°, that places the ommatidia with their R7 cells pointing randomly to either pole. Also, it appears that it is retarded rotation that arrests some of the fz ommatidia with their R1, R2 and R3 cells parallel or at 45° angles to the anterior-posterior axis in adult eyes. In wild-type, the broken symmetry of R3 and R4 cells is apparent at the four-cone cell stage (Fig. 2F). In fz eye discs, four-cone cell stage clusters still had symmetrically positioned R3 and R4 cells (Fig. 2G).

We examined a later stage of ommatidial development in fz mutant pupae using anti-elav antibody (Fig. 2K-M). By this stage, most PR clusters had almost completed their 90° rotations and were often oriented antiparallel to each other as in wild type, although some were still arrested at a 45° angle or had not rotated (Fig. 2L,M). Most clusters exhibited asymmetry due to R3 and R4 cells’ positional displacement while others still had symmetric R3-R4 pairs. In many, the anterior cell was displacing the posterior one as seen in wild-type. However, in some PR clusters, it was the posterior cell of the R3-R4 pair that displaced the anterior one (Fig. 2M). We also examined pupal retinas using cobalt sulfide staining to outline the cell boundaries. In wild-type, the four cone cells are surrounded by the two primary pigment cells, and all are arranged above the PR clusters in a stereotypical orientation that is symmetric about both the anterior-posterior (A-P) and dorsal-ventral (D-V) axes (Fig. 2J). In fz retinas, the number of pigment and cone cells was almost normal but their symmetry about both axes was abolished (Fig. 2N).

The equator is first detected in the morphogenetic furrow of wild-type eye discs; new columns of ommatidia are initiated at the equator and grow as new clusters are added to the peripheral ends (Wolff and Ready, 1991a). This equator-out progression is reflected in elav-stained eye discs. The leading column of elav-expressing clusters in the furrow is restricted to the midline while more posterior columns extend to the lateral ends of the discs (Fig. 2B). However, in the furrow of fz eye discs, the leading column of elav-expressing clusters is often arranged haphazardly with several advanced clusters in the peripheral dorsal or ventral regions of the furrow (Fig. 2C). It is not clear from these discs if the central ommatidia are initiating more slowly than normal or if the peripheral ommatidia are initiating more rapidly than normal. This result indicates a requirement for fz to initiate the equator-outwards progression of neuronal differentiation in the morphogenetic furrow.

In summary, the defects in developing fz eyes that we have observed are as follows. Posterior to the furrow, PR clusters rotate in either direction randomly regardless of their dorsoventral localization, and some exhibit retarded rotation while a few display accelerated rotation. It is not always the anterior cell of the presumptive R3-R4 pair that displaces the posterior cell and becomes an R3 cell. Later, the cone and primary pigment cells fail to adopt their normal polarities along the A-P and D-V axes. Based on these observations, the polarity phenotype seen in fz adult eyes can be interpreted as the result of randomized direction of ommatidial rotation, insufficient degree of rotation, and incorrect asymmetry established between R3 and R4 cells (Fig. 1H). Thus, fz is required for the correct rotational direction, the precise 90° turn, and the correct asymmetry arrangement of R3 and R4 cells. Noticeably, fz is not required for the synchronous movement of PR cells within individual clusters since PR cells in each fz mutant ommatidium still rotate as a unit.
spiny legs (sple), prickle-spiny legs (pk-sple) and dishevelled (dsh) also affect ommatidial polarity

To gain further insight into the mechanism of mirror-image symmetry formation, we examined other polarity mutants. Mutations in the sple and dsh genes were seen weakly to roughen the eye and disrupt ommatidial polarity (Gubb, 1993; Theisen et al., 1994). A third gene, pk, affects tissue polarity but has no mutant eye phenotype (Gubb, 1993; R. W. C., unpublished data). However, a pk-sple double mutant gives rise to a strongly roughened eye, suggesting that sple and pk act redundantly in eye development (Gubb and Garcia-Bellido, 1982). Sections of sple, pk-sple and dsh mutant eyes showed a disturbed ommatidial polarity with each ommatidium having the normal arrangement of PR cells (Fig. 3). Together with fz these polarity mutants can be categorized into two classes based on the adult eye phenotypes. One class, which consists of fz, pk-sple and dsh, exhibits all three aspects of polarity phenotype seen in fz adult eyes. Among them, pk-sple had the strongest eye phenotype (Fig. 3A). The other class, which consists of sple, exhibits only one aspect of the polarity phenotype seen in fz eyes. Although sple ommatidia were still arranged in antiparallel arrays and the equator was still detected, some of the ommatidia were oriented such that their R7 cells were pointing away, rather than towards the equator (Fig. 3C). Anti-elav staining of sple eye discs revealed that the disorientation was due to incorrect direction of rotation as in fz mutants (data not shown).

**fz has non-autonomous effects on eye development**

fz could control ommatidial polarity by acting within each ommatidium, or by mediating ommatidial interactions with surrounding cells. To distinguish between these two possibilities, we conducted mosaic analysis by generating clones of fz− cells in wild-type eyes. Sections through the clones and surrounding wild-type tissue of 15 mosaic eyes revealed a complexity of effects. Consequently, the results of this analysis have been categorized according to the effects on degree of rotation, direction of rotation, and R3/R4 cell positioning.

First, fz appears to act within an ommatidium to control its 90° rotation. Ommatidia with a mixture of mutant and wild-type PR cells were found at the borders of clones. When one or more PR cells were wild type within a mosaic ommatidium, the 90° rotation of that ommatidium was complete 99% (232/235) of the time (Fig. 4A,C,E). Complete rotation was not correlated with any particular fz+ PR cell. Genotypically mutant ommatidia within the clones had a frequency of incomplete rotation similar to homozygous fz mutants even if they were close to a clone boundary (data not shown). Therefore, if neighboring wild-type ommatidia were responsible for the rescued phenotype of mosaic ommatidia, their effect must be short-ranged. A more likely explanation is that the rescued phenotype of mosaic ommatidia was achieved by having one or more wild-type PR cells within each ommatidium, and each ommatidium rotated as an autonomous unit.

Second, fz appears to have a non-autonomous effect on the direction of ommatidial rotation that is graded along the equatorial-polar axis. Some large fz− clones disrupted the polarity of neighboring wild-type ommatidia on the polar side of the clones but only rarely on the equatorial side of the clones (Fig. 4B). In most cases, the disrupted ommatidia adopted an incorrect rotational direction. This directed non-autonomy was seen in clones that were exclusively in the dorsal or ventral half of the eye. Clones that were centered about the equator affected wild-type ommatidia on both sides of the clones. The effects were generally in the range of one or two rows distance from the clone boundary, which is a few cell diameters. We also observed that the rotational direction of mosaic ommatidia at clone boundaries was differentially affected by wild-type neighbors. We found that 87% (80/92) of mosaic ommatidia along equatorial borders were phenotypically wild type, compared with 43% (61/143) of mosaic ommatidia along polar borders (Fig. 4A,C,E). This directional non-autonomous rescue is consistent with the equator to polar effect that mutant tissue had on wild-type ommatidia.

It was possible that the genotypes of PR cells within the mosaic ommatidia were contributing to the rotational

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**Fig. 3.** Tangential sections of pk-sple+ (A), dsh+/dshv26 (B) and sple− (C) mutant eyes. Anterior is to the left, and dorsal is up. Arrowheads indicate the orientations of PR clusters. Arrows show examples of reversed positioning of R3 and R4 cells. The circled cluster in A is oriented at a deflected angle from its neighbors, disrupting the anti-parallel arrays of ommatidia. The equator can still be detected in sple− eyes, and is outlined in C.
mutant phenotype is partially penetrant and we were able to biasing effects that we had observed in sple to adopt an R3 cell positional identity.

73% (8/11) of the few ommatidia in which the fz in an anterior position. In 96% of these ommatidia (77/80), the fz activities in R3 and R4 cells influences the choice of rotational direction such that the cell in an R3-R4 pair with greater fz activity will be anterior to the other cell following the rotation. A correlation between the direction of rotation and differential borders were three-fold more likely to have R3 anterior to R4 than ommatidia along polar borders (Fig. 4E). This non-autonomous rescue of the R3/R4 cell positioning phenotype of equatorial ommatidia is consistent with the directional non-autonomy seen affecting rotational direction. In mosaic ommatidia, a role for the fz gene could be detected in the R3 and R4 cells themselves. Ommatidia in which both R3 and R4 cells were genotypically mutant displayed a mutant R3/R4 cell positioning phenotype 24% (18/74) of the time. In contrast, ommatidia in which both R3 and R4 cells were genotypically wild type displayed such a mutant phenotype only 7% (5/69) of the time. Analysis of mosaic ommatidia also revealed that relative fz activities in the R3 and R4 cells influences which positional fate each cell adopts. We examined mosaic ommatidia in which only one cell of the R3-R4 pair was fz*.

To determine which cells require the sple gene for ommatidial polarity, we performed mosaic analysis by producing clones of sple- cells. There was no evidence of the non-autonomous or biasing effects that we had observed in fz- clones. Since the sple mutant phenotype is partially penetrant and we were able to assign photoreceptor identities in mutant ommatidia, we only examined mosaic ommatidia that had rotated incorrectly. Far fewer mosaic ommatidia exhibited a mutant phenotype than genotypically mutant ommatidia. At the borders of seven clones, fourteen mosaic ommatidia with mutant polarity were scored. In all cases, the presumptive R4 cell was sple- and, in almost all cases, the presumptive R3 and R5 cells were sple+ (Table 1). Thus, removal of sple from R3, R4 and R5 cells can lead to incorrect rotational direction. If any one of these cells is sple+, the ommatidium almost always rotates normally. Therefore, sple function in either R3, R4 or R5 cell appears to be sufficient to drive an ommatidium in the right direction.

Table 1. Summary of mosaic studies with sple

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<th>Presumptive PR cells in phenotypically mutant ommatidia</th>
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frizzled and pattern formation in Drosophila eye

To localize fz gene expression, we performed in situ RNA hybridization of eye imaginal discs. An antisense RNA probe synthesized from a fz cDNA template hybridized to wild-type, but not fz mutant eye discs (Fig. 5). fz mRNA was uniformly detected anterior to the furrow. Immediately anterior to the furrow the level of expression sharply increased in a narrow band of cells (Fig. 5A,B). The staining intensity was reduced within the furrow before becoming intense again immediately posterior to the furrow. Approximately six columns posterior to the furrow, the concentration of fz mRNA decreased to a low level. In regions of fz expression, the level of fz mRNA was uniform from dorsal to ventral poles, with no evidence of a graded or localized distribution in clusters of cells. It appeared that fz was expressed in all cells within these regions.

The significance of this pattern of fz expression for the formation of mirror symmetry was unclear and can be considered in three ways. First, is the dynamic expression posterior to the furrow necessary for placing ommatidia in the correct sequence of morphogenetic movements? Second, is the expression anterior to the furrow necessary for establishing mirror-symmetric prepatternning of the disc? Third, is the low level of expression within the furrow necessary for organizing the ommatidial polarity? Therefore, we examined the effect of replacing the normal pattern of fz expression with one where the protein is expressed in the furrow and in all cells posterior to the furrow. The glass-responsive promotor GMR (Hay et al., 1994) was fused to a partial fz cDNA and fz mutant flies were transformed with the construct. The GMR promoter drives heterologous gene expression in the morphogenetic furrow and in all cells posterior to the furrow in a manner that is dependent on the Glass transcription factor (Hay et al., 1994). In situ hybridization of eye discs from transformed larvae confirmed that fz mRNA was produced in a pattern identical to the glass gene (data not shown). In particular, there was no detectable hybridization in cells anterior to the furrow. We examined the eyes of transformed adult flies and found them to be almost completely rescued (Fig. 1G). Therefore, expression of fz mRNA anterior to the furrow is not required for prepatternning of the eye. Moreover, dynamic expression posterior to the furrow does not place ommatidia in some morphogenetic sequence necessary for pattern formation.

fz expression in the furrow and in all cells posterior to the furrow is sufficient to establish mirror symmetry in the eye.

fz regulates the expression of nemo and other genes

A good candidate for mediating the effects of fz on ommatidial
rotation is nemo, a gene encoding a protein kinase (Choi and Benzer, 1994). nemo is required to complete ommatidial rotation from 45° to 90° with the same non-autonomous requirement as we observed for fz; normal gene function in one or more PR cells is sufficient to induce complete rotation. To determine if nemo expression is regulated by fz, we generated fz mutant flies that carry an enhancer trap in the nemo gene. Expression of β-galactosidase from the enhancer trap resembles the expression pattern of nemo transcripts (Choi and Benzer, 1994). The expression of β-galactosidase was greatly reduced in fz eye imaginal discs, especially in the morphogenetic furrow (Fig. 6A,D). To study further the interaction between fz and nemo, we examined the eyes of nemo, fz double mutants. Interestingly, there was a large number of ommatidia that did not rotate at all compared to either nemo or fz mutants alone, suggesting that fz acts redundantly with nemo to regulate the entire turning of an ommatidium (Fig. 6B,E). This suggests that fz and nemo function synergistically in directing rotation.

To assess further mirror-symmetric pattern formation in fz mutant eye discs, we monitored the expression pattern of an enhancer trap, WR122, that drives the E. coli lacZ gene. In a wild-type eye disc, the expression of the lacZ gene is symmetrically distributed in a domain about the equatorial midline posterior to the morphogenetic furrow in both differentiated and uncommitted cells (Fig. 6C). The expression is broad close to the furrow and is gradually restricted to the equator at the posterior of the disc. In a fz mutant disc, almost no lacZ expression was detected (Fig. 6F), demonstrating that fz is required for mirror-symmetric gene expression about the equatorial axis in eye discs.

**DISCUSSION**

A striking feature of Drosophila eye development is the coordinated regulation of morphogenesis through progression of the morphogenetic furrow and morphogenetic movements of developing ommatidia. Morphogenesis of ommatidia is...
essential for establishing a mirror-symmetric pattern in the adult eye, which involves three movements in each ommatidium. First, ommatidia rotate either clockwise or counterclockwise 90° according to their dorsal-ventral location in the eye. Second, the equatorial-most cell in an R3/R4 pair becomes the anterior-most cell as each ommatidium rotates, and it adopts the R3 cell positional fate while the other cell adopts the R4 cell positional fate. Third, the R8 cell migrates from its initially central position to a position between the R1 and R2 cells. The \textit{frizzled} gene is required for the first two processes.

The non-autonomous effects seen with \textit{fz} mutant clones suggest that a bidirectional signal originates from the equatorial region of an eye disc and travels to the dorsal and ventral poles. This signal is directly or indirectly mediated by \textit{fz}. The \textit{fz} gene encodes an integral membrane protein with seven transmembrane domains and an extensive extracellular domain (Vinson et al., 1989). Its overall topology resembles the product of the \textit{boss} gene which functions as the ligand for the sevenless receptor in the compound eye (Zipursky and Rubin, 1994). \textit{fz} may function like \textit{boss} as a tethered ligand or it may function as a receptor for a polarity signal. However, there is no localized or graded distribution of \textit{fz} mRNA along the equatorial-polar axis. Moreover, the distribution of \textit{fz} protein is uniform in the wing disc where it mediates a directional non-autonomous effect on hair cell polarity (Park et al., 1994). Certain \textit{fz} alleles have cell-autonomous effects on wing hair polarity suggesting that \textit{fz} encodes a receptor (Vinson and Adler, 1987). Thus, it seems likely that \textit{fz} indirectly mediates this bidirectional signal, perhaps functioning as a receptor needed to generate this signal.

This model relies on the existence of an equatorial boundary that would serve as the origin for signals mediated by \textit{fz}. This boundary does not reflect a lineage restriction since marked clones of cells can span the equator (Becker, 1957; Ready et al., 1976). However, such a boundary might reflect restricted gene expression as has been observed in the \textit{Drosophila} wing (Diaz-Benjumina and Cohen, 1993; Williams et al., 1993). Indeed, expression of the \textit{mirror} gene is restricted to the dorsal half of the developing eye disc and terminates precisely at the equator (H. McNeill and M. Simon, personal communication). We find that \textit{fz} clones centered about the equator affect the polarity of wild-type ommatidia to the same degree as clones in other regions of the eye. Thus, \textit{fz} is not likely required to establish an equatorial boundary. Rather, it mediates Fig. 5. \textit{In situ} hybridization of \textit{fz} mRNA in wild-type (A,B) and \textit{fz} mutant (C,D) eye discs using an anti-sense \textit{fz} RNA probe. Anterior is to the left and dorsal is up. (B,D) Higher magnifications of areas within the boxes in A and C, respectively.

Fig. 6. \textit{fz} regulates the expression of the \textit{nemo} and enhancer trap \textit{WR122} genes. Anterior is to the left, and dorsal is up. (A,D) Anti-\(\beta\)-galactosidase antibody staining of \textit{nemo}^{P1} (A) and \textit{nemo}^{P1}, \textit{fz} mutant (D) eye discs. Arrows indicate the morphogenetic furrows. (B,E) Tangential sections of \textit{nemo}^{P1} (B) and \textit{nemo}^{P1}, \textit{fz}KD4a (E) mutant eyes. Almost all of the ommatidia are arrested at a 45° angle in B. In E, there are many unrotated ommatidia, one of which is circled. (C,F) \(\beta\)-galactosidase activity staining of \textit{WR122} enhancer trap (C) and \textit{WR122}, \textit{fz} mutant (F) eye discs. Morphogenetic furrows are indicated by arrows.
a symmetric signal that originates from the equatorial boundary subsequent to its formation.

Several lines of evidence suggest that the sequential pattern formation mediated by fz is associated with progression of the morphogenetic furrow. The fz gene is dynamically expressed anterior and posterior to the furrow although expression of fz within and posterior to the furrow is sufficient for its normal function. Moreover, fz is required for expression of the nemo gene and for center-outward progression of neuronal-specific gene expression in the morphogenetic furrow. Finally, additional evidence has come from experiments ectopically expressing hedgehog in the eye (Heberlein et al., 1995). Ommatidia formed by an ectopic morphogenetic furrow moving in the opposite direction to the normal furrow exhibit an opposite chirality to normally formed ommatidia. This suggests that polarity of ommatidia is controlled by the direction in which the furrow progresses.

In fz, the ommatidia frequently fail to complete a full rotation, a phenotype also observed in nemo mutants (Choi and Benzer, 1994). In fz mutant eye discs, nemo expression is greatly reduced, suggesting that fz regulates nemo to promote complete 90° rotation of ommatidia. Consistent with this observation, both nemo and fz act non-autonomously within an ommatidial unit; any single nemo+ or fz+ photoreceptor cell in an ommatidium is sufficient to trigger 90° rotation of the entire group. However, they do not simply form a linear regulatory pathway since a nemo fz double mutant has a much more severe rotational degree phenotype than the single mutants alone. It is likely that, in addition to acting in a linear pathway, they may regulate rotation by redundantly acting on a common effector or different effectors.

Our mosaic analysis provides evidence that the signal is capable of influencing polarity over a range of several cell diameters. However, this does not necessarily mean that it normally acts over such a range. Indeed, our mosaic data indicate that the presence or absence of fz protein in the presumptive R3 and R4 cells greatly influences both their choice of positional fates and the direction of ommatidial rotation. When the border between fz+ and wild-type cells creates a mosaic pair of R3-R4 cells, it is almost always the wild-type cell that adopts an R3 cell positional fate and the mutant cell that adopts the R4 cell fate. The presence or absence of fz protein has been measured by these cells prior to their choice of positional fates, and fz is required for the mechanism that allows cells to choose between the two alternatives. This in part resembles the mechanism for cell fate choice mediated by Notch in the epidermis (Heitzler and Simpson, 1991). Moreover, the presence or absence of fz protein measured in R3 and R4 cells influences the direction of rotation that the entire ommatidium takes; ommatidia with mosaic R3 and R4 cells rotate such that the fz+ cell usually occupies an anterior position after the rotation. The importance of R3 and R4 cells in determining the direction of rotation is supported by observations that mutations in the fat facets and argos genes lead to extra R3/R4 cells in their ommatidia, and these ommatidia exhibit orientation defects (Fisher-Vize et al., 1992; Freeman et al., 1992). This suggests that the proper spatial arrangement of R3 and R4 plays an important role in establishing ommatidial polarity. These results place R3 and R4 cells as critical determinants of rotation, and fz function in either one of these cells is sufficient for directing rotation of an entire ommatidium in most cases.

The mosaic results suggest that both the presumptive R3 and R4 cells are receiving positional information. It appears that a cell with more fz activity than its neighbor has an increased propensity to become an R3 cell and a cell with less activity has an increased propensity to become an R4 cell. Our mosaic experiments cannot exclude the possibility that the fz protein in one cell stimulates it to adopt an R3 cell fate, whereby it physically moves the other cell to an R4 cell position. However, we favor a model in which fz mediates mutually instructive signals sent between neighboring cells. In normal development, presumptive R3 cells are equatorial to their presumptive R4 neighbors before rotation. This suggests that equatorial cells may normally have greater fz activity than their polar neighbors. If neighboring cells instruct each other through short-range signals that are dependent on fz, cells would be more able to signal their polar neighbors, and less able to signal their equatorial neighbors. The positional information in this graded signaling might instruct cells about their positional fates and about direction of rotation. This model is consistent with the bidirectional non-autonomy observed in fz mosaics since cells at the equatorial clone borders would...
follow the natural gradient of signal activity, and cells at polar borders would follow an inverse gradient of signal activity.

Several models can be proposed to explain formation of eye mirror-symmetry. In one model, fz mediates a series of short-range local interactions that polarize cells (Park et al., 1994). In another model, fz functions as a receptor for polarity morphogens within or near the morphogenetic furrow and graded along the equatorial-polar axis (Fig. 7). In both models, fz regulates the timing of neuronal development and the expression of secondary polarity genes. These genes may be expressed uniformly along the equatorial-polar axis as with the nemo gene or in a restricted manner as with the WR122 marker gene. There are known polarity genes, such as four-jointed, that are expressed in a symmetric gradient centered about the equator (M. Brodsky and H. Steller, personal communication). The second model builds upon a pattern polarity model devised for insect epidermal development (Lawrence et al., 1972). We favor this model since transplantation studies in the Oncopeltus eye suggest that ommatidium polarity develops by a similar mechanism to epidermal pattern formation (Lawrence and Shelton, 1975). Furthermore, it supports the notion that cells along an axial plane differentiate a stable gradient of gene expression, and some of these genes might encode secondary signals that locally impart positional information to neighboring cells through different receptors (Lawrence et al., 1972). The symmetric expression of such genes may be a response to a gradient of fz activity. Thus, clones of fz- cells would fail to express these local-acting signals and would locally disturb wild-type ommatidia on one side of the clone whereas the wild-type ommatidia would locally rescue fz- cells on the other side of the clone. Lawrence et al. (1972) observed that 180° rotations of Rhodnius epidermis resulted in asymmetric patterns that suggested a directional non-autonomous effect following the morphogen gradient. The directional non-autonomous effects in fz clones would suggest that cells make morphogenetic choices based on the relative levels of local-acting signals between neighboring cells.

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