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Independent regulation of anterior/posterior and equatorial/polar polarity in the *Drosophila* eye; evidence for the involvement of Wnt signaling in the equatorial/polar axis

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SUMMARY

The *Drosophila* retina is made from hundreds of asymmetric subunit ommatidia arranged in a crystalline-like array with each unit shaped and oriented in a precise way. One explanation for the precise cellular arrangements and orientations of the ommatidia is that they respond to two axes of polarized information present in the plane of the retinal epithelium. Earlier work showed that one of these axes lies in the anterior/posterior(A/P) direction and that the polarizing influence is closely associated with the sweep of the Hedgehog-dependent morphogenetic wave. Here we present evidence for a second and orthogonal axis of polarity, and show that it can be functionally separated from the A/P axis. Further, we show that the polarizing information acting in this equatorial/polar axis (Eq/Pl) is established in at least two steps – the activity of one signaling molecule functions to establish the graded activity of a second signal.

Key words: *Drosophila*, Retina, Ommatidia, Polarity, Axis, Signaling

INTRODUCTION

Planar polarity (Nübler-Jung, 1987) is a characteristic displayed by epithelia in which the cells are all coordinately aligned so, for example, they may project their hairs or bristles in the same direction. In such a situation, cells separated by many hundred cell diameters are able to coordinate their behaviour, raising the question of how such long-range organization is established during development. It may be that distant cells are able to communicate with each other and so coordinate their behaviour or, alternatively, cells may organize their behavior independently of each other by responding to a distant polarizing signal. The phenomenon of planar polarity therefore provides an attractive system with which to study the mechanisms of long-range developmental organization.

Planar polarity has been extensively investigated in the insect (largely hemiptera) ectoderm through surgical manipulation techniques (e.g. Piepho, 1955; Lawrence, 1966; Stumpf, 1967; Nübler-Jung, 1987) and recently by molecular and genetic manipulations in *Drosophila* (e.g. Gubb and Garcia-Bellido, 1982; Vinson and Adler, 1987; Struhl et al., 1997). The eye of *Drosophila* differs from many other epithelia in that planar polarity is not evident by the orientation of single cells but rather by the shape and orientation of the ommatidia, which are small clusters of cells.

Ommatidia are small groups of cells arrayed precisely and interlocked to form a retina of crystalline-like structure. The ommatidia contain a number of asymmetries, but the most obvious is the shape assumed by the rhabdomeres of the photoreceptors. The rhabdomeres in cross-section appear as large blob-like structures (Fig. 1A-C), and the so-called outer rhabdomeres (those corresponding to photoreceptors R1-R6) form an asymmetric trapezoidal shape when viewed in cross-section. The trapezoids occur as one of two different chiral shapes (color coded here as red and blue for simplicity, Fig. 1A-C). Those in the dorsal hemisphere are all of one type and are the mirror reflection (the other chiral form) of those in the ventral hemisphere (Dietrich, 1909), (Fig. 1C). The two forms meet at the line of pattern inversion termed the equator and in keeping with this global metaphor the dorsal and ventral extremities of the eye are called the poles. In the right eye the red form is found dorsally, but in the left eye the blue form is found dorsally, reflecting the mirror symmetries of the left and right eyes (Fig. 1D). Thus, the chiral shapes of the ommatidia do not correlate with a dorsal or ventral location; rather, they respect other positional information present within the eye.

Epithelia are largely cellular monolayers and we envisage them as two-dimensional sheets. Conceptually, the uniform orientation of cells in a two-dimensional sheet requires only a single organizing signal – the *wind* to align all the weather vanes or the *magnetic field* that uniformly orders the monopoles. But the ommatidia are asymmetric structures within the sheet and so require two pieces of shape-organizing information. A number of models can be proposed to explain how ommatidial fields containing the same chiral (color) shape can be achieved but the simplest is that there are two orthogonal planar polarities in the developing retina (Wehrli and Tomlinson, 1995). In this model, one planar polarity runs in the A/P axis of the retina and the other

runs between the equator and the pole (Eq/Pl) (Fig. 1D-2,3). The combined directional signals of the two polarities then direct each ommatidium in one eye half into the same shape (Fig. 1D-4). The mirror reflection of the Eq/Pl signal about the equator directs the ommatidia of the dorsal and ventral eyes into the opposite shapes, and the mirror reflection of the A/P signal between the right and left eyes inverts the shapes between one eye half and its corresponding hemisphere in the other eye (Fig. 1D-4).

Evidence for the two-planar polarity model (we call this cruciform planar polarity) came from experiments in which the morphogenetic wave that normally traverses the retinal epithelium from posterior to anterior in late larval development was reversed. When the wave was induced to run backwards (from anterior to posterior), then a concomitant reversal of the A/P axis of the ommatidia was observed. Here the ommatidia were still oriented correctly in the Eq/Pl axis, but were reversed in the A/P axis (Heberlein et al., 1995; Ma and Moses, 1995; Strutt et al., 1995; Wehrli and Tomlinson, 1995). Thus, since A/P information could be reversed without interfering with Eq/Pl polarity, then this argued in favor of the separate polarities. However, other results (Chanut and Heberlein, 1995; Strutt and Mlodzik, 1995) argued that the moving wave front, in addition to organizing the A/P polarity, could also provide the Eq/Pl information. This suggested that the signals polarizing the Eq/Pl axis were intimately associated with A/P-organizing mechanism, arguing against the idea that two separate polarizing mechanisms are superimposed to direct ommatidial chirality. To resolve this issue, we designed experiments to test whether Eq/Pl information can be manipulated without affecting A/P polarity.

We show here that Eq/Pl polarity can be manipulated without disturbing the A/P axis which we offer as further evidence for the two separate polarizing mechanisms. The wave front that lays down the A/P polarity is propagated by a mechanism that utilizes both the Hedgehog and TGF-β-type of secreted factors. We therefore looked at other secreted molecules and present evidence here that Eq/Pl polarity appeared to be controlled by a Wnt signaling mechanism. The Eq/Pl-organizing mechanism appears to be controlled primarily by Wg (or another Wnt) signaling that controls the expression of a secondary (unidentified) signaling molecule. We propose that the graded activity of this secondary molecule in the Eq/Pl axis of the retina provides the positional information for polarizing the ommatidia in this axis.

MATERIALS AND METHODS

Histology

Eyes were processed for sectioning and analysis following Tomlinson and Ready (1987).

Constructs and misexpression

white$^+$ flip-out cassettes

The initial *wg* experiments used a flip-out cassette containing the mini-*white* gene (Klemenz et al., 1987); this construct was a gift of Gary Struhl. Since this frequently gave only low level pigmentation in the eyes, we constructed a version expressing the *white* gene more strongly. Here the *white* cDNA (Pepling and Mount, 1990) was subcloned from the 5′ end of the coding sequence to the *Apa*I site (immediately upstream of the translational stop) and joined to the 3′ end of the mini *white* gene from the *Apa*I site to the end. The GMR enhancer element with the hsp70 minimal promoter (Hay et al., 1994) was placed immediately 5′ of the coding sequence and the whole construct was flanked by two FRT sequences.

wg and Wnts

The *wg* coding sequence carrying the tubulin trailer was subcloned into a transformation vector containing the Tubulin-α1 promoter (Basler and Struhl, 1994) and the mini-*white* flip-out cassette was inserted between the *wg* coding sequence and the tubulin promoter. Similar constructs were made for Wnt-2, Wnt-3 and Wnt-4 cDNAs (Eisenberg et al., 1992; Russell et al., 1992; Graba et al., 1995). Ectopic expression of the proteins was induced by crossing the transformed lines to flies carrying *flipase* under the transcriptional control of the hsp70 promoter and these were heat-shocked at various times.

Activated Armadillo

A truncated *arm* coding sequence (missing the codons for the N-terminal 20% of the protein (Zecca et al., 1996) was subcloned into the tubulin vector described above. Two derivative constructs were made by removing the tubulin promoter and inserting either UAS or the *sevenless* enhancer (two sev enhancers followed by the hsp70 promoter; Basler et al., 1991). Into each of the three constructs, the *white*$^+$ flip-out cassette was inserted between the promoter element and the coding sequence. The UAS construct was driven by GAL4 under the transcriptional control of the *arm* promoter (Sanson et al., 1996).

Mosaic analysis

arrow

All data shown were produced with the *arr*^{2} allele and the repolarizing effects have been reproduced with *arr*^{G6} and *arr*^{G68}. Clones were induced following Wehrli and Tomlinson (1995) in flies of the genotype *y, w,* hsp-*flipase*; FRT42D *arr, bw, sp*/FRT42D *w*$^+$(47A). *arr*^{2} was obtained from the Bloomington Stock Center and *arr*^{G6} and *arr*^{G68} *were* a gift of J. Jiang and G. Struhl (unpublished).

arm

arm^{XM19} (Wieschaus et al., 1984), *arm*^{25B} (Riggleman et al., 1989) and *arm*$^{H8.6}$ were all examined in clones in the eye. Partially rescued *arm* clones were generated in the following flies:

y, arm^{XM19}*, w*; BCD7, FRT42D, *bw*/FRT42D P{arm$^+$}#E, P{w$^+$}60C; FL122/+

BCD7 is an *arm* rescue construct inserted at 36A that only partially rescues the *arm* phenotype (Peifer et al., 1991). P{arm$^+$}#E is an *arm* genomic rescuing construct (gift of M. Peifer). FL122 is a hsp70-*flipase* on the third chromosome (gift of Gary Struhl).

shaggy

X-ray clones were induced in females of the following genotype – *w*^{1118}, *sgg*^{M11}/ +. *sgg*^{M11} is described in Perrimon and Smouse (1989). *sgg*$^-$ *wg*$^-$ clones were induced in flies of the following genotype:

sgg^{M11} *w*; Dp(1;2)sc^{19} y$^+$, Dp(1;2) w^{+70h} (*sgg*$^+$ *w*$^+$), FRT39E/*wg*^{CX4} FRT39E; FL122/+.

dishevelled

Clones were induced in females of the following genotype – *w*^{1118}, *dsh*^{V26}FRT18A/FRT18A; FL122/+. *dsh*^{V26} is described in Klingensmith et al. (1994). We further determined that the open reading frame between nucleotides 496 and 1040 is deleted. The nucleotide sequence across this junction is ggtaccaatcTacggcgg. A single thymidine is inserted in place of the deleted region. This results in a frame shift after amino acid 94 of Dsh, thus the product lacks the C-terminal 529 amino acids. C-terminal to the truncated Dsh protein, the shifted frame adds 111 amino acids of non-Dsh protein sequence.

RESULTS

Misexpression of Wg reverses Eq/Pl polarity

The A/P polarity in the retina is laid down with the sweep of the morphogenetic wave that is driven by the TGFβ and Hedgehog

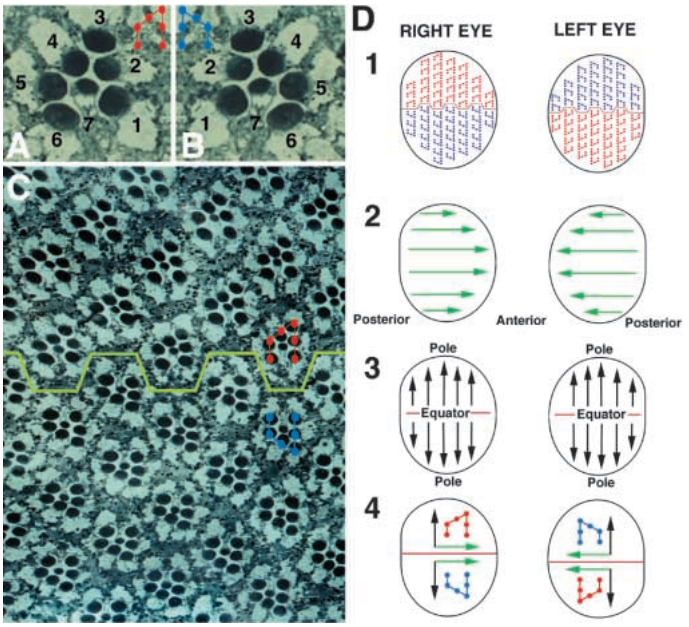


Fig. 1. Two orthogonally positioned axes of polarity are apparent in the crystalline lattice *array* of ommatidia in the *Drosophila* eye. (A,B) Ommatidia occur in two chiral shapes color coded here as red and blue. (C) In one half of the eye all ommatidia are of the same chiral shape and are the mirror reflection of those in the other half. The two forms meet at the line of pattern inversion called the equator (green line). Photograph taken from Ready et al. (1976). (D1) The red chiral shape is found dorsally in the right eye but ventrally in the left eye. The blue shape occurs in the complementary distribution. (D2) The right and left eyes are mirror reflections of each other so the A/P axis in one points in the opposite direction to the other. (D3) Each eye is polarized in the Eq/Pl axis with axis inversion occurring at the equator. (D4) The combined vectoral components of A/P and Eq/Pl define two chiral arrangements. The dorsal right is identical to the ventral left, but rotated through 180 degrees. Similarly, the ventral right is a simple rotation of the shape in the dorsal left.

families of secreted factors (Heberlein et al., 1993; Ma et al., 1993). Assuming that secreted factors would also be involved in Eq/Pl polarity, we therefore looked for a role of the another major class of secreted proteins, the Wnts, in organizing the Eq/Pl axis. Wg was ectopically expressed in clonal patches under the transcriptional control of the Actin5C promoter (Struhl and Basler, 1993) and significant changes in retinal polarity were observed (Treisman and Rubin, 1995; Tomlinson et al., 1997). The flip-out cassette used in these experiments was *yellow*$^+$, which is not a useful marker in the retina and cells ectopically expressing it could not be detected. Also, it appeared that cells ectopically expressing Wg did not differentiate and only a scar was present to mark their position in the adult retina. We were concerned that the polarity effects observed may have been related to a secondary effect of the scarring rather than as a direct result of the Wg misexpression. We changed the construct of Struhl and Basler (1993) by substituting the Tubulin-α1 promoter (for the Actin5C promoter) and replaced the *yellow*$^+$ flip-out cassette with a (mini)*white*$^+$ flip-out cassette.

Misexpression of Wg with the Tubulin-α1 promoter

We ectopically expressed Wg in the developing eye using the Tubulin-α promoter and marked the clones by the loss of the pigment-conferring *white* gene. Under these conditions, we observed two significant differences to the earlier experiment described above. First, large *white* patches of healthy ommatidia were generated with associated polarity inversions and no scarring occurred. This demonstrated that the polarity inversions resulted from the Wg misexpression rather than some secondary defect related to the scarring of the tissue. Second, although significant changes in retinal polarity were associated with the clones, the distance over which the effect was exerted was diminished from a maximum of 6 or 7 ommatidial rows in the Act5C-wg scars down to a maximum of about 1 or 2 for the Tub-wg patches. Small clones rarely showed polarity phenotypes, only those containing about 20 ommatidia or above had associated effects on chirality.

Ectopic Wg clones had two distinct features in respect to their polarity effects. First, the aberrant polarity was asymmetrically distributed with relation to the clone. Changes in polarity occurred at a polar position relative to the center of the clone extending into the wild-type tissue lying outside the clone on the polar side. The ommatidia in the equatorial region of the clone and in the bordering wild-type tissue remained unaffected. Fig. 2B shows the effect of a Wg-expressing clone and the asymmetrical distribution of the polarity reversals is evident as the 'color change' of the ommatidia. Note that the ommatidia are inverted in the Eq/Pl axis (rather than A/P) as they still maintain their A/P orientation but now point down to the equator rather than up to the pole. Second, the potency of the Wg-expressing clones to induce polarity reversals showed a change along the extent of the Eq/Pl axis and suggested that the retina has a graded sensitivity to misexpression of Wg, with maximal polarity-reversal effects at the equator and minimal effects at the pole. We scored 30 clones containing 20 ommatidia or above and we classified these into four different types depending upon their position in the eye. We observed 3 clones that spanned the midline of the eye and these caused a major reorganization in the equatorial region. They induced polarity reversions leading to two equators separated by a medial pseudo-equator (Fig. 2C; see Fig. 8C for an explanatory diagram). 18 clones lay between the equator and the pole (Fig. 2B, see Fig. 8B for an explanatory diagram) and, of this class, those closer to the equator tended to induce greater polarity inversions than those closer to the pole. 6 clones were restricted to the polar region and showed no polarity inversions (Fig. 2C). The final class of clones were large clones that extended from the pole to more than half-way to the equator. These clones showed the incorrect chiral form of ommatidia at their midpoint, but had the correct form above and below (data not shown).

These results demonstrate that Wg has an activity potent to change polarity in the Eq/Pl axis of the eye. Whether Wg normally functions to organize the Eq/Pl axis or whether it mimics another activity is discussed later.

The roles of the elements of the Wg transduction mechanism in Eq/Pl polarity

Since misexpression of Wg produced significant polarity effects, we then examined the roles of proteins associated with the Wg transduction pathway. A number of proteins have been implicated in Wg signaling. These included Dishevelled (Dsh), Shaggy (Sgg, otherwise known as Zw3) and Armadillo (Arm). More recently the product of the *arrow* (*arr*) gene has been

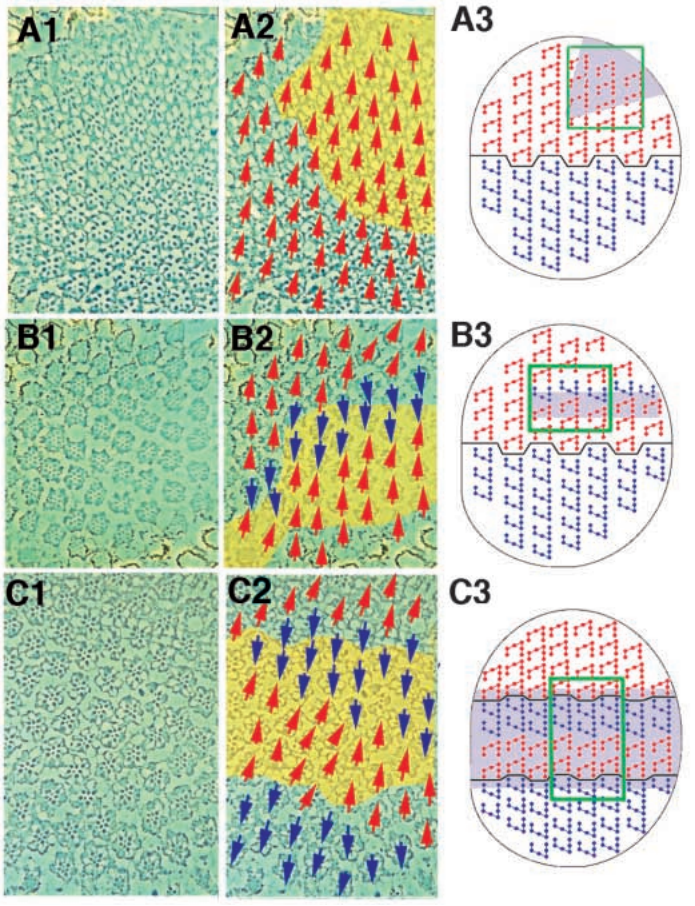


Fig. 2. Polarity reversals induced by ectopic expression of Wg. Wg was ectopically expressed under the control of the tubulin promoter and marked by the excision of a *white*$^{+}$ flip-out cassette. The strength of the *white*$^{+}$ in this experiment was low and it is difficult to see the pigment in the sections shown. Clones of cells misexpressing Wg show two effects. They induce polarity reversal on their polar side and they are maximally potent to do this in the equatorial position. In the left column (1), a section is shown. In the middle column (2), the polarities of the ommatidia are depicted and the extent of the clone is indicated by the yellow area. The right column (3) shows a schematic diagram of the eye from which the section was taken, indicating the position of the clone and the green box indicates the tissue shown in the section. (A) A section through a polar clone shows no polarity inversions. (B) A clone positioned roughly midway between the equator and the pole shows that polarity inversions occur in the polar regions of the clone and in wild-type tissue lying polar to the clone. This clone is explained diagramatically in Fig. 8B. Note that in the centre of the clone the red and blue ommatidia meet, but presenting their 'pointed' (R3/4) sides to each other rather than the normal 'flat' (R1/6/7) sides as found at a normal equator (see Fig. 1). Following Campos-Ortega and Gateff (1976), we refer to these as pseudo-equators. (C) Clones in the equatorial region produce the strongest effects and also affect both halves of the eye. Two equators now occur separated in the midline position by a pseudo-equator. A further explanation of this is shown in Fig. 8C.

placed in the *wg* signaling pathway on a number of criteria. First, mutant embryos display a segment polarity phenotype (Nüsslein-Volhard et al., 1984). Second, not only does the segment polarity phenotype appear like *wg*, but in all other tissues examined (gut, leg, wing) *arr* phenotypes phenocopy

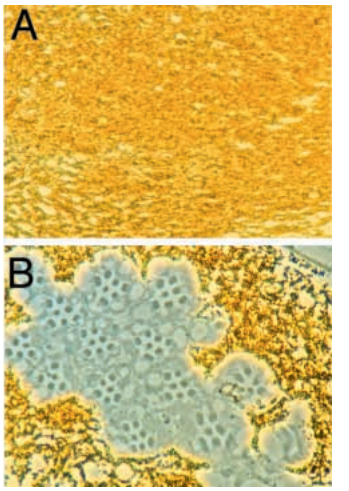


Fig. 3. *arr* lies downstream to Wg in an eye epistasis experiment. (A) When Wg is expressed under GMR transcriptional control a very small eye appearing to consist almost entirely of pigment cells forms. (B) A clone of *arr*^{2} marked by *white* induced in this background rescues the retinal tissue and healthy ommatidia form.

eg mutants (S. Dougan, L. O'Keefe, K. Caldwell and S. DiNardo, pers comm.). Third, an epistasis experiment performed in the eye places *arr* downstream of Wg. To demonstrate this, we misexpressed Wg in the developing eye using the GMR enhancer element (Hey et al., 1994). The GMR enhancer drives at high levels in cells posterior to the morphogenetic furrow and this induced a very small eye consisting almost entirely of pigment cells. We then induced *arr* mutant clones in these eyes and observed an autonomous rescue of the ommatidia (Fig. 3).

To examine whether the Wg pathway normally regulates the Eq/Pl polarity in the eye, we manipulated all four of these Wg transduction elements (Dsh, Sgg, Arm and *arr*).

Analysis of *arrow*

In the eye, *arr* mutant clones had two distinct effects that occurred at roughly equal frequency. First, *arr* clones induced ectopic differentiation of the retina ahead of the morphogenetic furrow, in a similar manner to *ptc* clones or ectopic expression of Hedgehog (see Wehrli and Tomlinson, 1995; Heberlein et al., 1995; Strutt et al., 1995). Ectopically differentiating patches of retinal tissue cause a radial wave of morphogenesis to propagate in the epithelium with concomitant A/P repolarization. Thus any Eq/Pl repolarization that occurred with these clones was obscured by the effects of the wave. The reason why *arr* clones were able to induce ectopic differentiation is discussed later and, since analysis of these clones does not aid in the investigation of the Eq/Pl axis, we will not describe them further. Second, *arr* mutant clones that did not induce ectopic differentiation showed clear repolarizing effects in the Eq/Pl axis and this is the class that we describe below.

arrow clones cause non-autonomous polarity inversion in the Eq/Pl axis

In the eye, *arr* clones had three significant features with regard to their influence on the Eq/Pl axis and these occurred in both dorsal and ventral halves of the eye. First, they had clear non-autonomous effects that caused the inversion of Eq/Pl polarity in neighboring and distant wild-type ommatidia. Second, these polarity inversions were only found on the equatorial side of the patch and the polar side remained unaffected. The polarity inversion on the equatorial side of the clone was the opposite to that seen with ectopic Wg which induced the inversions on

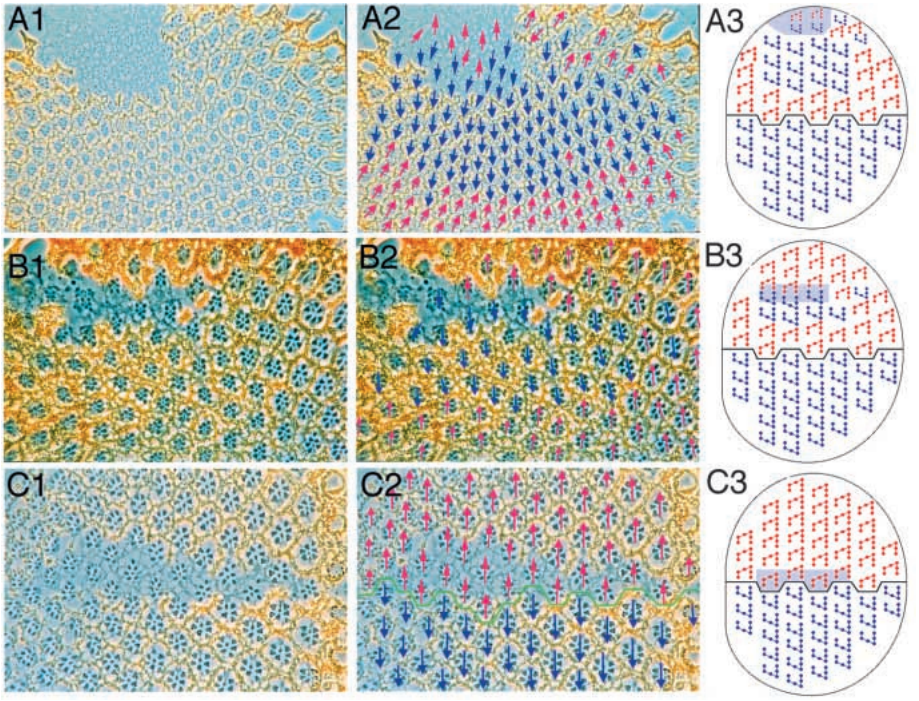


Fig. 4. *arr* clones cause position-dependent repolarization in the Eq/Pl axis. *arrow* clones show two distinct features. They induce polarity reversals on the equatorial side and they have maximal effects at the poles and minimal effects at the equator. (A) A clone in the polar region has a long-range repolarization effect. The section shown in A1 is labeled in detail in A2 and is schematized in A3. The *arr* clone is evident by the absence of pigment. In the dorsal right eye, all ommatidia should be of the red type but the effect of the clone is to induce an Eq/Pl reversal over several ommatidial rows. Note that within the clone the ommatidia organize with the blue form pointing down and the red type pointing up, thereby creating an ectopic equator. (B) A clone positioned in an intermediate Eq/Pl position still repolarizes in the Eq direction but over a shorter distance. Note that ommatidia polar to the clone are of the correct chiral shape and as described for A, an ectopic equator forms within the clone. (C) Clones at the equator do not display repolarizing activity.

the polar side. Third, the extent of polarity inversions induced by *arr* clones was position-dependent. At the poles, the clones exerted their maximal influence into the wild-type tissue, inverting the polarity over many ommatidial rows. This effect diminished progressively with the distance of the clone from the pole, disappearing at the equator (Fig. 4A-C).

Within the clone, the chiralities of the ommatidia were not randomly arranged. In the equatorial region of a clone, the ommatidia are inverted into the inappropriate chiral form found in the neighbouring wild-type tissue. But, in the polar regions of the clone, the ommatidia are of the correct chiral form (the form found polar to the clone) and thus within these patches of *arr* tissue, ectopic equators are present (Fig. 4A-C).

arr clones activate an equatorial marker

The enhancer trap Eq-1 (Sun et al., 1995) displays differential *white*$^{+}$ activity in the Eq/Pl axis – pigment is expressed at high levels at the equator and this grades off rapidly towards the poles (Fig. 5A). Thus the presence of the pigment marks tissue at the equator. In eyes carrying (unmarked) *arr* mutant clones, we saw ectopic expression of the equatorial marker (Fig. 5B). To demonstrate that the ectopic expression of the marker correlates with *arr* mutant clones, we sectioned these eyes and observed the chirality changes characteristic of an *arr* clone (data not shown). As a complementary experiment, we induced clones of *arr* marked with *white* in flies carrying the Eq-1 enhancer. When we sectioned such clones that were distant from the equator, we saw the ectopic expression of the equatorial enhancer evident as a low level of pigment within the cells of the clone (Fig. 5D). Hence this equatorial marker becomes ectopically expressed in *arr* clones.

The non-autonomy of the *arr* clones is significant since it argues that *arr* functions to regulate the activity of a downstream signaling molecule that we term factor-X. From this we propose two distinct elements in the Eq/Pl-organizing mechanism. First, there is the step that organizes the polarity signals and, second, there is the process by which the ommatidia 'read' those signals. If the signals cannot be read by the ommatidia then we predict that chiral shapes within a clone will be randomized. Conversely if a gene product is used in the polarity-organizing step then mutant clones will show non-autonomous effects outside the clone. If a gene product is used in both steps, then ommatidia within a clone would be randomized and non-autonomous polarity inversions would be evident in wild-type tissue outside the clone. From this perspective, the non-autonomy of *arr* clones placed this gene in the polarity-organizing mechanism, but the orderly array of the ommatidia within the clones suggests it was not involved in the read-out step. Since *arr* appears to transduce the Wg signal then our results suggested that Wg signaling through *arr* may function to regulate the activity of the subservient signaling molecule, factor-X (see Fig. 8).

We then asked whether experimental manipulation of other Wg transduction molecules would confirm this view.

Analysis of Armadillo

In the conventional view of Wg signaling, the loss of Arm prevents the transduction of the signal, whereas activation of Arm can mimic the receipt of the Wg signal. If Arm, like *arr* mediates the Wg regulation of factor-X activity, then we could make two predictions. First, loss of Arm should mimic *arr* and result in a repolarization on the equatorial side of a mutant patch. Second, since activation of Arm occurs in response to Wg signaling then, if we expressed an activated form of Arm, it should behave like misexpression of Wg and cause polarity inversions on the polar side of the mutant patch.

Reduction of *arm* function changes polarity on the equatorial side

We induced *arm* clones marked with *white* in the eye using a strong allele (arm^{XM19}) and also two weaker alleles (arm^{25B} and $arm^{H8.6}$). Only extremely small and infrequent clones were

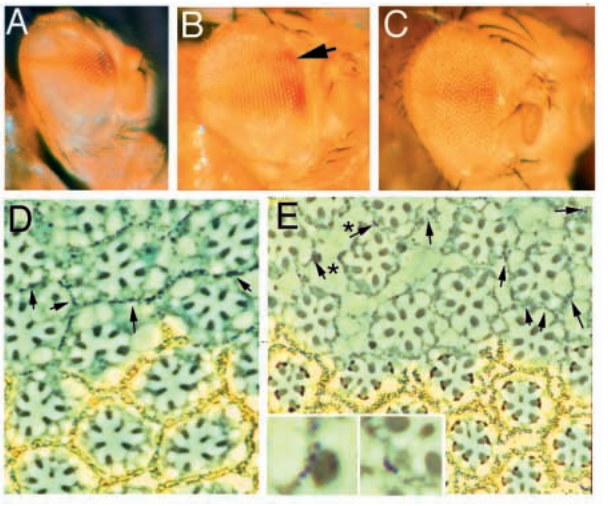


Fig. 5. Expression of an equatorial marker occurs in eyes in which *arrow* clones have been induced. (A) The enhancer trap line Eq-1 shows pigment expression in the equatorial region of a wild-type eye. (B) In eyes in which *arr* clones have been induced the marker occurs ectopically. In the equatorial region of the eye, the normal expression can be seen, but above (dorsal) ectopic expression (arrow) is present. (C) The Eq-1 enhancer remains unchanged in a *fz* null fly. (D) *arr* clones show the ectopic expression of the Eq-1 marker when distant from the equator. The lower part of the section shows the wild-type tissue in which the pigment in the secondary and tertiary pigment cells is present as the yellow lattice. In the upper part of the section, the clone is indicated by the absence of the yellow lattice but pigment is still evident in these cells at a low level (arrows). This is the pigment arising from the ectopic activation of the Eq-1 marker. (E) In a *dsh* clone distant from the equator, the ectopic expression of the Eq-1 marker is significantly reduced compared to *arr* clones but still evident (arrows). Inserts show a magnified view of the region of the two arrowheads marked with asterisks.

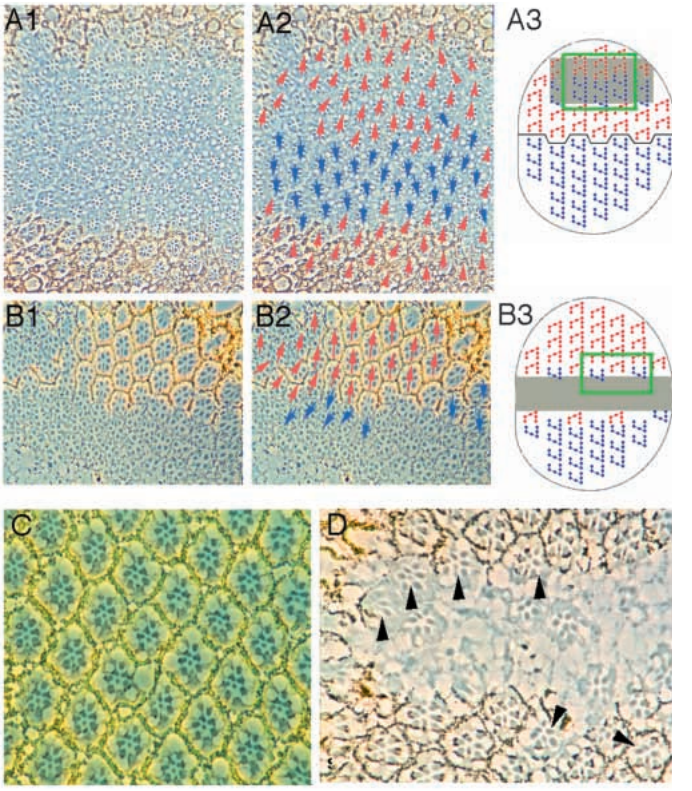


Fig. 6. Polarity phenotypes induced by manipulation of Armadillo. Arm shows effects in the generation of the polarizing signals but not in their read-out. (A,B) Sections shown to the left (1) have the ommatidial chiralities and orientations shown in the middle (2) and a summary diagram indicating the position of the clone and the area shown in the section (green box) is to the right. (A) Partially rescued *armadillo* clones induce polarity inversions in their equatorial side. (B) Activated *arm* induces polarity inversions on the polar side. The unpigmented area in the lower half of the section is where UAS-activated Arm has been transcriptionally activated by arm-Gal4. Ommatidia in the centre of the clones are severely disrupted but at the interface with the wild-type tissue normal ommatidia form and display the polarity inversions. The section shown is from a large clone that extends across the midline and a similar effect occurs on the other half of the eye. With the exception of the degeneration of the tissue that occurs in the clone, this midline clone appears very similar to the midline misexpression of Wg (Fig. 2C). (C,D) High levels of expression of wild-type or activated Arm in the early ommatidia using the *sevenless* enhancer do not disturb chirality. (C) A section through an eye carrying 4 copies of Sev-Arm shows no polarity defects. (D) As with the UAS-activated Arm the Sev-activated Arm also causes ommatidial degeneration but at the interface with the wild-type tissue normal ommatidia form and do not show any chirality changes.

ever observed. They were more frequent with the weaker alleles and tended to be in the equatorial rather than the polar regions of the eye and, when sectioned, *white* photoreceptors were not observed. Sometimes however, short-range Eq/Pl polarity inversions were associated with these clones.

To generate viable clones, we made use of BCD7, a genomic *arm* rescue construct (Peifer et al., 1991) that expresses only weakly due to a position effect. We induced arm^{XM19} clones in the presence of the BCD7 insertion and observed large clones with associated Eq/Pl polarity inversions. We regarded these as clones of a pseudo-hypomorphic *arm* allele. Some clones showed evidence of the ectopic differentiation of the retinal tissue that we had observed also with *arr* clones and the significance of this will be addressed in the Discussion. More importantly, in terms of the theme of this paper, the *arm* clones frequently showed the polarity inversions on their equatorial side. Fig. 6A shows such a clone and the polarity reversal is evident as the ectopic array of blue ommatidia in the lower half (Eq) of the clone. The polarity inversions were usually contained within the mutant patch, but when the clones were induced in dorsal regions clear non-autonomy could be detected and wild-type ommatidia up to two or four ommatidial rows distant from the clone showed chirality changes. We were unable to assay the expression of the equatorial marker Eq-1 in these clones since the BCD7 partial rescue construct is marked with $white^{+}$ and this would obscure any ectopic expression of the marker. From these results, we inferred that Arm, like *arr*, is involved in the transduction of the signal that leads to the regulation of the secondary signal (factor-X).

To test further the proposal that Arm function regulates factor-X activity, we now expressed an activated form of Arm, which we predicted would mimic Wg and lead to polarity inversion on the polar side of the clone.

Clones of activated Arm change polarity on the polar side

To examine the effects of activating Arm rather than reducing its function, we misexpressed a N-terminal truncated version of the protein known to elicit activation of the Wg signaling pathway (Zecca et al., 1996). We engineered a UAS construct that allowed the expression of this 'activated' form of Arm using the GAL4 system (Brand and Perrimon, 1993) when an intervening white^{+} flip-out cassette was removed. We then used flipase to induce *white* clones that expressed the activated Arm under the transcriptional control of a ubiquitous GAL4 line (AG11 – Sanson et al., 1996) In the center of these clones no photoreceptors formed but towards their periphery mutant ommatidia containing reduced numbers of photoreceptors were evident. To score the chirality of an ommatidium requires all eight photoreceptors to be present and so the polarity of the tissue in these regions could not be assessed. But, at the interface with the wild-type tissue, normal ommatidia formed and here we observed that polarity inversions only occurred at the polar interface with the wild type tissue and not in the corresponding equatorial position. Also these polarity inversion effects only occurred in clones induced in the equatorial regions and not in the more polar positions. Thus activated Arm clones behaved like ectopic expression of Wg but in a weaker manner – they induced polarity defects on the polar side and clones were more potent to induce polarity inversions in the equatorial rather than polar regions. Also the failure to form photoreceptors within the center of the clones appears similar to the effect seen with misexpression of Wg with the Actin5C promoter, which causes the scarring of the tissue.

These results suggested that both *arr* and Arm were involved in regulating the activity of the secondary signal – factor-X. If Sgg also was involved in this process, and since cells lacking *sgg* normally behave as though they had received the Wg signal, then we predicted that *sgg* clones in the eye would appear like both ectopic Wg expression and activated Arm in that they would cause polarity inversions on the polar side of the clone.

Analysis of Shaggy

shaggy clones induce polarity reversals on the polar side of the clone

When *sgg*^{M11} clones were induced in the eye, scars occurred similar to those seen when Wg was misexpressed with the actin5C promoter and no *white* photoreceptors were present within the scarred tissue. However, outside the scarred area, ommatidial chirality was inverted on the expected polar side of the clone (Fig. 7C). Since the *sgg* clones phenocopied the effects of ectopic Wg, we were concerned that *sgg* clones might cause polarity effects by ectopically transcribing *wg*. We therefore induced *sgg* and *wg* double mutant clones and observed that the phenotype remained unchanged (data not shown). Thus ectopic Wg-expressing clones, patches of activated Arm and clones of loss of *sgg* function all behave in a similar manner and induce repolarization on their polar side. This result is in marked contrast to the *arr* and *arm* reduced-function clones which repolarized on their equatorial side.

Separating the polarizing mechanism from the polarity read-out

The results presented so far have related to the mechanism that

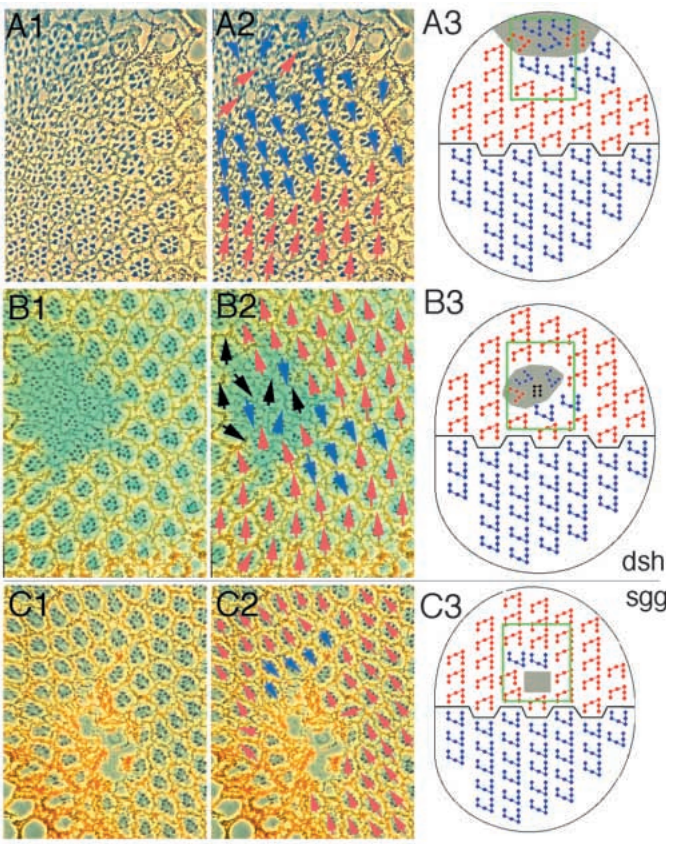


Fig. 7. *dsh* and *sgg* clones induce polarity inversions. (A,B), *dsh*^{V26} clones, (c) *sgg*^{M11} clone. Sections shown to the left (1) have the chiralities of the ommatidia shown in the middle (2) and a summary diagram indicating the position of the clone in the eye and the part shown in the section (3). (A) *dsh* clones at the poles show polarity errors within the patch (resulting from Dsh's role in the polarity read-out mechanism) and polarity inversions in the wild-type tissue lying equatorial to the clone. (B) Clones away from the equator rarely show any polarity effects outside the clone. The section shown here is one of the infrequent example when non-autonomous effects are evident. Note that a third form of ommatidia are present with this *dsh* clone. This is the symmetrical form (color-coded black) and is characteristic of read-out errors (see Tomlinson et al., 1997). (C) *sgg* clones cause degeneration of the retinal tissue leaving only a scar of pigment cells. But the non-autonomous effects of the clone are evident in the polarity reversal on its polar side.

organizes the polarizing influence within the Eq/Pl axis of the retina. Mutations such as *frizzled* (*fz*) and *dishevelled* (*dsh*) appear to randomize the chirality of the ommatidia. That is, the developing ommatidia appear unable to determine their appropriate chirality (red or blue) and apparently randomly adopt one of the two chiral forms (red or blue) or a third, symmetrical (black) form (Theisen et al., 1994; Zheng et al., 1995; Krasnow et al., 1995; Tomlinson et al., 1997). Thus these mutations appear to prevent the ommatidia from reading the polarizing signals correctly.

We therefore asked whether we could determine which of the genes we were examining were involved in both the polarity read-out step and the preceding polarizing mechanism. Since Dsh was clearly involved in the read-out mechanism and was also usually required for Wg signaling (Siegfried et al.,

1994), we asked whether Dsh was also involved in the earlier Wg-related step that was sensitive to *arr*, Arm and Sgg.

Analysis of Dishevelled

dishevelled null clones induce limited polarity reversals on the equatorial side

We examined a large number of dsh^{V26} null clones in the eye looking for non-autonomous effects. Since *arr* and *arm* clones had shown their greatest potency in the polar regions, we examined *dsh* clones in these positions and observed clear non-autonomous effects (Fig. 7A) with polarity inversion evident in the tissue lying equatorial to the clone. Clones elsewhere in the eye rarely showed non-autonomous effects, but when they did then the polarity inversion was on the equatorial side (Fig. 7B). Thus *dsh* clones induce polarity reversals on the equatorial side, which is consistent with a role for Dsh in regulating the activity of the inferred factor-X, but the weakness of the effect suggests that Dsh is functional only partially here.

One explanation for the weakness of the Dsh function detected here was that residual gene function persisted in the allele (dsh^{V26}) that we used. This allele had previously been reported to contain a gene-internal deletion (Klingensmith et al., 1994). To further characterize the molecular lesion in this allele, we sequenced the breakpoints and established that 3′ to the deletion the coding sequence is out of frame (see Materials and Methods).

Since Dsh functions in the read-out mechanism the chirality within the clones are random and the formation of ectopic equators as in *arr* or *arm* clones therefore could not be assessed. However, we were able to use the equatorial enhancer trap (Eq-1) as a marker to test for such equatorial quality in *dsh* clones.

dsh clones weakly express the Eq-1 marker

When dsh^{V26} clones were induced in a fly carrying the Eq-1 marker, ectopic expression of the equatorial marker was evident, but significantly reduced compared with *arr* (Fig. 5D). Thus both with the extent of the polarity inversions and the ectopic expression of the equatorial marker, *dsh* gene function appears to play a limited role in this initial polarizing mechanism.

Which proteins are used in the read-out step?

We expected the phenotype of mutations in the read-out mechanism to be similar to *fz* and *dsh*, that is the clones would contain ommatidia of random chirality. However, *sgg* clones do not differentiate as retinal tissue and therefore cannot be scored for chirality. With Arm, by contrast, we were able to make clones that contained ommatidia able to ‘read’ the polarizing signals as evident from their orderly arrangement into two domains of red and blue ommatidia (Fig. 6A). However, since these were effectively hypomorphic clones, then the residual gene function may have complemented the role of *arm* in the read-out step. Thus it was unclear to us whether Arm and/or Sgg operated in the read-out step.

Another way to probe the read-out mechanism is to use the *sevenless* enhancer element (Basler et al., 1991) by misexpressing proteins and assaying for chirality effects. The *sevenless* enhancer is active early in ommatidial development when the polarity read-out step occurs and drives transcription at a high level. When wild-type copies of the *frizzled* (*fz*), *dishevelled* (*dsh*) or *shaggy* (*sgg*) genes are expressed in this way then the ommatidia adopt random chiralities resulting probably from a compromised read-out mechanism (Tomlinson et al., 1997). Thus from this previous analysis, data suggested that Sgg was involved with Fz and Dsh in the polarizing read-out mechanism. We now asked whether mis-expression of Arm could similarly affect ommatidial chiralities.

Misexpression of Arm under *sevenless* enhancer control does not disturb ommatidial chirality

When Arm was expressed under *sevenless* transcriptional control, we saw no evidence for chiral changes, even when four copies of the transgene were present in the fly (Fig. 6C). We then tested activated Arm under *sevenless* control and again saw no evidence for chirality problems. The analysis with the activated Arm was complicated by the fact that the construct induced severely disrupted ommatidia, frequently devoid of photoreceptors and the retinal tissue appears to be made largely of pigment cells. However, when expressed in clones, at the interface with the wild type tissue genetically mutant photoreceptors form. The arrowheads in Fig. 6D point to phenotypically wild type ommatidia that are either made completely of mutant cells or are mosaics of mutant and wild-type cells. These ommatidia were always of the appropriate chiral form, and we infer that Arm does not influence the chiral choice mechanism in a similar manner to Fz, Dsh and Sgg.

Our results suggested that Arm functioned in the polarizing mechanism but was not involved in the read-out step. We were unable to express *arr* with the *sevenless* assay and test for a role in the read-out step since the gene is not available. We then asked whether we were able to assay any role for Fz in the polarizing mechanism. *fz* clones show non-autonomous effects but these are highly short-range and limited to the polar side of the clones (Zheng et al., 1996). The significance of this non-autonomy is unclear but, since it occurs on the polar side of clones, we inferred that it is not involved in the mechanism that regulates factor-X activity. Since the Eq-1 enhancer becomes ectopically expressed in *arr* clones, we therefore place this marker downstream of the primary organizing mechanism. To test whether Fz is involved in the primary organizing mechanism, we examined whether the Eq-1 enhancer was affected in *fz* mutant eyes.

Placing Frizzled downstream of the polarizing signals

When the Eq-1 enhancer is crossed into a *fz* null fly, its expression pattern remained unchanged (Fig. 5C). If Fz is involved in the polarizing step then we would have expected to see changes in the expression of this equatorial marker as we observe with *arr* and *arm*. Since we do not, we place the Eq-1 expression upstream of *fz* and downstream of the primary polarizing mechanism.

Does Wg normally function as the primary organizer of the Eq/Pl axis?

The experiments that we have described demonstrate the ability of Wg to polarize the Eq/Pl axis of the retina. Additionally we have demonstrated that elements of the Wg transduction mechanism appear to mediate this polarizing activity by regulating the activity of a secondary signal – factor-X.

We now asked whether Wg functions to organize the Eq/Pl axis during normal eye development or whether its ectopic effects mimic the activity of the bona fide organizing molecule.

wg^+ is expressed in the tissues bordering the presumptive polar regions of the eyes disc ahead of the morphogenetic furrow, but not in the main body of the developing retina (Ma and Moses, 1995; Treisman and Rubin, 1995). So its expression domain makes it a likely candidate for an Eq/Pl-organizing molecule. We first removed *wg* gene function from the eye in clonal patches and, consistent with the absence of its transcription from this tissue, we observed only infrequent and minor patterning defects (data not shown). To look for a role of the polar expression of Wg, we removed *wg* gene function during larval life by shifting flies carrying the wg^{ts} allele to the restrictive temperature. This experiment was not informative in defining a role for Wg in the Eq/Pl axis for two reasons. First, loss of *wg* gene function in this manner can cause major reorganizations of the head capsule tissue, leading to severe distortion of the retina and therefore preventing analysis of the Eq/Pl polarity. Second, loss of *wg* gene function induces ectopic morphogenetic waves to move in the Eq/Pl axis (Ma and Moses, 1995: Treisman and Rubin, 1995), obscuring any concomitant reversal in the Eq/Pl axis.

We then asked whether any of the three other identified fly Wnt proteins (*Drosophila* Wnt-2, Wnt-3, Wnt-4; Eisenberg et al., 1992; Russell et al., 1992; Graba et al., 1995) could also cause the polarity reversal seen with Wg. We induced clones in the eye of each of the three Wnts expressed under the control of the tubulin promoter. None of the three were able to phenocopy the effects of Wg expressed under these conditions. This suggests that Wg or another untested protein is responsible for organizing the Eq/Pl axis.

DISCUSSION

The results that we present in this paper make five major points.

(1) The graded activity of Wg (or a closely related molecule) over the Eq/Pl axis functions as the primary organizing mechanism of polarity.

(2) The graded activity of the primary organizing influence functions to set up the graded activity of a secondary molecule we refer to as factor-X.

(3) This graded activity of factor-X likely represents the polarity information that is 'read' by the ommatidia.

(4) The primary polarizing mechanism is mediated by Arm Sgg and Dsh and so appears as a Wg transduction, although Dsh appears to be only weakly used. The read-out of factor-X utilizes Fz, Dsh and Sgg, but apparently not Arm.

(5) The polarizing mechanism for the Eq/Pl axis is separable from the establishment of A/P polarity and thus we propose that the *Drosophila* retina is an epithelium polarized in two orthogonal axes.

Is Wg the primary organizer of the Eq/Pl axis?

The misexpression of Wg in the developing eye has two important features. First, the associated polarity reversals occur exclusively on the polar side (rather than the equatorial side) and the retina is more sensitive to ectopic Wg at the equator than at the poles. From this we infer that the normal polarizing influence has a graded activity that is high at the poles and low at the equator and that the direction of the gradient supplies polarized information in the Eq/Pl axis (Fig. 8A-C).

The most likely molecule responsible for this graded activity is Wg itself. It is transcribed immediately adjacent to the developing retina in the polar regions (Ma and Moses, 1995; Treisman and Rubin, 1995) and, from this position, we envisage that Wg can diffuse from its site of secretion and achieve a graded distribution in the Eq/Pl axis of the retina (a similar proposal was previously made by Treisman and Rubin, 1995). Wg is known to influence cells many diameters away from its source of secretion (Zecca et al., 1996) and assuming that this signaling occurs early when the retina is small then it is possible for Wg to achieve a graded influence over the entire retina. However, we have been unable to demonstrate a requirement for *wg* in the Eq/Pl axis and the phenomena that we observe when we misexpress Wg may result from it mimicking the activity of another Wnt molecule. To investigate this we misexpressed the other identified fly Wnts in a manner similar to Wg and observed no polarity inversions.

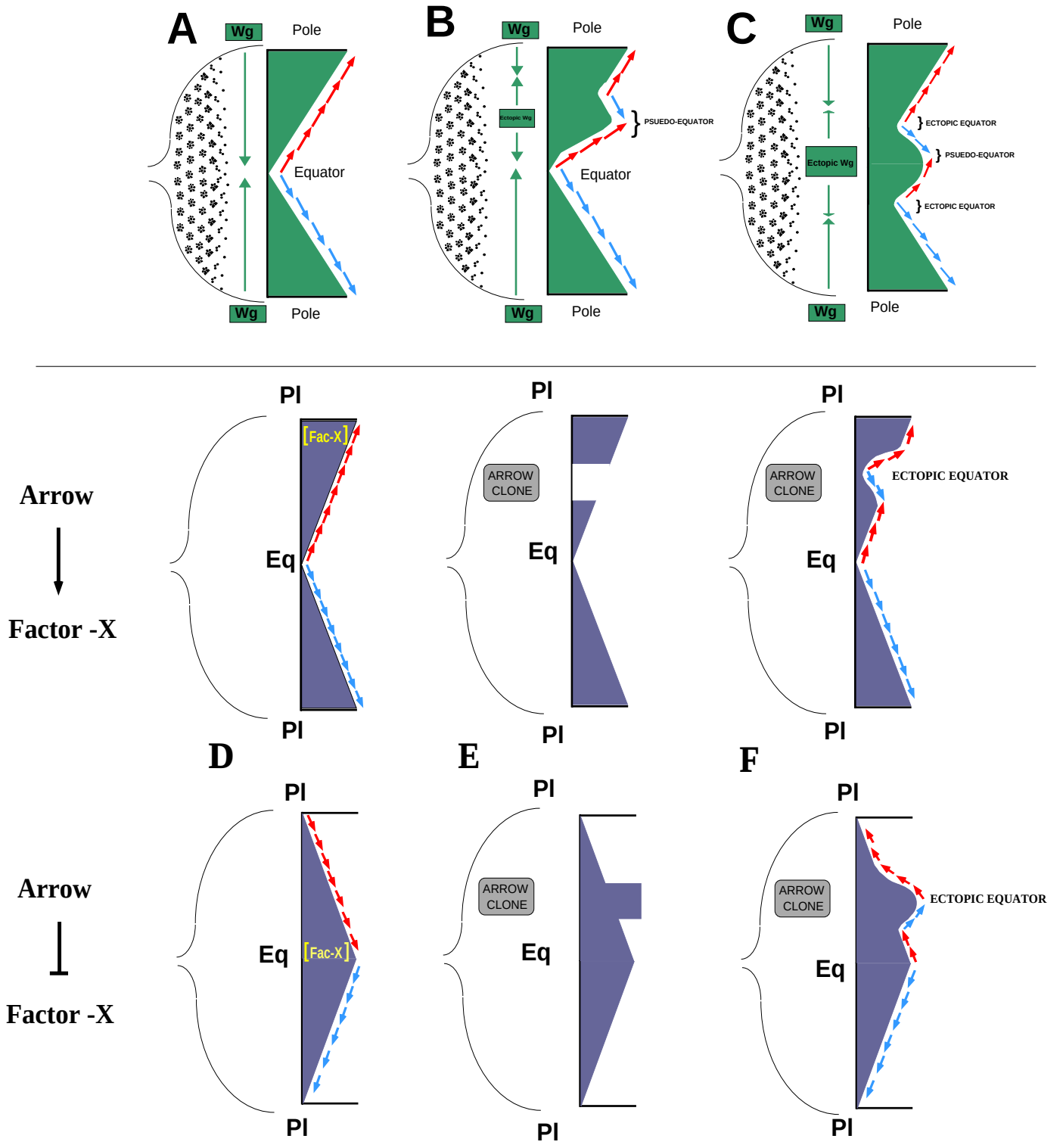
Evidence from recent publications support a role for Wg in organizing the Eq/Pl axis. Reduction or ectopic expression of Wg can cause a reorganization of the eye disc as visualized with markers that are normally restricted in the Eq/Pl axis. Reifegerste et al. (1997) show that two equatorial markers (Eq-1 and PD) become expressed in polar regions of the eye disc when Wg signaling is reduced. Eq-1, the equatorial marker that we describe here, is ectopically activated in *arr* clones (Fig. 5) and this provides indirect evidence that Wg organizes the Eq/Pl axis. Heberlein et al. (1998) using a number of markers different from those described above show clear evidence of a role for Wg in directing differential gene expression in the Eq/Pl axis. Thus, although our analyses using ommatidial chirality as a meter of polarity have failed to confirm a role for Wg in organizing the Eq/Pl axis, the effects of ectopic Wg expression that we present here, combined with the results of Reifegerste et al. (1997) and Heberlein et al. (1998), suggest that Wg is the most likely candidate for the primary organizing molecule.

The Wg-related signaling appears to controls a second secreted molecule with ability to polarize the Eq/Pl axis

Clones of mutations in *arr*, *arm* and *dsh* to a variable extent induce polarity inversions on their equatorial side. Why they show different potencies will be discussed below, but the critical observation is that mutations in these recognized transducers of the Wg signal induce non-autonomous effects, consistent with them regulating the activity of a secondary signaling factor. We term this secondary signal as factor-X. Not only do *arr*, *arm* and *dsh* clones specifically affect the equatorial side, they are also more potent in achieving this at the pole rather than the equator. Thus we infer that factor-X activity is graded in the Eq/Pl axis but we do not have sufficient information to determine whether it is high at the equator and low at the poles, or vice-versa. Fig. 8D-F shows our interpretations of the effects of *arr* clones under these two different scenarios. If factor-X is positively regulated by *arr* then the activity distribution will be high at the pole and low at the equator. Conversely if *arr* negatively regulates, then factor-X will be high at the equator.

The variable effects on factor-X activity of the transducers of the Wg-like signal

arr mutant clones display the strongest non-autonomous effects on Eq/Pl polarity whereas *arm* and *dsh* clones are considerably



G

POLARIZING STEP

READ-OUT STEP

Wg(?) — [Dsh, arr, Sgg, Arm] → FAC-X — [Fz, Dsh, Sgg] → Chirality

Fig. 8. Models for the roles of Wg and Factor-X in organizing Eq/Pl polarity. The upper level (A-C) depicts the model for Wg function and the middle level (D-F) shows how the Wg-related signal may function to achieve a graded distribution of factor-X activity in the Eq/Pl axis. The lower level (G) depicts the two step cascade that organizes polarity. (A) Wg is expressed in positions flanking the poles and not in the eye itself (Ma and Moses, 1995; Treisman and Rubin, 1995). Polarizing effects from Wg would influence the retina ahead of the advancing wave front and establish a high/pole to low/equator distribution. (B) When Wg is ectopically expressed in the eye, a supernumerary high point of Wg signal is induced the local Wg distribution is disturbed causing polarity reversals on the polar side. Note that this leads to the formation of a pseudo-equator with the clone. (C) When Wg is ectopically expressed at the midline, the entire equatorial region is reorganized and polarity inversions occur in both halves of the eye. The midline is now represented by a pseudo-equator, flanked either side by the polarity reversals that induce ectopic equators at the junction with the wild-type tissue. (D-F) The upper row shows the distribution of factor-X activity if it is positively regulated by *arr*, and the lower row shows the situation for negative regulation. In either case, loss of *arr* function causes the clone in a cell autonomous way, to adopt the equatorial condition for factor-X. Diffusion then distributes factor-X activity in a graded manner and in both scenarios the polarity reversal will now be seen on the equatorial side of the clone and ectopic equators form at their centers.

less potent. We propose that these effects result from both differential usage of these molecules and from the strength of alleles used. We were unable to generate scorable null *arm* clones in the eye. This most likely resulted from cell-lethality problems associated with the structural role Arm plays in the cell. We therefore used a partial rescuing construct to supply low levels of wild-type Arm protein (Peifer et al., 1991). In this balanced situation, we presume that enough Arm protein is available to rescue the essential structural function but insufficient to fully regulate the activity of factor-X. Hence the clones of *arm* that we analyzed in the eye were far from null and accordingly we propose that this accounts for the weakness of their affect. Conversely, the dsh^{V26} allele that we used is very strong and probably null (Klingensmith et al., 1994; this paper) and yet it showed significantly reduced non-autonomous effects compared to *arr*. We therefore suggest that this Wnt transduction mechanism is only partially Dsh-dependent and that another molecule supplies the function not provided by Dsh.

An alternative hypothesis is that the weak effects that we observe with the reduced Arm levels represent the full extent of Arm function in this pathway and that Arm and Dsh play similar roles mediating significantly less polarity information than *arr*.

The read-out of planar polarity and the molecular nature of factor-X

The Wg-related signal functions to produce graded activity of factor-X in the Eq/Pl axis of the developing retina and we propose that it is the direction of change in factor-X activity that is read by the emerging ommatidia. The *sevenless* enhancer assay allows us to probe for a protein's function in the read-out step by overexpressing it at a critical stage of ommatidial development. By this method, we have implicated Fz, Dsh and Sgg in the read-out mechanism but not Arm.

The Wnt receptors are Frizzled class proteins and Fz itself has been shown to bind Wnts (Bhanot et al., 1997). This then raises the possibility that factor-X is a Wnt and, if this is the case, then its second messenger system appears to be *arm* independent. Further evidence for the Fz transduction pathway being not of the standard Wnt type comes from the work of Strutt et al. (1997) that suggests that Fz signaling is mediated by the GTPase RhoA.

Multiple roles of the Wg signaling mechanism in eye development

The work of Ma and Moses, (1995) and Treisman and Rubin, (1995) assigned to Wg a critical role in regulating where ommatidial differentiation was initiated in the disc. The dorsal and ventral Wg expression (the polar expression in the tissue bordering the eye) functions to suppress precocious ommatidial differentiation in these positions and thereby prevent the formation of ectopic waves of eye patterning running in the dorsoventral axis. Furthermore, Treisman and Rubin (1995) suggested that Wg also acts to suppress premature initiation of the ommatidial differentiation program in the main body of the retina ahead of the advancing wave front. That is, a long-range influence of Wg at the poles would prevent the retinal tissue from differentiating prior to the arrival of the inductive morphogenetic wave. Evidence for this came from ectopic expression of Wg in the eye and the effect of *sgg* clones both of which suppressed ommatidial differentiation. We too have observed these effects and offer further evidence in favor of this model. First, the expression of activated Arm mimics ectopic Wg in that it has potent abilities to prevent ommatidial differentiation, whether expressed constitutively or under *sevenless* control. We note however that there is significant rescuing non-autonomous effect of the wild-type tissue surrounding the clones. Second, clones of *arr* and *arm* (transducers of the Wg signal) frequently appeared to trigger the precocious differentiation of the ommatidia causing similar effects to loss of *ptc* or *pka* or premature expression of Hedgehog (Heberlein et al., 1995; Ma and Moses, 1995; Strutt et al., 1995; Wehrli and Tomlinson, 1995). Thus there is mounting evidence that the Hedgehog signal from the advancing wave front antagonizes a Wg or Wg-like repression to elicit timely differentiation of the ommatidia.

Cruciform planar polarity in the *Drosophila* eye

The planar polarity in the *Drosophila* retina is evident as the chirality and orientation of the ommatidia. A two-dimensional organization is required to generate the ommatidial array, whereas a single piece of directional information can orient hairs or bristles in standard planar polarity. We proposed earlier (Wehrli and Tomlinson, 1995) that ommatidial chirality was directed by two independent and orthogonal signaling mechanisms, one lying in the A/P axis and the other Eq/Pl (Fig. 1). The manipulation of the morphogenetic wave that sweeps anteriorly across the developing retina frequently caused inversions in the A/P axis without affecting the Eq/Pl axis. However, other data suggested that sometimes both the A/P and Eq/Pl axes could be affected by the reversal of the morphogenetic wave. Why different effects can be observed is not clear and, in order to resolve this issue, we began an investigation of the Eq/Pl axis. The manipulations of the Eq/Pl polarity that we describe here argue that this axis is organized independently of the A/P axis and we propose that two signaling systems impose separate planar polarities on the retina. Reifegerste et al. (1997) infer the presence of a second

polarizing axis in the retina that is independent of the A/P polarity and Heberlein et al. (1998) conclude that there is Eq/Pl information distinct from and ahead of the advancing wave front. Since the resulting axes lie at right angles to each other, we describe this phenomenon as 'cruciform planar polarity'. We envisage that a group of cells destined to form an ommatidium respond to each axis of information and the shape of the ommatidium that forms depends upon the combined action of the two signals that the cells experience (Fig. 1D).

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