

Cellular polarity, mitotic synchrony and axes of symmetry during growth. Where does the information come from?

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ABSTRACT The polarization of cells during development is discussed with relationship to synchronized cell divisions and lineage restrictions. A tessellation model is proposed to explain the generation of the precise hexagonal array of ommatidia in the eye. This model allows the assembly of highly organized structures from localized cellular interactions. There is no requirement for a precise genetic description of the adult organism. Instead a sequential set of reiterated cellular interactions generates increasingly complex structures. The polarity patterns observed in adult cuticular bristles and hairs reflect accurate control of the shape of terminally differentiating cells rather than fine-grained positional information.

KEY WORDS: *polarity, chirality, tessellation, compound eye, prickle-spiny-legs*

Introduction: Turing's Problem

One of the core problems in developmental biology during the 20th century has been to understand how morphogenetic information is encoded. An adult is clearly more complex than its embryo. The developmental program, however, passes through an "information bottleneck" in which only a single copy of DNA is carried. Turing (1936) considered the properties of a machine that could read information encoded on a tape. The information to build the machine itself could well be encoded, but it was not possible for the tape to encode a blueprint of the machine which included a copy of the tape itself. During biological replication, the Turing machine problem is avoided as the fertilized egg inherits both a copy of the genetic information and a highly organized cellular structure. The increase in complexity during development, however, can not be explained as a simple consequence of re-iterating the "hard-copy" information encoded in DNA with each successive cell division. Instead, developmental information must be generated from sequential interactions between growing cells. Given that the starting point in every generation is a single cell, in the initial stages of development most of the interactions will occur between neighboring cells. Such localized interactions are likely to remain critical even when the behavior of large numbers of cells is integrated within a developing organism. Many of the key gene products will control conserved cellular processes that are regulated precisely in single cells such as yeasts (Verde *et al.*, 1995) and have secondarily become incorporated into morphogenetic pathways.

As a corollary to this view I would like to suggest that adult cells with the same developmental fate have the same positional infor-

mation. Provided that the growth of imaginal discs is controlled to the extent that regions containing similar cells have the correct size and shape, there is no need for individual cell fates to be specified. This would greatly reduce the information requirement. It is only within fine-scale fields, such as the ommatidium of the eye, or at the boundary between vein and non-vein tissue in the wing, that additional information is necessary. Within these fields, cells adopt differential fates with respect to their immediate neighbors. At no stage during development is there a precise genetic description of the adult organism.

Compartments and the allocation of regional fate

Two of the key advances in *Drosophila* developmental genetics in the later half of this century have been the identification of the fate-determining homeotic (e.g., Lewis, 1978) and segmentation genes (Nüsslein-Volhard and Wieschaus, 1980). García-Bellido and co-workers have contributed a number of ideas to this field, largely based on study of the development of the imaginal wing disc. The first observation was that somatic clones of wild-type cells could be given a growth advantage in a *Minute (M)* background (García-Bellido *et al.*, 1973). Surprisingly, the wild-type (*M⁺*) cells did not fill the whole wing but followed a straight line, the antero-posterior (A/P) compartment boundary. Provided that clones were induced after the first division post-blastoderm they did not cross the A/P compartment boundary. Later in development, during the second larval instar, an additional dorso-ventral (D/V) boundary is set up, which separates the dorsal and ventral surfaces of the wing. These results, together with the compartmen-

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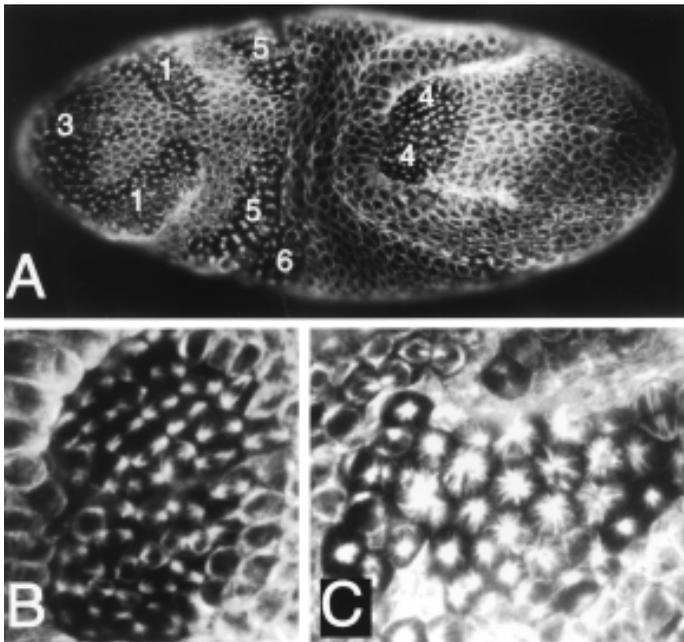


Fig. 1. After nuclear cycle 13 nuclei divide within synchronized mitotic domains. The mitotic pattern is reproducible and precisely aligned to the anteroposterior and dorsoventral axes of the egg. In at least some mitotic domains the cells share specific spindle orientations and shapes (A) View of dorsal surface showing domains 1,3,4,5 and 6, courtesy of Victoria Foe (B) Enlarged view of domain 4. (C) In domain 9, instead of dividing parallel to the embryonic surface, the spindles are oriented end on. As a consequence half the daughter cells are placed within the embryonic head. Reproduced from Figure 17B of V. Foe, 1989.

specific phenotypes of several homeotic mutations, formed the basis for the selector gene hypothesis (García-Bellido, 1975), which postulated that developmental pathways were controlled within progressively smaller regions by the sequential activation of “selector” genes. This hypothesis has been confirmed with the identification of first *engrailed* (Morata and Lawrence, 1975) and more recently *apterous* as genes controlling A/P and D/V (Diaz-Benjumea and Cohen, 1993) fate, respectively.

The continued sub-division of imaginal discs into progressively smaller compartments (García-Bellido, 1975), however, has not been confirmed. This raises the question of what is the advantage of dividing the wing blade into four compartments, each containing thousands of cells of several different types. It is not immediately clear how this might simplify the problem of allocation of specific fates to individual cells. Before considering this question, I shall review the problem of cellular polarity in the embryonic and adult patterns. What are the underlying principles that allow the homeotic and segment-polarity genes to control not only embryonic segmentation, but the growth of imaginal discs (Wilkins and Gubb, 1991; Couso *et al.*, 1993) and, possibly, the final allocation of cell fate and polarity in the adult organism?

Cellular polarity and cell division patterns

A critical element in generating cellular diversity during development is polarized communication between cells. Such communica-

tion, however, requires that the cells themselves be polarized. Polarization precedes blastocyst formation in mammals and gastrulation in *Drosophila*. Within an epithelium, cells must be polarized along an initial, apico-basal, plane or they could not remain associated in a sheet (Gubb, 1993). This remains true whether or not the epithelial cells are visibly differentiated from each other in the plane of the epithelium. The first indication of apico-basal polarization in *Drosophila* is at the cellular blastoderm stage, when cells form a uniform layer at the boundary of the egg. At this stage the mitotic spindles are oriented normal to the apico-basal plane of individual cells (Foe, 1989). This alignment of spindle axes parallel to the plane of an epithelium is a general feature of epithelial cells. Centromeres separate to opposite poles of a cell rather than along the apico-basal axis. This implies a direct link between spindle orientation and, at least, apico-basal polarity.

In *Drosophila*, the initial embryonic divisions are syncytial (reviewed in Wilkins, 1993). Nuclei migrate to the surface of the egg by the tenth division and by cycle 13 the mitotic spindles lie parallel to the surface. Spindles pack together, but are not precisely aligned, so that there are occasional slight gaps between them (Fig. 5; Foe, 1989). Within the next three divisions, embryonic development is completed to give a pharate larva. These three differentiative divisions are initiated in a series of mitotic domains (Foe, 1989). Within each domain mitosis starts in one, or a few, interior cells and spreads outwards towards the domain boundaries. The spindle orientations are no longer random within the plane of the epithelium, but aligned with characteristic patterns (Foe, 1989; Fig. 1). The divisions in domain 9 are particularly intriguing as spindle orientations are perpendicular to the embryonic surface. This division segregates two lineages; half the daughter cells remain on the embryonic surface, while the other half form a new layer inside the presumptive embryonic head. The other mitotic domains also appear to represent cell fate boundaries (Cambridge *et al.*, 1997). Clearly, the timing and orientation of spindle formation are precisely controlled in the embryonic stages during which differentiation of specialized cell types takes place. It is tempting to postulate that these observations might reflect a general principle: when cells within an epithelium divide in a synchronous wave their mitotic spindles tend to align with respect to each other. Such a rule could generate complex patterns by linking the cell-cycle oscillators of individual cells.

Cellular polarity and cell fate

The initial information for patterning of the antero-posterior axis of the embryo is provided by a gradient of the bicoid (BCD) protein. Alterations in the dose of the maternal *bcd* transcript cause the embryonic fate map, including the mitotic domains, to expand or contract along the antero-posterior axis (Driever and Nusslein-Volhard, 1988; Foe and Odell, 1989). Although the BCD gradient provides positional information this is only of a very rough, regional nature. Allocation of precise fates to cells along the antero-posterior axis of the embryo requires the interaction of a whole cascade of maternal and zygotic gene products. It is the segment polarity genes, at the bottom of this cascade, that are responsible for allocation of precise cellular fates at the blastoderm stage (reviewed in Wilkins, 1993).

The phenotypes of the segment polarity mutants correspond to deletions and mirror-image duplications of parts of the segmental

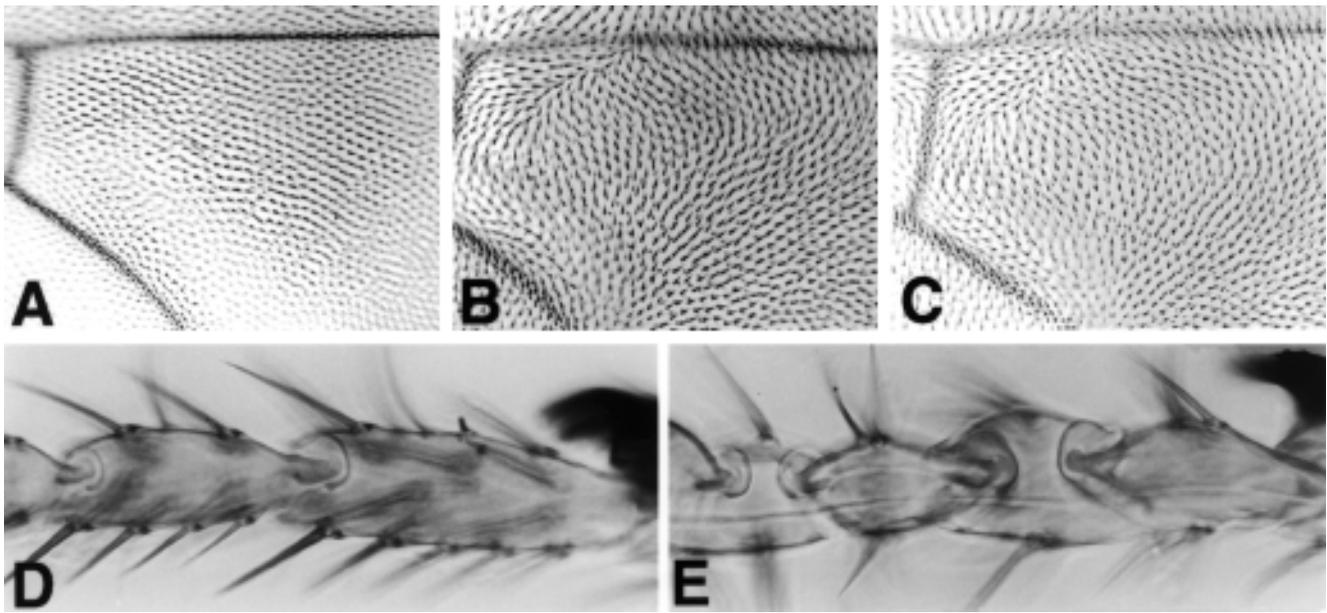


Fig. 2. Wing and tarsal phenotypes. The region distal to the posterior cross vein in a wild-type wing is shown in (A). Hairs are aligned along the length of the wing. The hair polarity pattern on the dorsal surface of the same region of *pk* wings is shown in (B) and (C). Note how similar the pattern is between the two wings. The distal tarsal segments of *sple* legs show mirror-image duplications; compare the wild-type pattern (D) with the *sple* pattern (E). The pattern transformations correspond to loss of the central region of each segment and their replacement with a mirror-image duplication of the distal (socket) and proximal (ball); leaving the adult flies with too many joints, but a functional ball and socket structure.

pattern. A critical point is that it is not simply the polarity of cells within each segment that is affected, as indicated by the denticle belt orientation, but the fate of cells is altered to give mirror-image duplications. It is as if altering the polarity of a cell can affect both the polarity and the fate of its neighbor. The segment polarity gene products form a heterogeneous group ranging from transcription factors, membrane-bound receptors, protein kinases and diffusible factors and the interactions between them have been studied intensively in a number of laboratories. In some sense the transcription factor *Engrailed* (*En*) (Fjose *et al.*, 1985) and the diffusible factors *Wingless* (*Wg*) (Rijsewick *et al.*, 1987) and *Hedgehog* (*Hh*) (Lee *et al.*, 1992) are key, although it is probably more reasonable to think of the set of segment polarity gene products as an interacting module that sets up a segmentally repeating pattern. The scale of this pattern is small, about 3.5 cells per segment at the blastoderm stage, so that models invoking localized cell-cell interactions are attractive (Martinez-Arias *et al.*, 1988; Bejsovek and Wieschaus, 1993). An alternative view is that cell fate within a segment is determined with response to a graded signal such as *Wg* (Lawrence *et al.*, 1996).

There is an additional set of genes which affects the polarity of cuticular structures in adult flies (Gubb and García-Bellido, 1982). Mutant alleles of these “tissue polarity” genes cause discrete changes in the orientations of bristles and hairs, mirror-image duplications in the tarsi, reversals of ommatidia in the eye and multiple hairs in the wing (reviewed in Adler, 1992; Gubb, 1993). In general the tissue polarity genes that have been cloned encode novel classes of membrane-bound molecules (Vinson *et al.*, 1989; Park *et al.*, 1996; Collier and Gubb, 1997) that are implicated in *Hh* and *Wnt* signaling pathways in terminally differentiated cells (Bhanot *et al.*, 1996; Struhl *et al.*, 1997). The *prickle-spiny legs* (*pk-sple*)

gene differs from these in encoding a conserved LIM domain protein (Gubb *et al.*, unpublished). The wing and tarsal phenotypes of different mutant alleles of *pk-sple* are shown in Figure 2. The *pk* mutant wing hair polarity pattern is striking in being very fine-grained and invariable. Similar polarity patterns are shown in *sple* legs and, in addition, mirror-image duplications in the tarsal segments (Held *et al.*, 1986). The tissue polarity mutations might affect the cellular response to an underlying pre-pattern, but to explain the invariability of the mutant patterns such a pre-pattern would itself have to be very precise. This is not a comfortable conclusion. Again it demands that cells encode a very detailed genetic description of the adult and that imaginal disc cells can interpret their positions with extreme fidelity.

Morphogenesis of the compound eye: high fidelity for the minimum of information

The problem in later development, therefore, is to understand how pattern formation occurs across the much larger numbers of cells within imaginal structures. The information requirement is perhaps most extreme during development of the eye. The precision with which this structure is assembled forces the question of whether there might be a mechanism to generate precise cellular orientations that is independent of fine-grained positional information.

The compound eye consists of an hexagonal array of ommatidia, each one containing a number of specialized cell types. Unlike in other regions of the adult body, the last two cellular divisions occur as synchronized waves. These synchronized divisions are separated by a line of cells constricted in the apico-basal plane, known as the morphogenetic furrow (see Wolff and Ready,

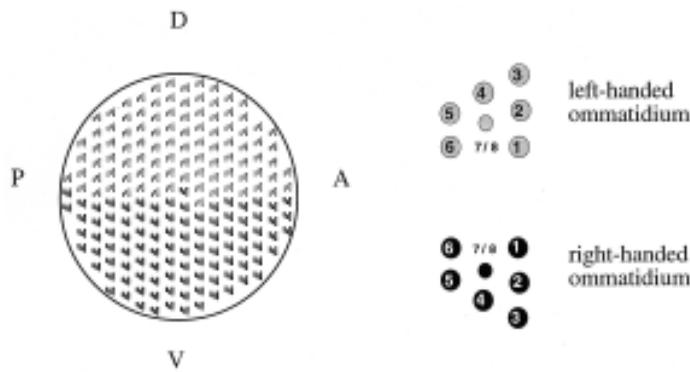


Fig. 3. Organization of ommatidia. The eye is divided into dorsal and ventral hemispheres by an axis of mirror-symmetry, the equator. In both hemispheres ommatidia are oriented with photoreceptors R1 and R6 towards the equator; R1, R2, and R3 towards the anterior (A) and R5 and R6 towards the posterior (P). The different chiral types in the dorsal and ventral hemispheres can be thought of as “left-handed” and “right-handed” reflections around the equatorial axis.

1991). The furrow is initiated at the posterior margin of the eye-antennal disc in the early second larval instar and travels anteriorly during the remainder of larval development. Behind the advancing furrow undifferentiated cells are recruited into ommatidial preclusters in a precise sequence. The first cell type to be recruited is the R8 photoreceptor followed by the R2 and R5 and the R3 and R4. In this way, the initial alignment of the emerging preclusters is determined with respect to the morphogenetic furrow (Wolff and Ready, 1991).

The link between this process and embryonic polarity is that furrow progression is controlled by segment polarity genes, in particular *hedgehog* (*hh*) (Ma *et al.*, 1993). Normal furrow progression requires the induction of *hh* expression in posterior cells (Heberlein *et al.*, 1993; Ma *et al.*, 1993). The direction of furrow progression does not reflect polarized transmission of information between cells, but is a consequence of the furrow being initiated at the posterior margin of the eye. When expressed ectopically, *hh* can initiate ectopic furrows which expand uniformly to generate a ring (Heberlein *et al.*, 1995). At this stage, eye disc cells are apolar in the plane of the epithelium, at least by the criterion that the *Hh*-propagated signal expands uniformly in all directions. As the R cells begin to differentiate, the polarized transmission of signaling molecules between particular cell types does occur (Tomlinson *et al.*, 1987; Zipursky *et al.*, 1992). It is as if passage of the furrow resets cellular polarity. Undifferentiated cells are, almost by definition, apolar.

Following the passage of the furrow, the ommatidial pre-clusters rotate through roughly 90° in opposite directions in the dorsal and ventral halves of the eye. This rotation is the first manifestation of an axis of mirror-symmetry along the “equator” (Dietrich, 1909), with ommatidia in the dorsal and ventral eye having opposite chiralities (Fig. 3). During normal eye development, the morphogenetic furrow passes along the presumptive equator recruiting ommatidia from the centre outwards (Wolff and Ready, 1991). This sequential recruitment of ommatidial units prevents stacking flaws within the hexagonal array (Gubb, 1993) which is critical to the function of the adult eye. Such precision would not be required within most regions of the body. Although differentiated structures, like the bristle cells and associated cell types, are common they are

surrounded by a background of undifferentiated cells and do not need to fit together exactly.

Not only does the topography of the furrow provide the initial alignment of preclusters, but their centre-outwards recruitment means that the position of the equator is always in the direction of the previously recruited ommatidium in the row (Fig. 4). In this situation, the reversal of axes at the equator would be a direct consequence of the centre-outwards recruitment of ommatidia (Gubb, 1993). As the ommatidia complete their rotation they become hexagonal and fit against the previous ommatidium in the row. The importance of this stacking process is indicated by the phenotype of the *nemo* mutation (Choi and Benzer, 1994). In *nemo* mutants, the last 45° of rotation is blocked and ommatidia pack in a cuboidal lattice in the adult eye. Mutant ommatidia contain the correct R cell types, despite being square and aligning at roughly 45° to the equator.

The eye phenotypes of tissue polarity mutants

Several of the tissue polarity mutants, including *dishevelled*, *frizzled* and *starry night*, give a rough eye phenotype associated with disrupted packing of the corneal lenses. Within the eye,

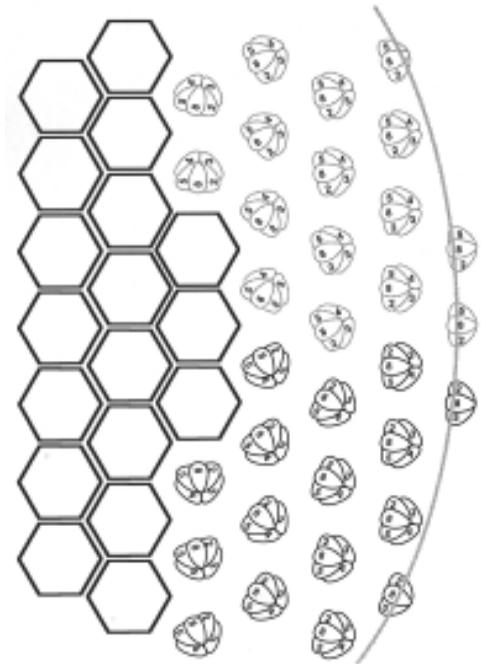


Fig. 4. Morphogenetic movements in the eye disc. Pre-cluster cells are recruited by the passage of the morphogenetic furrow (thick grey line) from posterior to anterior across the eye disc. The orientation of the pre-clusters is aligned along the morphogenetic furrow with the R8, R5 and R2 cells beginning to express the neural-specific marker *Elav* before the R4 and R3 cells. The R1, R6 and R7 cells are recruited following a second division after passage of the furrow, but have been omitted from this diagram. As the furrow progresses from posterior to anterior, dorsal pre-clusters (grey outline) rotate anticlockwise, while ventral pre-clusters (black outline) rotate clockwise. After rotating through 90°, ommatidia fit precisely into a hexagonal array. In this diagram the curvature of the furrow has been exaggerated to illustrate the correct centre-outwards recruitment of rows of ommatidia.

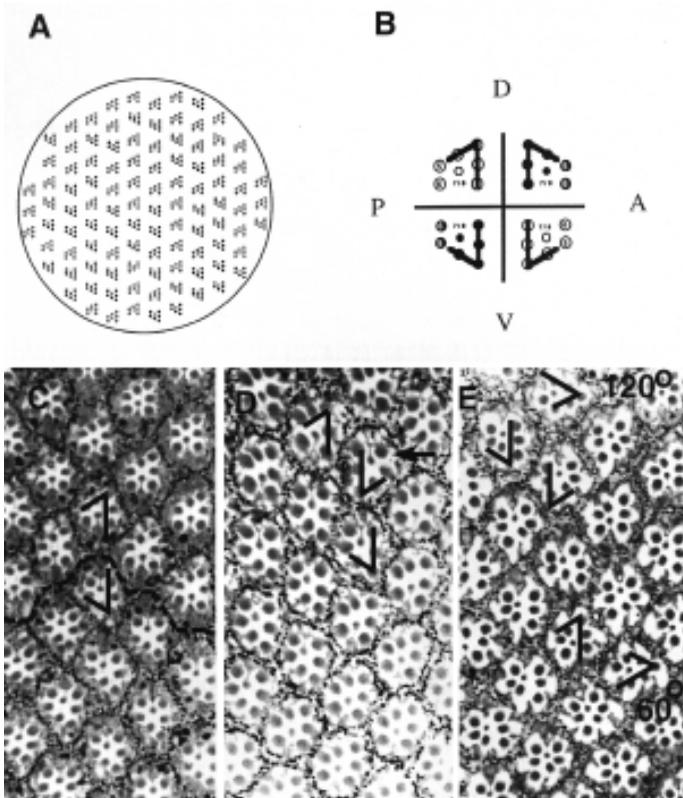


Fig. 5. Organization of ommatidial arrays. (A) In the *sple* eye both hemispheres of the eye contain both chiral forms. Ommatidia with normal chirality retain normal polarity with R1 and R6 aligned to the equator. Ommatidia with reversed chirality are oriented with R1 and R6 photoreceptors towards the pole. (B) In the normal eye, the alternative chiral forms correspond to reflections around the equatorial (A-P) axis. Reflections around the polar (D-V) axis are not seen in the normal eye, but would result in R5 and R6 being aligned to the anterior rather than posterior. (C) Section of wild-type eye showing the equator (black line). (D) Dorsal section of *sple* eye. Ommatidia with normal chirality are oriented as normal with R1 and R6 facing towards the equator. Ommatidia with reversed chirality are oriented R1 and R6 to pole. Note the rare A-P reversed ommatidium (arrow). (E) Section of ventral region of *pk-sple*¹³ eye showing regular hexagonal packing remains regular, with both A-P (arrows) and D-V reflections. Note ommatidia rotated to precisely 120° and 60°.

ommatidia are mis-rotated and can be either left- or right-handed in either hemisphere (Gubb, 1993; Theisen *et al.*, 1994; Zheng *et al.*, 1995; Choi *et al.*, 1996). This phenotype is shown in *pk-sple*¹³ eyes (Fig. 5E). A weaker phenotype is given by *sple* alleles, which are unique in having ordered rows of corneal lenses within a perfect hexagonal array including a mixture of both chiral forms (Fig. 5D). About a third of the ommatidia in *sple* eyes have the wrong chirality for the hemisphere in which they are found. Significantly, these ommatidia are oriented with their R1 and R6 photoreceptors facing the pole rather than the equator (Fig. 5D). What appears to have occurred is that a proportion of the preclusters have rotated in the wrong sense through 90° to give ommatidia that have reversed both their chirality and their dorso-ventral (D-V) orientation (Figs. 5 and 7). Interestingly, there is no disruption in polarity at the boundary between two ommatidia with reversed polarity (Fig. 5D).

It has been suggested that ommatidia orient to the slope of a

gradient of positional information with a peak at the presumptive equator (Lawrence and Shelton 1975; Wehrli and Tomlinson 1995; Zheng *et al.*, 1995; but see also Chanut and Heberlein, 1995; Ma and Moses, 1995; Strutt *et al.*, 1995). The slope of such a gradient would be bi-directional so that ommatidia would orient with R1 and R6 photoreceptors towards equator in both hemispheres of the eye. On this model *sple* pre-clusters which rotate in the wrong direction would adopt a final alignment which is reversed to the slope of the gradient. While such a model is possible, an alternative interpretation is suggested by the somewhat more extreme phenotype of *pk-sple*¹³ eyes. In this mutant the hexagonal packing of ommatidia is correct over much of the eye although there are regions which are slightly rough. In both hemispheres of the eye about a third of the ommatidia show reversed chiral forms, but in regions of the eye that remain smooth both A-P and D-V reversed forms can be identified (Fig. 4E). In addition about 5% of the ommatidia stack within the hexagonal array with an orientation of 60° or 120° to the equator (Fig. 4E). This result would not be predicted from a gradient model.

A simple hypothesis is that ommatidia cease to rotate when they fit against the previous hexagonal ommatidium in the row. At the equator ommatidia would rotate through 90° and align normal to the direction of furrow progression. Subsequent centre-outwards

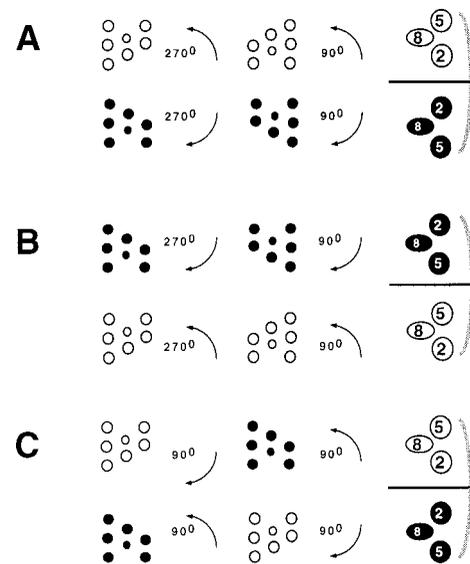


Fig. 6. Sense of rotation of pre-clusters and ommatidial chirality. Behind the advancing furrow (curved grey line), pre-clusters rotate anti-clockwise above the equator and clockwise below the equator. If rotation and chirality are coupled, as in a wild-type eye, a 90° rotation will give ommatidia oriented with R1 and R6 towards the equator. (A) To align ommatidia with R1 and R6 towards pole, and reverse the normal A-P orientation, would require rotation of the pre-cluster through an additional 180°. (B) Pre-clusters with incorrect specification of equatorial and polar R cell fates would generate reversed chiral forms oriented with R1 and R6 towards pole, after rotation through 90°. Rotation through a further 180° would orient ommatidia tail-towards-equator. (C) Pre-clusters in which rotation and chirality were uncoupled could give ommatidia with either chirality, in either orientation with respect to the equator, after rotation through 90°. The forms illustrated correspond to pre-clusters that have adopted the opposite handedness to that expected from their direction of rotation.

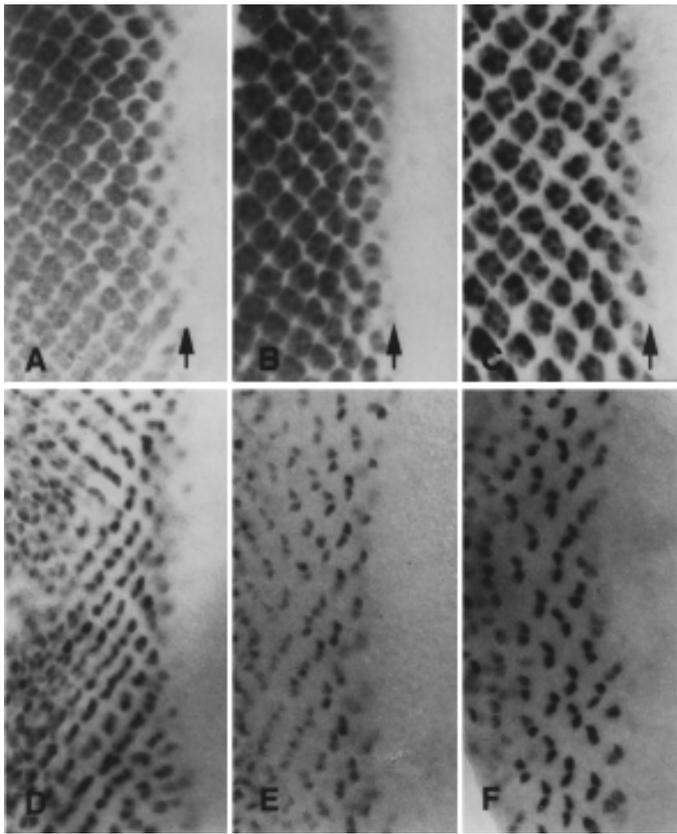


Fig. 7. Antibody staining of mutant eye discs. *Elav* stained discs of wild-type (A) *sple*¹ (B) and *pk-sple*¹³ (C). In both mutants the initial orientation of pre-clusters follows the topography of the furrow (arrow) as in the wild-type. Subsequent rotation is less precise in the mutant discs and adjacent pre-clusters that appear to be rotating in different directions are often seen (arrowheads). The rotation defect is more clearly seen with the *Sal* antibody, which shows the R3 and R4 cells; wild-type (D), *sple*¹ (E) and *pk-sple*¹³ (F). Note the R3 and R4 cells form a shallow V which is directed towards the furrow in the initial rows. A-P reversals of this V shape are not seen in the mutant discs.

growth would maintain this alignment, like sticking tiles on a bathroom wall. A “tessellation” mechanism of this type would provide mechanical transmission of an equatorial signal. In *sple* and *pk-sple* eyes, an hexagonal array of ommatidia of both chiral forms could assemble without localized disruptions at the boundaries of ommatidia with reversed polarity. Occasional ommatidia that rotated through significantly less than 90° would be corrected to a 60° orientation by the stacking mechanism.

This view does not imply that there is no equatorial morphogen. On the contrary, antagonistic polar and equatorial morphogens would be required to maintain the topography of the advancing furrow. It is not necessary, however, to postulate polarized transport of such molecules along the polar axis, nor that they form a long range gradient. It is more likely that such molecules, like Hh, will diffuse uniformly in all directions from the leading edge of the furrow.

If preclusters require to read both their initial alignment and equatorial orientation from the topography of the furrow, does it matter which of the R cells are mutant for *sple* and *pk-sple*? To

determine the cellular autonomy of the *sple*¹ and *pk-sple*¹³ mutations, somatic mosaics were made using the yeast FRT/FLP recombinase system (Xu and Rubin, 1993). In *sple*¹ mutants the only cell type that is critical is the R3, which is in the anterior equatorial position when the precluster emerges from the furrow (see also Zheng *et al.*, 1995). The genotype of both the anterior and posterior equatorial (R2 and R3) photoreceptors affects ommatidial chirality in *pk-sple*¹³ (Fig. 8, Table 1). The topography of the furrow is normal in these mutants (Fig. 6), suggesting that the mutant phenotype results from failure to specify equatorial and polar cell fate correctly.

In addition to the D-V reversed chiral forms, about 1% of the ommatidia in *sple* alleles and 15% of ommatidia in *pk-sple* alleles have reversed chirality with their R1 and R3 cells facing the posterior margin of the eye. These A-P reversed ommatidia can be oriented towards either the equator or the pole. Such reversals could not result from a 90° rotation unless the direction of rotation and ommatidial chirality are uncoupled (Fig. 5). That these reversals are more frequent in *pk-sple* than *sple* eyes might well be correlated with the requirement for *pk-sple* expression in both anterior and posterior equatorial R cells. Whether that is the case or not, it is intriguing that the direction of rotation and chirality can be uncoupled in these mutant eyes. Interestingly Ma and Moses (1995) found regions with unrotated, but otherwise normal, ommatidia in *wg*^{ts} eyes. These results imply that although there is an absolute correlation between the sense of precluster rotation and the chirality of ommatidia in wild-type eyes, the development of a chiral ommatidium does not require rotation to occur.

On this view of eye development, the passage of the morphogenetic furrow initiates the polarization of a field of undifferentiated cells. Provided that pre-clusters rotate through roughly 90° a “tessellation” mechanism ensures that a precise hexagonal array of ommatidia is formed. The model does not require signaling of polarity information during the final stages of morphogenesis, nor that individual cells have precise positional information within the eye.

TABLE 1

PHOTORECEPTOR GENOTYPE OF MOSAIC OMMATIDIA

<i>sple</i> ¹ : Reversed chirality (N=36)	<i>sple</i> ¹ : Normal chirality (N=39)
R1 R2 R3 R4 R5 R6 R7 R8	R1 R2 R3 R4 R5 R6 R7 R8
63 69 91 63 60 49 52 53	56 59 51 62 69 54 49 36
<i>pk-sple</i> ¹³ : Reversed chirality (N=50)	<i>pk-sple</i> ¹³ : Normal chirality (N=50)
R1 R2 R3 R4 R5 R6 R7 R8	R1 R2 R3 R4 R5 R6 R7 R8
52 84 88 68 60 58 42 29	52 50 52 46 46 52 54 55

The % of each type of photoreceptor cell that were mutant in mosaic ommatidia. (N is number of ommatidia of each class scored). Note that even when all cells are mutant, in homozygous eyes, only about one third of the ommatidia show reversed chirality. For this reason Fisher’s exact test (Sokal and Rohlf, 1981) was used to determine statistical significance. R cell genotype differs significantly from random in reversed compared to normal ommatidia only for the R3 cell in *sple*¹ mosaics (P-value = 0.0002) and for the R2 (P=0.0005), R3 (P=0.0001) and possibly R4 (P=0.0279) cells in *pk-sple*¹³ mosaics. The genotype of R1, R5, R6, R7 and R8 cells has no significant effect on chirality in either mutant.

Growth and lineage restrictions

The imaginal discs in *Drosophila* are set aside during embryogenesis and divide repeatedly during the larval stages. Mature discs contain many thousands of cells and have the topographical shapes necessary to generate individual adult structures such as the wing, eye, leg or haltere. Cells within each disc remain very similar, however, until specification of the final adult pattern takes place during the last few cell divisions. García-Bellido (1975) suggested that the factors controlling disc morphogenesis included mitotic rate, mitotic orientation and differential cellular affinities. It seems clear that the region-specific control of division rate and spindle orientation could go a long way to explain the generation of discs with specific sizes and shapes. At some level these are the processes that must be controlled by the homeotic and segment polarity genes during imaginal disc growth.

The possibility that anterior and posterior cells might have differential cell affinities was also suggested by García-Bellido (1975). The more specific idea that the straight line boundary at the A/P compartment boundary could result from differential affinities of A and P cells was suggested by Lawrence and Morata (1976). Like the boundary between oil and water, the A and P cells would separate into discrete populations. There are two problems with this idea as it stands. Firstly, the A/P boundary in the leg (Steiner, 1976) is far from straight, despite marking the confrontation between A and P cells. Secondly, the boundary between oil and water is only straight when the two liquids have different densities. In this situation, it is the uniform action of gravity that causes the phases to separate. If the liquids had the same density they would tend to minimise their boundaries of contact, leading to globules of one liquid within the other. Similarly, populations of A and P cells with differential affinities would tend to form smoothly curved, rather than straight-line, compartment boundaries. Such smooth, curved boundaries are observed with clones of both *patched* and *cubitus interruptus*, which affect the *hh*-signaling pathway between A and P cells (Dominguez *et al.*, 1996; Chen and Struhl, 1996).

Cellular polarity and emergent patterns

An alternative view is that compartment boundaries represent lines along which cells are in some sense polarized. The simplest possibility would be that the spindle orientations were aligned parallel to compartment boundaries. In this case, clones would elongate parallel to the boundary and generate a lineage restriction. This is an attractive hypothesis, particularly since Foe (1989) has demonstrated that precise control of spindle orientation takes place during embryogenesis. There is little evidence, however, for preferential spindle orientations at compartment boundaries, although they have not been ruled out rigorously (Blair, 1995).

If divisions at compartment boundaries do show random spindle orientation, then maintenance of a straight line would require cellular rearrangements (See also Blair, 1995, for alternative models). This would require that cells show polarized cell affinities. Not only must A and P cells at the boundary have differential cell affinities, but the ligands responsible must be restricted to the cell surfaces facing the compartment boundary. Strong support for the involvement of polarized cell affinities in compartmentalization is provided by the observation that *smoothened* clones "sink" into the posterior compartment leaving their twin sister clones in the

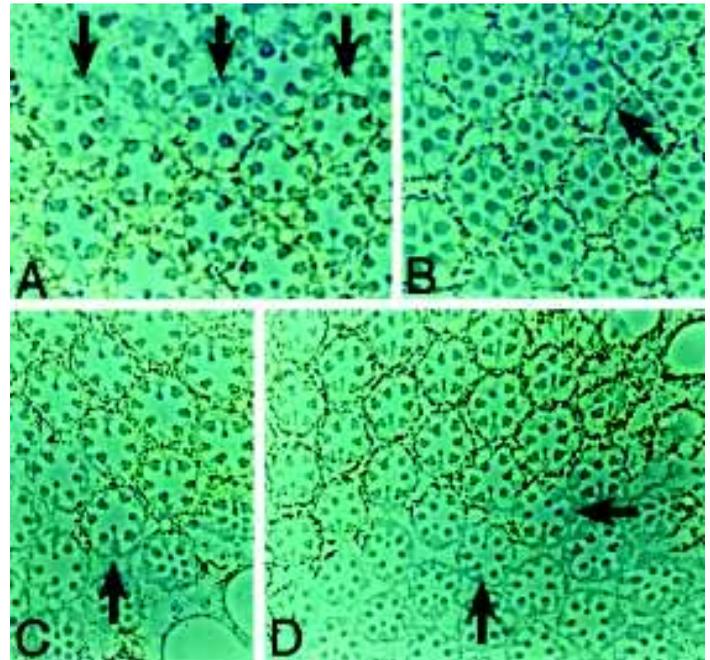


Fig. 8. Sections of mosaic eyes. Homozygous polarity mutant cells are marked with white and lack eye pigment. **(A)** Mosaic *sple* ommatidia with reversed chirality (arrows). The ommatidia shown are mutant for R1, R2, R3, R7; R1, R3, R5, R6, R7 and R2, R3 and R4, respectively from left to right. **(B)** Mosaic *pk-sple*¹³ ommatidium rotated 60° anti-clockwise from normal position and fitting precisely within hexagonal array. Note complete lack of disruption in polarity of surrounding ommatidia (the equator can be seen between wild-type ommatidia immediately to the right of the rotated ommatidium). **(C)** D-V reversed *pk-sple*¹³ mosaic ommatidium (arrow) mutant for R2 and R6. **(D)** D-V (vertical arrow) and A-P (horizontal arrow) reversed ommatidia. In neither case is the polarity of the wild-type ommatidium on the polar (dorsal) side of the reversed ommatidium affected.

anterior compartment (Blair and Ralston, 1997; Rodriguez and Basler, 1997).

These conclusions fit well with the suggestion that the function of compartments might be to control the size and shape of imaginal discs (Crick and Lawrence, 1975). The related ideas, that compartments represent discrete units of pattern formation and that compartment boundaries act as the boundaries for gradients of positional information (Crick and Lawrence, 1975, Struhl *et al.*, 1997) are more problematical. Not only would cells need to be able to register their position precisely, but each cell must interpret this position as a very fine-grained genetic address. This would require a prodigious amount of information which would have to be encoded in a way that could pass through the information bottleneck at the fertilised egg stage. As discussed for the eye, however, it is possible to generate fine-grained patterns, without requiring that the vast majority of cells know their precise position, except with respect to neighboring cells.

From this standpoint, the control of imaginal disc growth at compartment boundaries is an elegant solution. Many of the segment-polarity genes are used during later development to regulate imaginal disc growth. Instead of setting up a short-range standing wave, in which adjacent cells have different fates, the

differential expression of segment polarity genes during larval growth defines large populations of similar cells within each compartment. It is only at the compartment boundaries that there is an abrupt alteration in the levels of expression of the critical gene products by signaling mechanisms that have recently been elucidated (e.g., Guillen *et al.*, 1995; Zecca *et al.*, 1995; de Celis, this issue). Significantly, slow-growing clones survive at compartment boundaries although they are outcompeted by normal cells within the wing blade (Simpson, 1979). It is as if cells at a compartment boundary form a competitive pool that is separate from that of the internal cells. Whether or not this is the case, there is increasing evidence that the size and shape of imaginal discs is controlled by growth at the boundaries. Internal cells divide to fill in the gaps (Karlsson, 1984).

Cellular polarity in other regions

Given that tissue polarity mutants such as *pk-sple*, *dishevelled* and *frizzled* affect the polarity of bristles and hairs in other regions of the body it is likely that the underlying morphogenetic processes affected are analogous to what is happening in the eye disc. There is no evidence for synchronized waves of division during terminal differentiation in other discs. It is striking, however, that mature pupal wing disc cells form a hexagonal array with the prehair initiation site at the distal vertex (Wong and Adler, 1993; Eaton *et al.*, 1996). Prehair formation is initiated in the distal wing and spreads proximally, which might correlate with progressive alignment of the hexagonal array. Interestingly, the tissue polarity mutants affect the sub-cellular localization of the prehair initiation sites, but not the hexagonal shape of the pupal wing cells (Wong and Adler, 1993). A similar tessellation mechanism in the abdomen might avoid the problem, noted by Struhl *et al.* (1997), that cells in the anterior and posterior regions of the anterior compartment would have to align with opposite orientations to the postulated Hh gradient.

Occam's razor: for any two morphogenetic hypotheses, the one requiring cells to interpret the least information is most likely to be correct

During the embryonic and larval development of *Drosophila* a relatively small number of key gene products are used to regulate growth. As suggested by García-Bellido (1975), the critical factors appear to be region-specific control of division rate, spindle orientation and cellular affinities. Quite how control of these processes relates to the cell lineage restriction at compartment boundaries remains elusive, despite the identification of many of the components of the signal transduction pathways. Towards the end of development the polarity of adult cuticular structures is dependent on precise control of cell shape changes. The resultant patterns have deceptively high fidelity.

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References

- ADLER, P.N. (1992). The genetic control of tissue polarity in *Drosophila*. *BioEssays* 14: 735-741.
- BEJSOVEC, A. and WIESCHAUS, E. (1993). Segment polarity gene interactions modulate epidermal patterning in *Drosophila* embryos. *Development* 119: 501-517.
- BHANOT, P., BRINK, M., SAMOS, C.H., HSIEH, J.C., WANG, Y. MACKE, J.P., ANDREW, D., NATHANS, J. and NUSSE, R. (1996). A new member of the *frizzled* family from *Drosophila* functions as a wingless receptor. *Nature* 382: 225-230.
- BLAIR, S.S. (1995). Compartments and appendage development in *Drosophila*. *BioEssays* 17: 299-309.
- BLAIR, S.S. and RALSTON, A. (1997). Smoothed-mediated Hedgehog signalling is required for the maintenance of the anterior-posterior lineage restriction in the developing wing of *Drosophila*. *Development* 124: 4053-4063.
- CAMBRIDGE, S.B., DAVIS, R.L. and MINDEN, J.S. (1997). *Drosophila* mitotic domain boundaries as cell fate boundaries. *Science* 277: 825-828.
- CHANUT, F. and HEBERLEIN, U. (1995). Role of the morphogenetic furrow in establishing polarity in the *Drosophila* eye. *Development* 121: 4085-4094.
- CHEN, Y. and STRUHL, G. (1996). Dual roles for Patched in sequestering and transducing Hedgehog. *Cell* 87: 553-563.
- CHOI, K.W. and BENZER, S. (1994). Rotation of photoreceptor clusters in the developing *Drosophila* eye requires the *nemo* gene. *Cell* 78: 125-136.
- CHOI, K.W., MOZER, B. and BENZER, S. (1996). Independent determination of symmetry and polarity in the *Drosophila* eye. *Proc. Natl. Acad. Sci. USA* 93: 5737-5741.
- COLLIER, S. and GUBB, D. (1997). *Drosophila* tissue polarity requires the cell-autonomous activity of the *fuzzy* gene, which encodes a novel transmembrane protein. *Development* 124: 4029-4037.
- COUSO, J.P., BATE, M. and MARTINEZ-ARIAS, A. (1993). A wingless-dependent polar coordinate system in *Drosophila* imaginal discs. *Science* 25: 484-489.
- CRICK, F.H.C. and LAWRENCE, P.A. (1975). Compartments and polyclones in insect development. *Science* 189: 340-347.
- DE CELIS, J. (1998). Positioning and differentiation of veins in the *Drosophila* wing. *Int. J. Dev. Biol.* 42: 335-343.
- DIAZ-BENJUMEA, F.J. and COHEN, S.M. (1993). Interaction between dorsal and ventral cells in the imaginal disc directs wing development in *Drosophila*. *Cell* 75: 741-752.
- DIETRICH, W. (1909). Die facettengaugen der dipteren. *Z. Wiss. Zool.* 92: 465-539.
- DOMINGUEZ, M., BRUNNER, M., HAFEN, E. and BASLER, K. (1996). Sending and receiving the *hedgehog* signal: Control by the *Drosophila* Gli protein *cubitus interruptus*. *Science* 272: 1621-1625.
- DRIEVER, W. and NUSSLEIN-VOLHARD, C. (1988). The bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* 54: 95-104.
- EATON, S., WEPF, R. and SIMONS, K. (1996). Roles for Rac1 and Cdc42 in planar polarization and hair outgrowth in the wing of *Drosophila*. *J. Cell Biol.* 135: 1277-1289.
- FJOSE, A., MCGINNIS, W.J. and GEHRING, W.J. (1985). A homeo box containing gene isolated from the *engrailed* region of *Drosophila* and the spatial distribution of its transcripts. *Experientia* 41: 816.
- FOE, V.E. (1989). Mitotic domains reveal early commitment of cells in *Drosophila* embryos. *Development* 107: 1-22.
- FOE, V.E. and ODELL, G.M. (1989). Mitotic domains partition fly embryos reflecting early cell biological consequences of determination in progress. *Am. Zool.* 29: 617-652.
- GARCÍA-BELLIDO, A. (1975). Genetic control of wing disc development in *Drosophila*. In Cell patterning (Eds. Porter and K. Elliot). Elsevier, Amsterdam. *Ciba Found. Symp.* 29.
- GARCÍA-BELLIDO, A., RIPOLL, P. and MORATA, G. (1973). Developmental compartmentalisation of the wing disk of *Drosophila*. *Nature New Biol.* 245: 251-253.
- GUBB, D. (1993). Genes controlling cellular polarity in *Drosophila*. *Development (Suppl.)*: 269-277.

- GUBB, D. and GARCÍA-BELLIDO, A. (1982). A genetic analysis of the determination of cuticular polarity during development in *Drosophila melanogaster*. *J. Embryol. Exp. Morphol.* 68: 37-57.
- GUILLEN, I., MULLOR, J.L., CAPDEVILA, J., SANCHEZ-HERRERO, E., MORATA, G. and GUERRERO, I. (1995). The function of *engrailed* and the specification of *Drosophila* wing pattern. *Development* 121: 3447-3456.
- HEBERLEIN, U., SINGH, C.M., LUK, A.Y. and DONOHOE, T.J. (1995). Growth and differentiation in the *Drosophila* eye coordinated by *hedgehog*. *Nature* 373: 709-711.
- HEBERLEIN, U., WOLFF, T. and RUBIN, G. (1993). The TGFbeta homolog *dpp* and the segment polarity gene *hedgehog* are required for propagation of a morphogenetic wave in the *Drosophila* retina. *Cell* 75: 913-926.
- HELD, L., DUARTE, C.M. and DERAKHSHANIAN, K. (1986). Extra tarsal joints and abnormal cuticular polarities in various mutants of *Drosophila melanogaster*. *Roux Arch. Dev. Biol.* 195: 145-57.
- KARLSSON, J. (1984). Morphogenesis and compartments in *Drosophila*. In *Pattern formation: a primer in developmental biology* (Eds. G.M. Malakinski and S.V. Bryant) Macmillan, New York. pp323-337.
- LAWRENCE, P.A. and MORATA, G. (1976). Compartments in the wing of *Drosophila*: a study of the *engrailed* gene. *Dev. Biol.* 50: 321-337.
- LAWRENCE, P.A. and SHELTON, P.M.J. (1975). The determination of polarity in the developing insect retina. *J. Embryol. Exp. Morph.* 33: 471-486.
- LAWRENCE, P.A. SANSON, B. and VINCENT, J-P. (1996). Compartments, *Wingless* and *engrailed*: patterning the ventral epidermis of *Drosophila* embryos. *Development* 122: 4095-4103.
- LEE, J.J., VON KESSLER D., PARKS, S. and BEACHY, P.A. (1992). SECRETION AND LOCALIZED TRANSCRIPTION SUGGEST A role in positional signaling for products of the segmentation gene *hedgehog*. *Cell* 71: 33-50.
- LEWIS, E.B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* 276: 565-570.
- MA, C. and MOSES, K. (1995). *wingless* and *patched* are negative regulators of the morphogenetic furrow and can affect tissue polarity in the developing *Drosophila* compound eye. *Development* 121: 2279-2289.
- MA, C., ZHOU, Y., BEACHY, P. and MOSES, K. (1993). The segment polarity gene *hedgehog* is required for progression of the morphogenetic furrow in the developing *Drosophila* eye. *Cell* 75: 927-938.
- MARTINEZ-ARIAS, A., BAKER, N.E. and INGHAM, P.W. (1988). Role of segment polarity genes in the definition and maintenance of cell states in the *Drosophila* embryo. *Development* 103: 157-170.
- MORATA, G. and LAWRENCE, P.A. (1975). Control of compartment development by the *engrailed* gene in *Drosophila*. *Nature* 255: 614-617.
- NUSSLEIN-VOLHARD, C. and WIESCHAUS, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287: 795-801.
- PARK, W.J., LIU, J., SHARP, E.J. and ADLER, P.N. (1996). The *Drosophila* tissue polarity gene *inturned* acts cell autonomously and encodes a novel protein. *Development* 122: 961-969.
- RIJSEWIJK, F., SCHUERMANN, M. WAGENAAR, E., PARREN P., WEIGEL, D. and NUSSE, R. (1987). The *Drosophila* homolog of the mouse mammary oncogene *int-1* is identical to the segment polarity gene *wingless*. *Cell* 50: 649-657.
- RODRIGUEZ, I. and BASLER, K. (1997). Control of compartmental affinity by *Hedgehog*. *Nature* 389: 614-618.
- SIMPSON, P. (1979). Parameters of cell competition in the compartments of the wing disc of *Drosophila*. *Dev. Biol.* 69: 182-193.
- SOKAL, R.R. and ROHLF, F.J. (1981). *Biometry*. New York: Freeman and Company. pp 730-736.
- STEINER, E. (1976). Establishment of compartments in the developing leg imaginal discs of *Drosophila melanogaster*. *Roux Arch. Dev. Biol.* 180: 9-30.
- STRUHL, G., BARBASH, D.A. and LAWRENCE P.A. (1997). *Hedgehog* acts by distinct gradient and signal relay mechanisms to organise cell type and cell polarity in the *Drosophila* abdomen. *Development* 124: 2155-2165.
- STRUTT, D.I., WIERSDORFF, V. and MLODZIK, M. (1995). Regulation of furrow progression in the *Drosophila* eye by cAMP-dependent protein kinase A. *Nature* 373: 705-709.
- THEISEN, H., PURCELL, J., BENNETT, M., KANSAGARA, D., SYED, A. and MARSH, J.L. (1994). *dishvelled* is required during *wingless* signaling to establish both cell polarity and cell identity. *Development* 120: 347-360.
- TOMLINSON, A., BOWTELL, D.D., HAFEN, E. and RUBIN, G.M. (1987). Localization of the *sevenless* protein, a putative receptor for positional information, in the eye imaginal disc of *Drosophila*. *Cell* 51: 143-150.
- TURING, A. (1936). On computable numbers, with an application to the Entscheidungsproblem. *Proc. Lond. Math. Soc.* 42: 230-265.
- VERDE, F., MATA, J. and NURSE, P. (1995). Fission yeast cell morphogenesis: identification of new genes and analysis of their role during the cell cycle. *J. Cell Biol.* 131: 1529-1538.
- VINSON, C.R., CONOVER, S. and ADLER, P.N. (1989). A *Drosophila* tissue polarity locus encodes a protein containing seven potential transmembrane domains. *Nature* 338: 263-264.
- WEHRLI, M. and TOMLINSON, A. (1995). Epithelial planar polarity in the developing *Drosophila* eye. *Development* 121: 2451-2459.
- WILKINS, A.S. (1993). Genetic analysis of animal development. 2nd ed. (John Wiley & Sons). New York.
- WILKINS, A.S. and GUBB, D. (1991). Pattern Formation in the Embryo and Imaginal Discs of *Drosophila*. What are the links? *Dev. Biol.* 145: 1-12.
- WOLFF, T. and READY, D.F. (1991). The beginning of pattern formation in the *Drosophila* compound eye: the morphogenetic furrow and the second mitotic wave. *Development* 113: 841-850.
- WONG, L.L. and ADLER, P.N. (1993). Tissue polarity genes of *Drosophila* regulate the subcellular location for prehair initiation in pupal wing cells. *J. Cell Biol.* 123: 209-221.
- XU, T. and RUBIN, G.M. (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* 117: 1223-1237.
- ZECCA, M., BASLER, K. and STRUHL, G.A. (1995). Sequential organizing activities of *engrailed*, *hedgehog* and *decapentaplegic* in the *Drosophila* wing. *Development* 121: 2265-2278.
- ZHENG, L., ZHANG, J. and CARTHEW, R.W. (1995). *frizzled* regulates mirror-symmetric pattern formation in the *Drosophila* eye. *Development* 121: 3045-3055.
- ZIPURSKY, S.L., KRAMER, H., CAGAN, R., HART, A. and VAN VACTOR, D. (1992). Induction of the R7 neuron in the *Drosophila* compound eye: the *bride of sevenless* and *sevenless* interaction. *Cold Spring Harbour Sym. Quant. Biol.* 57: 318-389.