Chapter 10. Ommatidial tessellation in the eye twin-field and the specification of fine-scale pattern.

While the shape of the wing blade is critical for its aerodynamic properties, the precise spatial organisation of differentiated cell types is essential for the function of the compound eye. Although there are no lineage discontinuities between the eye, head and antenna the eye field becomes divided into dorsal and ventral lineages [1] [2]. This D/V lineage restriction forms an AMS, like the A/P boundary of the wing. However, while this lineage restriction may regulate larval growth of the eye twin-field, it has only an indirect effect during the alignment of the ommatidial array. Instead, ommatidial recruitment is keyed to the advance of the morphogenetic furrow as a synchronised metachronal wave. Ommatidial units are aligned sequentially by a tessellation mechanism, with the last formed units defining the boundary of the eye twin-field [3]. The first column is initiated from the posterior margin towards the end of the first larval instar (L1) and consists of only three ommatidia (either 2D + 1V, or 1D + 2V). Subsequent columns align against the first, and progressively lengthen, with new columns initiated every 20 minutes throughout larval and pupal development [3]. The ommatidial columns reach a maximum (polar) extension before shortening until the A limit of the eye. Immediately behind the furrow, epithelial cells contract along their Ap/Ba axis, with apical nuclear displacement. Presumptive photoreceptor (R) cells are recruited sequentially, with the uniform spacing of the initial (R8) cells being determined by lateral inhibition, reviewed in [3]. Following R8 specification, the R2-R5 and R3-R4 photoreceptors are recruited progressively into a 5-cell pre-cluster (R8>R2 + R5>R3 + R4) with polarised signalling between adjacent R cells, reviewed in [5]. Starting with the initial three ommatidia, successive columns of pre-clusters rotate in opposite directions to either side of the equator. The final R1, R6 and R7 precursors are added after the initial rotation, following a second wave of division [3]. In the adult eye, the R3 and R4 cells are displaced to the corners of a trapezoid (Fig. 14), with R3 aligned towards the equator and R4 towards the poles. After rotating through 90°, the maturing pre-clusters align against previously formed ommatidia to give a hexagonal array. This centre-outwards recruitment ensures correct ommatidial alignment, but also generates a reversal in ommatidial chirality around the equator [6].

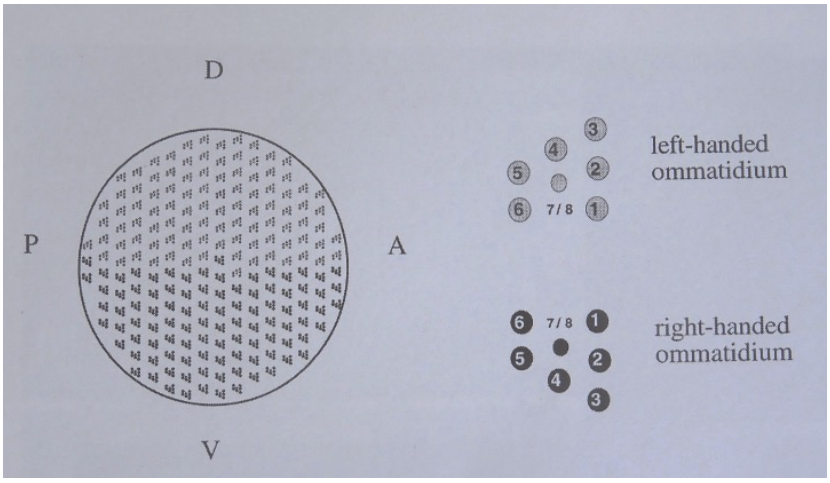

Fig. 14. The compound eye of *Drosophila.* An equatorial AMS separates D and V twin-fields of chiral ommatidia. Within each ommatidium, the R3 and R5 photoreceptors are displaced to the corners of a trapezoid, with R3 aligned towards the poles, and R1 and R6 aligned towards the equator. From Gubb, 1993.

The morphogenetic furrow is straight as it crosses the equator in the larval disc, but curves progressively backwards towards the poles [7]. This curvature is eliminated during metamorphosis, as the array of ommatidial pre-clusters is mapped across the hemispherical surface of the adult eye (Fig. 15). (This mapping distortion is analogous to the Mercator projection of continental land masses onto a 2D map of the globe.) In consequence, the ommatidial pre-clusters are aligned in trailing columns across the disc, with a shallow V centred along the equatorial mid-line [3].

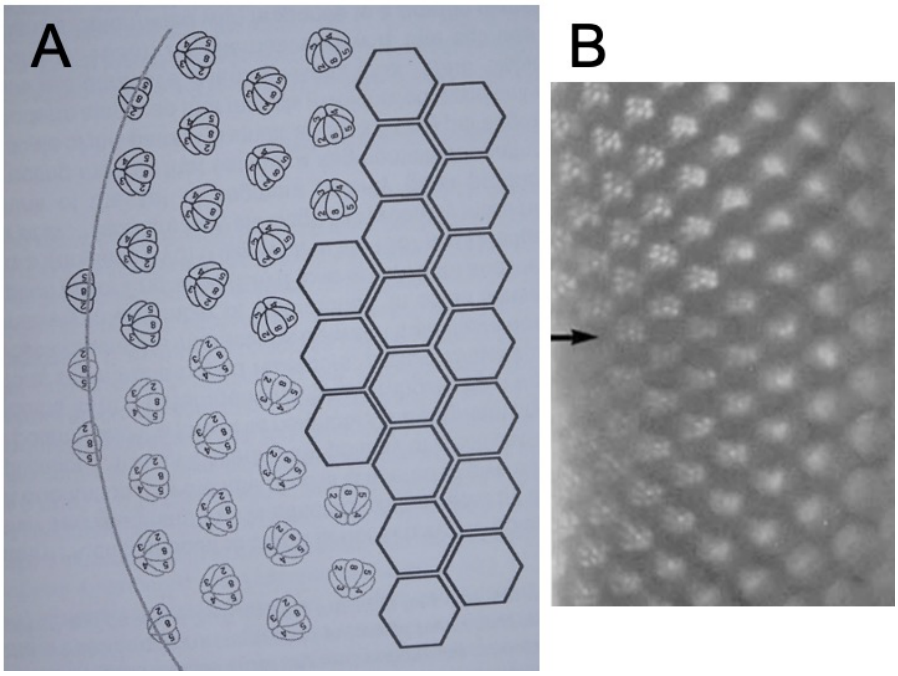

Fig. 15. Ommatidial recruitment and alignment. A. An initial column of 3 pre-clusters at the P boundary of the eye is followed by columns of increasing length to either side of the equator. After reaching maximum of about15 ommatidia, the columns become progressively shorter until the A limit of the eye. Pre-clusters rotate clockwise in the D twin-field and anticlockwise in the V twin-field (in the R eye). The initial R8 cell is specified along the advancing furrow, with R2 and R5 recruited to either side, followed by R2 and R5, R1 and R6 and the final (R7) photoreceptor (not shown). The advancing furrow is straight near the equator but progressively curved towards the poles, although drawn as a uniformly curved grey line in this figure. This curvature is eliminated during metamorphosis, as the array of hexagonal ommatidia is mapped across the hemispherical surface of the adult eye, from Gubb, 1998. **B.** Hemispherical compound eye, focused light-beam passing through R-cell light guides emerges through corneal lenses on the eye surface, black arrow indicates equator, from Gubb, 1993.

As each maturing ommatidium fits against the previous hexagonal unit its shape is adjusted by soap-bubble packing. Notably, aligning horizontal rows of ommatidia to the equator would give the same, uniform hexagonal array as aligning vertical columns to the poles. In this sense, aligning the R3 and R4 pre-cluster cells to the polar axis is equivalent to aligning R2 and R3 to the equatorial axis. By implication, R cell fate is specified with respect to both axes, which cannot be independent (see below, **11**). As in other imaginal discs, the epithelial cells have fluid shapes and irregular boundaries prior to terminal differentiation. Semi-rigid cell boundaries must form to allow mechanical coupling as the pre-clusters begin to rotate and the

morphogenetic furrow advances. The equatorial/polar recruitment of ommatidia is arrested at the G_1 checkpoint around the boundary of the eye twin-field [8].

In some respects, the progressive P > A recruitment of ommatidial columns resembles vertebrate segmentation, in which uncommitted cells are recruited (tail to head) to either side of the dorsal midline. In particular, R cell fate is independent of cell lineage [9]. The P > A recruitment of ommatidial pre-clusters is regulated via interactions between *dpp*, *wg* and *hh*; with a requirement for *hh* expression posterior to the furrow [10 11]. Small clones of *hh*-overexpressing cells can initiate more anterior ectopic furrows, which expand as rings with radial equators [11 12 13]. Similarly, ectopic furrows can be induced in transgenic wg^{ts}, or ptc^{-} somatic mosaics, but the pre-clusters may fail to rotate and give symmetrical ommatidia [14]. By contrast, ectopic expression of *dpp* anywhere within the disc triggers furrow initiation close to the A twin-field boundary [15]. Taken together, these results establish that ommatidial orientation and chirality are dependent on the topography of the advancing furrow. Neither *hh* nor *dpp* affect cell-fate directly, although both regulate cell-cycle progression, at the G_1, or G_2/M checkpoints, respectively [16]. The specification of R8 fate is via the N/Wg signalling pathway [17]. Hh is not detectable during the initial allocation of R8 fate, but is expressed in the R2 + R5 pair, followed by R3 + R4. The R1 + R6 precursors do not express Hh, but the Bar-H1 and H2 cognate TFs are expressed [18]. By contrast, the last (R7) photoreceptor fate is specified by the Rolled (Rl) MAP kinase [19]. During this final stage, the R7 cell comes to lie above R8 and ommatidial rotation stops. In principle, the apposition of the apical surface of R8 with the basal surface of R7 could form a “closed loop” rosette, with asymmetric morphogen partitioning and alternative fates. In this context, the Boss (Bride of sevenless) kinase, is expressed in R8 and activates the Sev (Sevenless) receptor tyrosine kinase in R7. Within this regulatory loop, Sev signal transduction may be dependent on the delayed transcription of *rl* from an extended (460 kb) TU. Ommatidial recruitment is regulated by the import of Fringed (Fng) and Dpp from the peripodial membrane of the eye-antennal disc. Transgenic *UAS-fng* overexpression in the peripodial membrane results in reduced furrow progression, with fewer ommatidia, aligned in a cuboidal tessellated array [20]. Thus, the delivery of excess Fng to the disc epithelium may trigger aberrant cell-cycle release and altered morphogenetic interactions. During normal development, sequential alterations in cell shape take place as the ommatidial pre-clusters rotate and the lateral interfaces between neighbouring R cells are remodelled [21 22 23]. By implication, Wg flux from the posterior D and V margins of the eye twin-field may be asymmetrically partitioned between R-cell pairs. In this system, asymmetric partitioning may allocate cell fate, set alternative transcriptional responses and restrict the metabolic range of differentiated cell types.

Summary:

R-cell fate is allocated behind the advancing morphogenetic furrow as ommatidial columns are recruited from P > A and equator to poles. This centre-outwards recruitment generates a chiral reversal to either side of the equatorial midline, with ommatidial pre-clusters rotating in opposite directions. Furrow progression is driven by interactions between Hh, Wg and Dpp (with Dpp imported from the peripodial membrane) and polarised signal transduction in the wake of the furrow. Progressive tessellation allows the 3D compound eye to be assembled from a 2D template, while automatically correcting planar mapping distortions. Co-ordinated cell shape changes may canalise morphogen partitioning between lateral cytoplasmic interfaces during pre-cluster rotation. The repetition of a recursive morphogenetic loop within each ommatidial pre-cluster allows assembly of a compound eye structure with minimal (additional) genetic complexity.

References:

1. Kenyon, K. L., Ranade, S. S. & Pignoni, F. Dpp, Notch, and Ey: control of tissue specification and proliferation in the Drosophila eye-antennal disc. *Dev. Biol.* **259**, 515 (2003).
2. Kumar, J & Moses, K. EGF receptor and Notch signaling act upstream of Eyeless/Pax6 to control eye specification. *Cell* **104**, 687–697 (2001).
3. Wolff, T. & Ready, D. F. The beginning of pattern formation in the Drosophila compound eye: the morphogenetic furrow and the second mitotic wave. *Development* **113**, 841–850 (1991).
4. Maung, S. M. Planar cell polarity in Drosophila. *Organogenesis* **7**, 165–179 (2011).
5. Dickson, B. & Hafen, E. *Genetic Dissection of Eye Development in Drosophila.* (1993).
6. Gubb, D. Genes controlling cellular polarity in Drosophila. *Dev. Suppl* 269–277 (1993).
7. Wolff, T. & Ready, D. F. Pattern formation in the Drosophila retina. *Dev. Drosoph. Melanogaster* 1277–1325 (1993).
8. Firth, L. C., Bhattacharya, A. & Baker, N. E. Cell cycle arrest by a gradient of Dpp signaling during Drosophila eye development. *BMC Dev. Biol.* **10**, 28 (2010).
9. Lawrence, P. A. & Green, S. M. Cell lineage in the developing retina of Drosophila. *Dev. Biol.* **71**, 142–152 (1979).
10. Heberlein, U., Wolff, T. & Rubin, G. M. The TGF homolog dpp and the segment polarity gene hedgehog are required for propagation of a morphogenetic wave in the Drosophila retina. *Cell* **75**, 913–926 (1993).
11. Ma, C., Zhou, Y., Beachy, P. A. & Moses, K. The segment polarity gene hedgehog is required for progression of the morphogenetic furrow in the developing Drosophila eye. *Cell* **75**, 927–938 (1993).
12. Heberlein, U., Singh, C. M., Luk, A. Y. & Donohoe, T. J. Growth and differentiation in the Drosophila eye coordinated by hedgehog. *Nature* **373**, 709 (1995).
13. Chanut, F. & Heberlein, U. Role of the morphogenetic furrow in establishing polarity in the Drosophila eye. *Development* **121**, 4085 (1995).
14. Ma, C. & Moses, K. Wingless and patched are negative regulators of the morphogenetic furrow and can affect tissue polarity in the developing Drosophila compound eye. *Development* **121**, 2279 (1995).
15. Pignoni, F. & Zipursky, S. L. Induction of Drosophila eye development by decapentaplegic. *Development* **124**, 271–278 (1997).
16. Vrailas, A. D. & Moses, K. Smoothened, thickveins and the genetic control of cell cycle and cell fate in the developing Drosophila eye. *Mech. Dev.* **123**, 151–165 (2006).
17. Baker, N., Han, D., Lee, E. C., Tallon, R. & Zitron, A. E. Determination and redundancy: The strange case of Notch, lateral inhibition, and R8 photoreceptor development in Drosophila. *J. Cell. Biochem.* **19A**, 346 (1995).
18. Rogers, E. M. *et al.* Pointed regulates an eye-specific transcriptional enhancer in the Drosophila hedgehog gene, which is required for the movement of the morphogenetic furrow. *Development* **132**, 4833 (2005).
19. Biggs, W. H., 3rd *et al.* The Drosophila rolled locus encodes a MAP kinase required in the sevenless signal transduction pathway. *EMBO J.* **13**, 1628–1635 (1994).
20. Gibson, M. C. & Schubiger, G. Peripodial cells regulate proliferation and patterning of Drosophila imaginal discs. *Cell* **103**, 343–350 (2000).

21. Longley, R. L. & Ready, D. F. Integrins and the development of three-dimensional structure in the Drosophila compound eye. *Dev. Biol.* **171**, 415–433 (1995).
22. Hayashi, T. & Carthew, R. W. Surface mechanics mediate pattern formation in the developing retina. *Nature* **431**, 647–652 (2004).
23. Larson, D. E., Liberman, Z. & Cagan, R. L. Cellular behavior in the developing Drosophila pupal retina. *Mech. Dev.* **125**, 223–232 (2008).