Drosophila Retinal Patterning

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The *Drosophila* eye is an outstanding model system for exploring fundamental mechanisms of growth and development. The adult eye is composed of a perfect hexagonal lattice of ~750 unit eyes, or ommatidia, each containing precisely 20 well-characterized cells. The eye develops from the eye/antennal imaginal disc, a flattened epithelial sac. During larval and pupal development, cells in the disc grow and undergo compartmentalisation, cell cycle arrest, differentiation, directed movement, and apoptosis, all utilising gene networks and signalling pathways similar to those in vertebrates. The genetic accessibility of *Drosophila*, together with the precision of eye development, makes the fly retina an extremely useful system with which to investigate the roles of genes and signalling pathways in development.

Introduction

Over the past forty years, the *Drosophila* eye has provided an outstanding model for tissue patterning and development. Beginning as a small number of identical cells in the embryo, the developing retina grows over a period of ten days to the complex and precisely ordered array of neurons and accessory cells that comprise the adult compound eye. Along the way, fundamental processes such as compartmentalisation, cell cycle arrest, cell differentiation and apoptosis occur, all using genes and signalling pathways similar to those in vertebrates. The *Drosophila* retina has provided, and continues to provide, a simplified system for elucidating these fundamental processes, with implications for human development and disease.

In structure, the vertebrate and invertebrate eyes appear very different. The vertebrate eye is a simple camera, consisting of a lens and a retina packed with photoreceptor neurons. By contrast, the *Drosophila* retina is a compound apposition eye composed of some 750–800 'ommatidia'; each ommatidium is a self-contained unit eye of 20 cells, complete with lens and a full complement of photoreceptors and support cells. Nevertheless, these two apparently divergent structures share many common regulatory genes and networks, and work in the developing fly eye has increasingly informed work in vertebrates. **See also**: **Photoreceptor Cell Development Regulation**

The structure of the adult *Drosophila* eye makes it a particularly useful tool for the discovery of new genes and their functions. All ommatidia mature identically, allowing developmental effects of an altered gene or signalling pathway to be assessed up to 800 times in each eye. Because the adult *Drosophila* eye is an easily viewed external structure, it is often used for genetic screens to identify genes involved in fundamental processes. Finally, the ability to culture many flies simultaneously under various conditions, or in the presence of possible therapeutic drugs, has provided a means to test medical treatments.

Because of the parallels to human pathways and the unique properties of its development, studies of the fly retina have revealed far more than how to create an eye. The simplicity and powerful molecular and genetic tools of the fly retina have made it one of the most successful model systems for exploring the signalling pathways that control tissue growth and differentiation. The purpose of this review is to provide an overview of these processes and pathways.

Structure of the Eye Imaginal Disc and Adult Eye

Anatomical structures in adult *Drosophila* are derived from larval imaginal discs, epithelia that grow during the three larval stages (or 'instars') and achieve their final shapes during pupal metamorphosis. The *Drosophila* retina is part of the 'eye/antennal imaginal disc', a flattened epithelial sac that gives rise to the eye, antenna, and other head structures such as ocelli, maxillary palps and cuticle (**Figure 1a**). The eye/antennal disc begins as a small group of precursor cells in the embryo that proliferate during the larval period to approximately 20 000 cells. The disc contains several regions with cells of different shapes. One side of the sac is a pseudostratified columnar epithelium (a monolayer of tall, slender cells in which the position of cells' nuclei are randomly distributed) that includes the cells that will become the

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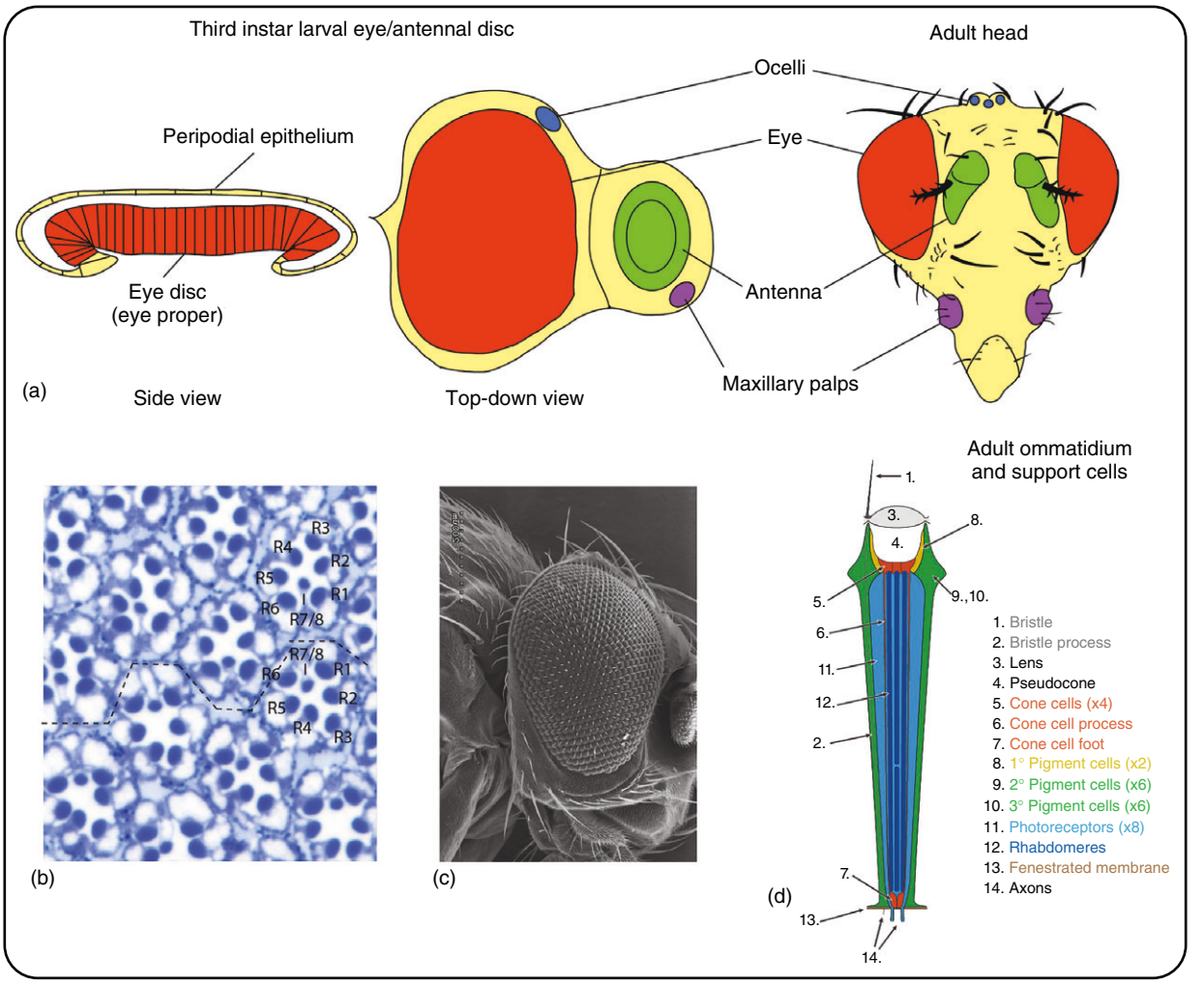

Figure 1 (a) The eye/antennal imaginal disc develops into structures in the adult head. Left, a side view of the disc showing the peripodial epithelium (yellow) overlying the eye disc underneath (red). Middle, a top-down view of the disc, showing regions that will develop into the eye, ocelli, maxillary palps, antenna and cuticle. Right, structures of the adult eye. (b) section of the adult eye shows rhabdomeres in individual ommatidia; photoreceptors are labelled. Note the mirror image arrangement of the rhabdomeres above and below the equator (dashed line). (c) scanning electron micrograph of the adult eye. (d) Side view schematic of cell arrangement in an adult ommatidium. Posterior is to the left in all figures.

eye. At the margins of this tissue are cuboidal epithelial cells that will contribute to the head cuticle around the eye. Finally, overlying these tissues and attached at the edges is a squamous epithelium, the 'peripodial epithelium' (PE), with large, flat cells that will also contribute to the head cuticle (Haynie and Bryant, 1986). For simplicity, here the terms 'eye disc' or 'eye primordia' will refer to the columnar epithelium that forms the eye itself, while 'eye/antennal disc' refers to the whole tissue. The cells throughout the eye/antennal disc undergo what appears to be coordinated proliferation throughout the first, second, and third instars – in other words, the PE, antennal and eye primordia all keep pace with one another. This suggests that there is communication between the different domains of the disc, and indeed, these domains appear to be linked to one another through secreted morphogens and membranous extensions and have profound influences on one another's development (see section titled 'The Peripodial Epithelium').

During larval and pupal development, cells differentiate to generate the final architecture of the adult eye. The ommatidia in the adult eye form a precise hexagonal array, with each ommatidium containing precisely 20 cells: 8 photoreceptors (R1–R8), four cone cells, and an array of pigment cells and bristle groups. The arrangement of these cells in the adult eye is shown in **Figure 1**. Photoreceptors have rhodopsin-containing extensions known as rhabdomeres. These are clustered in the centre of the ommatidia, arranged in a trapezoid that allows each photoreceptor to be identified by its position: R1–R6 rhabdomeres create the trapezoid border, while the small rhabdomeres of R7 and R8 stack on top of

one another in the centre (**Figure 1b**). Overlying the photoreceptors are cone cells that are responsible for secreting the lens that focuses light and covers the eye. Surrounding the photoreceptors and visually insulating each ommatidium from those surrounding it are the pigment cells. Finally, bristle groups containing a bristle and neuron are found at corners of the hexagons, alternating with tertiary pigment cells (**Figure 1c,d**).

Eye Disc Identity and Establishment of Developmental Axes

By what mechanism do the original cells set aside in the embryo know to become an eye, instead of another tissue? Cells within the eye disc are competent to develop as retinal cells owing to the activity of the retinal 'master regulator' genes, Eyeless (Ey) and Twin of Eyeless (Toy), members of the Pax-6 family of transcription factors. These master regulatory genes are conserved across metazoa, and mutations in Pax-6 family members prevent establishment of the retina in many species, resulting in Aniridia and Peter's Anomaly in humans, the Small Eye phenotype and mice, and the *eyeless* phenotype in *Drosophila*. Indeed, introduction of mammalian Pax-6 into the fly can rescue loss of *eyeless*, and overexpression can cause eyes to form ectopically, an extraordinary demonstration of the conserved nature of retinal development.

The eye imaginal disc grows throughout the three larval instars. Ey and Toy are expressed throughout first instar eye/antennal discs, but by the second instar are restricted to the posterior region, which will give rise to the retina; anterior cells, which will become the antenna, begin to express the transcription factor Cut (**Figure 2a**). In the first instar, all cells in the eye primordia appear equivalent. By the mid-second instar, however, the eye disc begins to express specific transcription factors that will distinguish its dorsal and ventral halves. The dorsal edge of the eye disc expresses the transcription factor Pannier (Pnr) (Singh and Choi, 2003). Pannier activates transcription of the diffusible protein Wingless (Wg), which drives expression of the homeodomain Iroquois transcription factors (Iro-C, comprised of Caupolican, Araucan, and Mirror) throughout the dorsal half of the eye disc (Maurel-Zaffran and Treisman, 2000; **Figure 2a**). Conversely, the ventral half of the disc expresses Unpaired (Upd), a ligand for the JAK/STAT pathway, which suppresses Wg activity (Gutierrez-Avino *et al*., 2009). By the end of the second instar, the ventral region trades Upd expression for expression of Sloppy-paired (Slp) 1 and 2, members of the forkhead transcription factor family. Slp and Iro-C gene families each suppresses expression of the other, thereby maintaining the dorsal/ventral division of the eye disc with complementary expression of these genes and their downstream effectors (Sato and Tomlinson, 2007).

The division of the eye disc into dorsal and ventral halves affects signalling within the disc: the transmembrane receptor Notch (N) begins to be expressed widely in the eye disc, while two of its ligands, the transmembrane proteins Delta (Dl) and Serrate (Ser), are expressed in the dorsal and ventral halves, respectively. Despite the broad expression of its ligands, N is not active throughout the eye disc. N activity is regulated by Fringe (Fng), a glycosyltransferase that is expressed only in the ventral half of the eye disc. Fringe adds *N*-acetylglucosamine to *O*-linked sugars on the extracellular domain of N; these additional sugars increase the ability of Dl to bind and activate N but decrease the ability of Ser (see review under Further Reading). The expression patterns of these ligands and Fng result in only a narrow band of activated N along the midline of the eye disc.

N activation along the midline drives proliferation of cells in the disc at long range (i.e. noncell autonomously) by activating expression of Eyegone, a Pax6 homolog, which, in turn, drives transcription of the JAK/STAT ligand Upd at the posterior edge of the eye disc (Reynolds-Kenneally and Mlodzik, 2005; Tsai and Sun, 2004). Upd diffuses throughout the disc, in the space between the eye disc and the peripodial membrane, and binds to JAK/STAT receptors to promote cell proliferation. One interesting theory proposes that cells continue to proliferate so long as the Upd concentration in the disc is maintained above a certain level; as the disc grows, and the space between the disc and the peripodial membrane grows, unpaired is diluted and the rate of proliferation drops, limiting the size of the eye (Vollmer *et al*., 2017).

In addition to Notch and JAK/STAT signalling, proliferation in the second instar eye disc is promoted by the activity of the master regulator Ey in conjunction with two other transcription factors, Homothorax (Hth) and Teashirt (Tsh). As development proceeds, Ey also directly and indirectly drives expression of another group of transcription factors and transcriptional co-activators that includes eyes absent (Eya), Sine oculis (So), and Dachshund (Dac) (Bessa *et al*., 2002). Known collectively as 'retinal determination genes', each of these contributes to the identity of this tissue as the eye. Loss of these genes causes conversion of the tissue to head cuticle, and overexpression can cause formation of ectopic eyes in other locations (see review under Further Reading). Suppressed in early second instar eye discs by Hth and Tsh, expression of Eya, So and Dac is required for cellular differentiation during the third instar; several factors, including EGFR signalling (Salzer *et al*., 2010) and Dpp secreted from the morphogenetic furrow help to alleviate their repression, allowing differentiation to proceed (Firth and Baker, 2009).

Photoreceptor differentiation

The end of the second instar marks a transition in the eye disc from a freely proliferating tissue to one undergoing a remarkably ordered sequence of differentiation. During this period and continuing through the third instar, a wave of differentiation sweeps across the eye disc, starting at the posterior edge and proceeding anteriorly (**Figure 2a**). Differentiation stems from the passage of the 'morphogenetic furrow', a visible indentation in the disc that results from changes in signalling and cell shape. As the furrow travels across the disc, it acts as a moving boundary between proliferating and differentiating cells, leaving in its wake the first glimpse of the ommatidial pattern of the adult eye. As cells differentiate, they produce diffusible factors that push the furrow further toward the anterior. Thus, passage of the furrow fuels its own movement.

The morphogenetic furrow is initiated by expression of the diffusible morphogen Hh at the posterior edge of the eye disc.

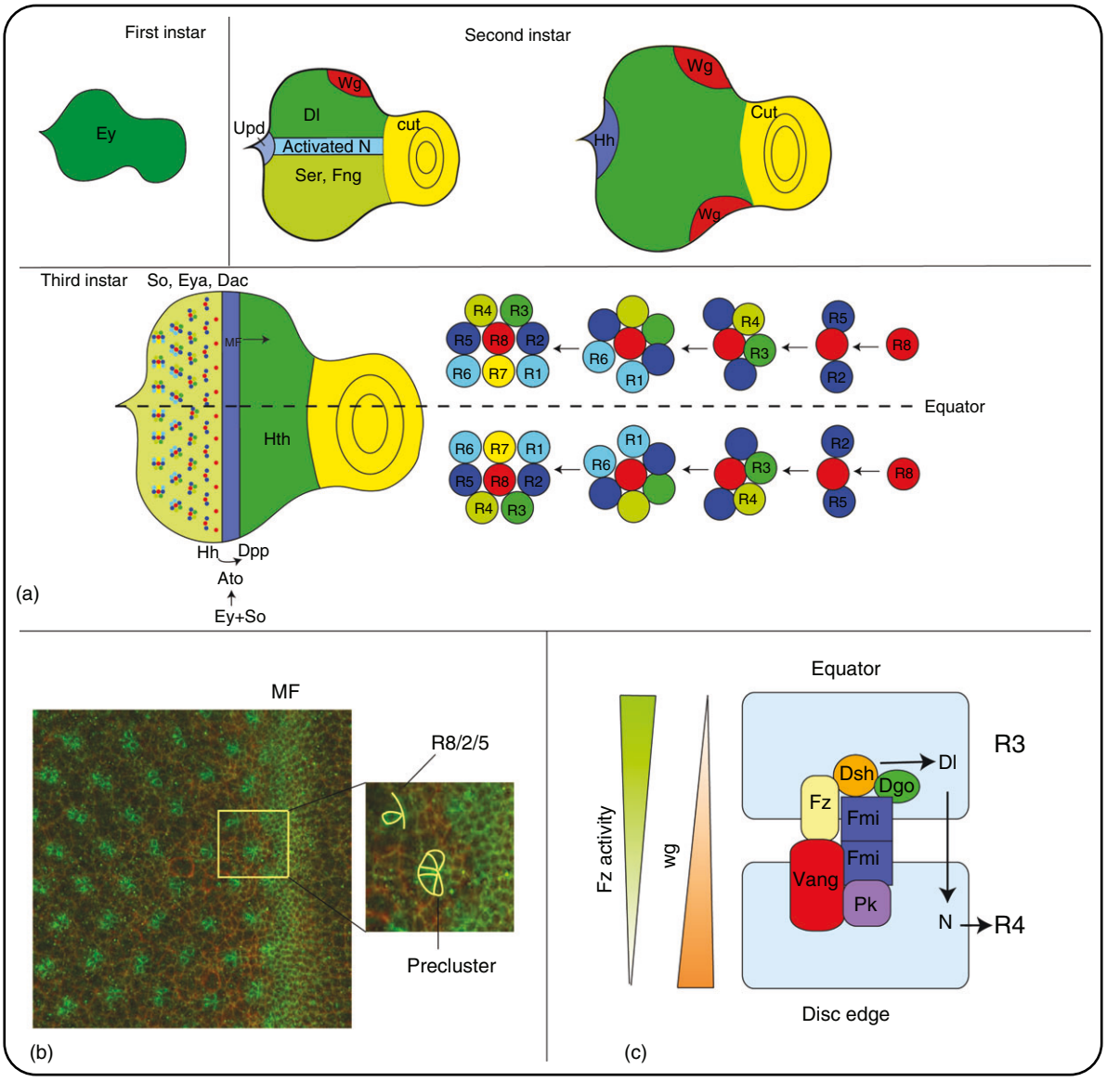

Figure 2 (a) Schematic of gene expression in the eye/antennal disc through larval development and the stepwise differentiation of the photoreceptor cells. Note that ommatidial clusters rotate as they mature and that they are mirror images above and below the equator (dashed line). (b) view of a third instar eye disc stained with antibodies against DE-Cadherin (green) and the cell adhesion protein Echinoid (red). DE-Cadherin outlines the small apical profiles of cells in the morphogenetic furrow (MF). As cells emerge from the furrow (left), DE-cadherin is highest on the membranes of cells in the ommatidial precluster. Slightly later (next row to the left), it is most visible on the membranes of R8, R2, and R5. (c) Schematic of gradients influencing R3 and R4 differentiation and ommatidial rotation. Wg is secreted from the edges of the disc; Fz activity is highest in cells closest to the equator. Higher Fz activity leads to expression of Dl, which activates N on the neighbouring cell, leading to R3 and R4 specification. Photoreceptors in (c) are arranged as they would be below the equator.

By the third instar, Wg expression has expanded to both the dorsal and ventral edges of the eye disc, where it inhibits cell differentiation and allows the eye margins to differentiate into head cuticle rather than the eye proper. As the disc grows and distance between the anterior and posterior edges increases, it is thought that the concentration of Wg at the posterior edge of the disc drops and releases its inhibition on the cells there. Expression of Upd at the posterior edge (described above) also inhibits *wg* transcription (Tsai *et al*., 2007). Reduced levels of Wg allow expression of the Odd-skipped family of transcription factors at the 'firing point', the intersection of the equator with the posterior edge of the eye disc (Bras-Pereira *et al*., 2006). Odd-skipped proteins, in turn, promote expression of Hh in cells at the firing point, initiating the morphogenetic furrow.

Hh acts through both cytosolic and transcriptional mechanisms. It alters actin polymerisation, reducing the apical profile of cells and creating the invagination that constitutes the morphogenetic furrow (**Figure 2a**). The small apical surface area of cells in the

furrow likely increases the number of cell transfers required for Hh to spread and restricts its effects to a few cell diameters (Benlali *et al*., 2000; Corrigall *et al*., 2007). Over this short distance, cells respond to Hh by initiating expression of several genes, including the long-range morphogen Decapentaplegic (Dpp) and the bHLH transcription factor Atonal (Ato). **See also**: **Hedgehog Signalling**

While Hh's direct actions do not extend far, its initiation of Dpp expression allows its indirect effects to extend for many cell diameters. In the anterior part of the eye disc, cells express Hth and are actively proliferating. When Dpp is secreted from cells in the morphogenetic furrow, it diffuses, causing a stripe of cells anterior to the furrow to arrest in G1 of the cell cycle. Hth expression decreases, and expression of Ey and retinal determination genes So, Eya, and Dac increase. Thus Hh and Dpp create a 'pre-proneural' zone that primes cells for differentiation. Within the furrow, the combined actions of Ey and So lead to expression of Ato (Zhang *et al*., 2006). Atonal is a proneural gene that is required for differentiation of the eye's first photoreceptor, R8. Although Atonal is initially present in all cells within the furrow, its expression is soon restricted to groups of 8–10 cells, known as proneural groups. These groups represent the first patterning present in the eye disc and will give rise, eventually, to the individual ommatidia that make up the adult eye. The spacing of the proneural groups is critical – if they are too close together, each ommatidium may lack the requisite 20 cells necessary for its function; if too far apart, the eye will contain fewer than normal numbers of ommatidia. Both the Notch and EGFR signalling pathways contribute to the winnowing of Atonal's expression and spacing of proneural groups (Baonza *et al*., 2001; Gavish *et al*., 2016). Over time, Atonal expression is further refined: Notch-mediated lateral inhibition occurs between neighbouring cells until only one cell continues to express Atonal and becomes R8. (Pepple *et al*., 2008). **See also**: **Neural Development: bHLH Genes**

While Atonal expression designates R8, other photoreceptors rely on signals emanating from R8 for their differentiation. There are no lineage restrictions – no cell is destined to differentiate as a certain cell type based on it parentage – instead, a cell's differentiated identity is determined by cell-to-cell interactions. As cells emerge from the morphogenetic furrow, they form distinctive clusters with R8 at their centre (**Figure 2b**). R8 begins to express two TGF-alpha orthologues, Spitz (Spi) and Keren (Krn), as well as their activating proteases, Rhomboid and Roughoid; secreted Spi and Krn then diffuse to activate Epidermal Growth Factor Receptor (EGFR) on adjacent cells. These cells differentiate as photoreceptors R2 and R5, which in turn also start to secrete Spi to neighbouring cells, resulting in recruitment of R3 and R4. Along with Spi, differentiated cells also secrete Argos (Aos), a long-range diffusible inhibitor that binds to and sequesters Spi to prevent EGFR activation in cells farther away (Klein *et al*., 2004). This is thought to inhibit premature differentiation of photoreceptors and to promote precise and reproducible ommatidial development.

As cells enter the morphogenetic furrow, they arrest in G1 of the cell cycle. However, there are not enough cells present in the eye disc at this point to provide all 20 cells each ommatidium needs to function. After differentiation of R3 and R4, still-undifferentiated cells undergo one more round of cell division, termed the 'second mitotic wave'. After this last round of cell division, EGFR-driven differentiation will continue for the rest of the photoreceptors (R1 and R6, then R7), the cone cells, and pigment cells (Freeman, 1996).

Cell fate specification and competence

EGFR signalling is employed for the differentiation of R1-7, yet these photoreceptors do not differentiate into identical cells. How is this specificity achieved? Each cell type requires a specific transcription factor, and these factors, or unique combinations of factors, appear to dictate a cell's particular identity. These include the homeobox protein Rough (in R2/5), the steroid receptor Seven-up (R3/4/1/6), the homeobox protein Bar H1 (R1/6) and Prospero (R7). These factors are expressed in a defined sequence that coincides with the changing competence of cells. For example, Atonal is expressed first (R8); as cells lose Atonal expression, they gain expression of Rough (R2/R5), then Seven-up (R3/R4, R1/R6), and so on. Loss in activity of one of these factors results in loss of a specific cell type, and ectopic expression is often sufficient to promote ectopic cells to the same fate. **See also**: **Neurogenesis in *Drosophila***

Detailed studies of the specification of the R7 photoreceptor neuron – the best-understood cell fate decision in the fly retina and a particularly successful model system – have revealed surprising subtlety and complexity in localised Ras signalling. The R7 precursor cell expresses two receptor tyrosine kinases, DER and Sevenless; these receptors are activated by Spitz and Bride of Sevenless (Boss), respectively. Both receptors activate the Ras signal transduction pathway and loss of either receptor's function results in loss of R7 differentiation. R7s are restored by constitutive activation of the Ras pathway. R7 specification also requires activation of Notch signalling, though Notch is thought to inhibit cell differentiation. A current model suggests that the dual activation of DER and Sevenless ratchets Ras-pathway signalling to a sufficient level that the cell can overcome this inhibition by Notch. Complete loss of Notch signalling, on the other hand, results in an R1-6 fate, suggesting that it activates expression of transcription factors that are specific to the R7 fate (Cooper and Bray, 2000; Tomlinson *et al*., 2019).

A gradient of differentiation

After its initiation at the firing point, movement of the morphogenetic furrow is fuelled by the differentiation of photoreceptors, creating a self-propagating loop. EGFR activation in differentiating photoreceptors causes expression of the transcription factor Ptd, which, together with the retinal determination gene sine oculus (So), drives expression of Hh (Rogers *et al*., 2005). The released Hh diffuses, inducing Dpp expression, and thereby reproducing changes in cell cycle and transcription factor expression in rows of cells further anterior. By this means, a new row of ommatidia emerges approximately every two hours. Each row contains preclusters and R8 cells that are staggered from those in the previous row, producing a lattice of differentiating ommatidia. Thus, a single mature larval eye disc preparation contains all early ommatidial differentiative events, which can be read in

a smooth progression from anterior (early events) to posterior (later events). A schematic of the larval eye disc is presented in **Figure 2**. After the end of the third instar and by the beginning of pupal development, all photoreceptors in the eye disc have differentiated and the pattern of ommatidia is in place.

The peripodial epithelium

One of the least understood aspects of eye development is how it is influenced by the overlying PE. For many years, the PE was thought to have little to do with patterning of the eye, as the PE itself differentiates into other head structures, and was thought to be primarily important in disc eversion during metamorphosis (Milner *et al*., 1984). More recent research has indicated this is far from the case: the PE appears to secrete many of the factors necessary for development of the eye disc, and both tissues appear capable of influencing signalling in the other; see Further Reading for review. For example, Hh, Dpp and Wg produced in the PE influence the dorsal/ventral patterning of Dl and Ser in the second instar eye disc. Conversely, Dpp produced in the eye disc is necessary for cell survival in the PE. Remarkably, even expression of Ey in the PE, but not the eye disc itself, has been suggested to be required for initiation of the MF by promoting Dpp expression in the PE (Baker *et al*., 2018). The morphogens produced in the PE are secreted, but not all are very soluble, leading to the question of how they travel the distance between the PE and eye disc. At some developmental stages, the PE and eye disc are in close contact with one another, so molecules may be able to simply diffuse. In other cases, however, long, microtubule-based extensions of the cell called 'translumenal processes' have been observed in the space between the tissues (Cho *et al*., 2000; Gibson and Schubiger, 2000). The precise function of these extensions is not clear, but they provide a mechanism by which the tissues could communicate with one another and influence one another's growth and development.

Planar cell polarity

As the morphogenetic furrow travels anteriorly, cells in the differentiating ommatidia begin to rotate as a group, starting four or five rows after the MF has passed and stopping when they have turned 90 degrees. Ommatidia in the dorsal and ventral halves of the eye rotate in opposite directions and become mirror images of one another. This is most easily seen by the trapezoidal arrangement of rhabdomeres of the photoreceptor cells (**Figure 1b**). The R3 rhabdomere is at the apex of the trapezoid and is furthest from the equator in both the dorsal and ventral halves. This arrangement of ommatidia is an example of planar cell polarity (PCP), the asymmetric orientation of cells that arises in many epithelia during development, and is also important for the orientation of feathers, bristles and other polarised structures.

Two systems help ommatidial cells sense the direction of the equator: The 'global' PCP system utilises the atypical cadherin proteins Fat (Ft) and Dachsous (Dac) and the Golgi-associated kinase four-jointed (Fj). Fj phosphorylates the extracellular domains of both Ft and Dac, altering their ability to bind one another. Fj and Dac are transcribed in gradients within the eye disc: Fj has strongest expression at the equator, while Dac is most highly expressed at the dorsal and ventral edges. This produces gradients of Ft:Dac binding that vary with distance from the eye disc midline and can be sensed by cells to provide directional information.

The other major signalling system is the 'core' PCP system, which includes the transmembrane proteins Fz, Van gogh/Strabismus (Vang), and Flamingo (Fmi), and the cytoplasmic proteins Dishevelled (Dsh), Prickled (Pk), and Diego (Dgo). These proteins cluster together at the plasma membranes of nascent R3/R4 cells, with Fz, Dsh, Dgo, and Fmi forming complexes on the sides of cells farthest from the eye disc equator, and Vang, Pk, and Fmi forming complexes closest to the equator. The chirality of the ommatidium is established by interactions between these complexes and is modulated by Fz activity: whichever cell of the R3/4 pair is closest to the equator experiences the highest Fz activation and differentiates as R3. This, in turn, promotes expression of Dl, which activates N on the adjacent cell. N activation designates this cell as R4 (**Figure 2c**) (Cooper and Bray, 1999). The correct designation of R3 and R4 is critical for the ommatidium to achieve its correct orientation; if cell fates are altered through genetic manipulations, chirality is also changed.

These data present a conundrum – Fz activity appears highest near the equator, yet its prospective ligand Wg is secreted from the dorsal and ventral edges of the disc. This puzzle may have been solved when it was found that Wg inhibits interactions between Fz and Vang on adjacent cells (Wu *et al*., 2013). In this model, cells near the equator would experience relatively lower Wg concentrations, and thus higher Fz/Vang 'activity', than their neighbours closer to the disc edge, allowing relative distance from the equator to be sensed within ommatidia.

In addition to establishing the orientation of R3/R4, the core PCP genes also influence the rotation of ommatidia. Movement of a group of cells necessarily requires changes in cell adhesion and cytoskeletal structure. Consistent with this, Fz signals through the cytoplasmic protein Dsh to activate Rho kinases, which can alter the actin cytoskeleton, and Fmi encodes an atypical cadherin that can link cells to one another. In addition, mutations in many other cytoskeleton and cell adhesion proteins also produce rotation defects, including Myosin II, E- and N-cadherins, and integrins. The EGFR pathway also appears important specifically for rotation, as mutations in pathway components alter the degree of turning, but not the specification of R3/4. Mutations in the EGFR pathway also interact genetically with mutations in cytoskeletal and adhesion proteins (see reviews under Further Reading).

Pupal Development

At the end of larval development, each photoreceptor cluster is surrounded by a pool of loosely disorganised undifferentiated cells. After four cells are recruited to be cone cells that will cap each ommatidium and two are recruited to become primary pigment cells (1°s), the surrounding cells are reorganized to generate the honeycomb interommatidial cell lattice of secondary pigment cells (2°s) that define the 'edges' of each hexagon, and tertiary (3°) pigment cells and bristle groups that sit at alternate

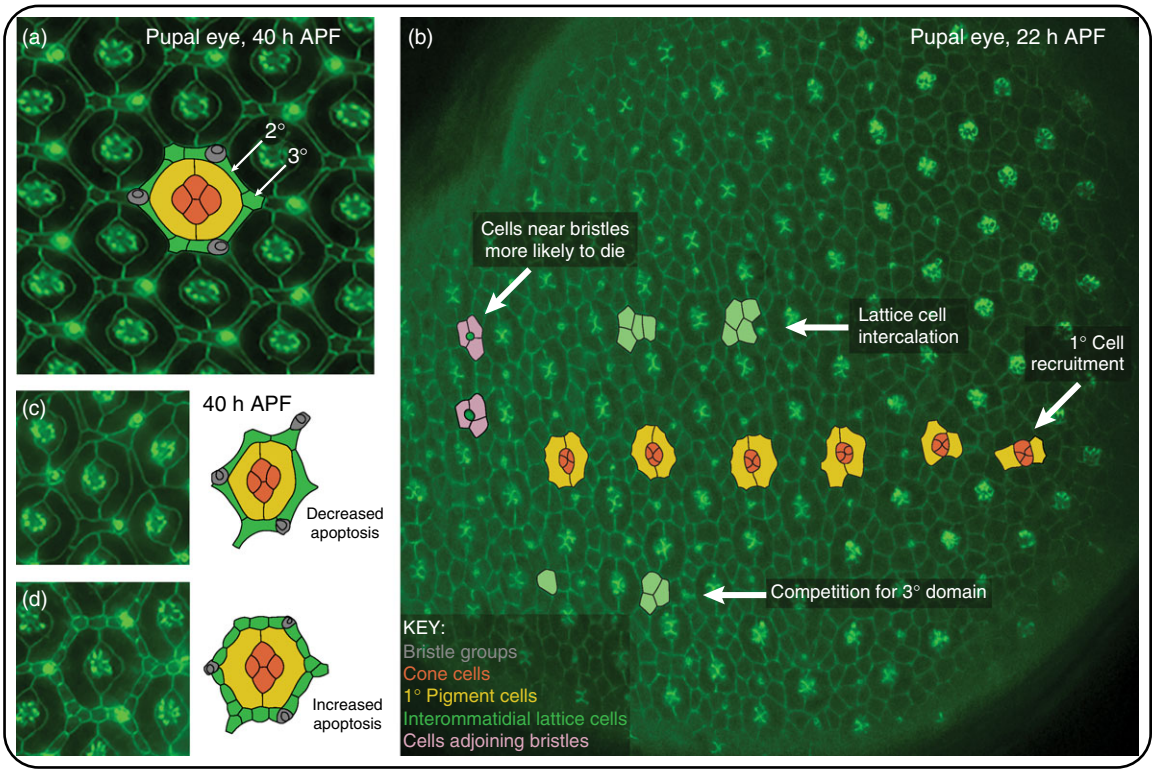

Figure 3 Pupal Development. (a) Small region of the eye at 40 h APF. The outlines of cells are visualised via detection of adherens junctions (in green).Source: From Johnson, RI. 2020. Adhesion and the Cytoskeleton in the Drosophila Pupal Eye. In Molecular Genetics of Axial Patterning, Growth and Disease in the Drosophila Eye. A. Singh and M. Kango-Singh, editors. Springer, Cham. 189–213 (b) The eye at 22 h APF, marked by a gradient of development so that step-wise changes in cell morphologies can be seen, as indicated. Cells have been colour-coded as per key.Source: Adapted from (Hellerman *et al.*, 2015). (c) Increased apoptosis of interommatidial cells or (d) decreased apoptosis still leads to mainly hexagonal ommatidia by 40 h APF.

vertices (**Figure 3a**; Cagan and Ready, 1989a); also see Further Reading. Since the 2° and 3° lattice cells become filled with pigment to optically isolate neighbouring ommatidia and provide mechanical support for each ommatidium, and since the 1° and cone cells generate and support the crystalline cone and corneal lens that focus light on each rhabdomere (**Figure 1d**), the correct organisation and architecture of these cells is essential for fly vision. How then does this ordered cell pattern emerge?

The shapes and organisation of cells in the eye ~20 h after the organism enters pupation (APF) reflect many mechanical processes that contribute to eye patterning (**Figure 3b**). Cells next to the anterior and posterior cones of each ommatidium are recruited as 1° cells and stretch to encircle the four cone cells. At about the same time, bristle groups emerge Intercalation and local movements reorganise cells into single rows around ommatidia. Cells then compete to inhabit and secure the 3° cell domains and apoptosis removes excess cells so that only six 2°s remain about each ommatidium. The defining shapes of the cone, 1°, 2° and 3° pigment cells are refined to generate the beautiful pattern observed by 40 h APF (**Figure 3a**).

A range of processes contribute to these morphological changes although few have been studied in detail. First, several classical signal transduction pathways contribute to cell fate specification. These include Notch signalling, activated in the two cells that become 1°s since the adjacent cone cells express *Delta* (**Figure 3b**) (Cagan and Ready, 1989b; Nagaraj and Banerjee, 2007). The cone cells, in turn, were specified a day earlier in the larval eye disc via a combination of Notch and DER activities and prolonged function of the Tramtrack (Ttk) transcription factor (Mavromatakis and Tomlinson, 2017). No signalling pathways have been associated with setting the 2° and 3° cell fates, which instead are the default fates adopted by cells remaining between ommatidia. Indeed the 2° and 3° cell types may be molecularly indistinguishable and their different morphologies simply due to a second factor: biophysical constraints imposed by the cells' positions. According to this idea, the final shape of 3°s is a function of forces received from three neighbouring ommatidia, whilst the 2°s simply expand to occupy the rectangular domain between two ommatidia with a 3° and bristle at each end. This model implies that 2° and 3° shapes are passive responses to forces received from surrounding cells, but we know at least two additional factors crucial for cell shape: the adhesive properties of each cell and organisation of the cytoskeleton (third and fourth factors, respectively).

Several classes of adhesion receptors are essential for correct eye patterning and mutations in their corresponding genes, or changes in their expression, disturb the arrangement and shape

of lattice cells, 1°s and/or cone cells. These include the orthologues of the Nephrin IgCAMs (Hbs, Rst, Sns and Kirre) that form heterophilic Hbs-Rst and Sns-Kirre complexes that accumulate at 1° cell : lattice cell adherens junctions, increasing the affinity of these different cell types for each other. This mechanism is described as 'preferential adhesion' and describes why lattice cells actively maximise the adherens junction interface with 1°s whilst minimising the junction between neighbouring lattice cells (Bao and Cagan, 2005; Bao *et al*., 2010). Accordingly, even when the number of lattice cells is severely reduced (for example, when the survival of lattice cells is genetically compromised) these cells still reach between ommatidia to separate them (**Figure 3c**). The Nephrin IgCAM complexes are also important earlier during patterning to secure lattice cells in place after intercalation, as revealed by live-imaging studies, and mutations in Nephrin IgCAM genes hence leave lattice cells grouped in multiple rows between the ommatidia (Larson *et al*., 2008). **See also**: **Immunoglobulin Superfamily and the Nervous System**

The classical cadherin E-Cadherin (E-cad) is essential for the integrity of the eye epithelium and removing E-cad or any other protein component of adherens junctions from the eye severely compromises viability of the tissue. **See also**: **Adherens Junctions**. However, since simply reducing or increasing E-cad does not greatly modify the final arrangement of cells, E-cad is not thought to have a prominent role in directing eye patterning. On the other hand, N-Cadherin, which is exclusively expressed in developing cone cells, contributes to their biophysical properties by increasing the strength of adhesion between them in comparison to adhesion between cones and neighbouring 1° cells. This results in the straight boundaries between neighbouring cones, the rounded shape of the cone cell : 1° cell interface that is characterised by lower adhesion, and the arrangement of the four cones in a conformation that minimises free energy which is similar to the way in which a group of four dish-soap bubbles arrange themselves (**Figure 3a**) (Hayashi and Carthew, 2004). However, the final arrangement of cone cells – with the dorsal and ventral cones in apposition and separating the anterior and posterior cones – is not the arrangement first established. Instead the anterior and posterior cones, which are recruited first, are initially in direct contact and the quartet then undergoes a classical T1-T2-T3 transition so that the dorsal and ventral cones touch (Bertet *et al*., 2004; Harris, 2018). What drives this transition has not been well studied in the eye, but targeted turnover and assembly of adherens junctions and changes in associated cytoskeletal structures are likely to be involved, as they are in T1-T2-T3 transitions in other epithelia.

The actin and myosin network (the fourth factor discussed here that contributes to eye patterning) is a crucial determinant of cell architecture. **See also**: **Actin and Actin Filaments**; **Myosins**. More accumulation of nonmuscle myosin II (Myo-II) is detected along curved cell interfaces in the pupal eye, including concave cone cell : 1° cell and 1° cell : lattice cell interfaces (Aigouy and Le Bivic, 2016; Chan *et al*., 2017). The idea here is that Myo-II activity introduces higher cortical tension and curvature at these membranes and there is some evidence that in cone cells N-cad has a direct role in preventing Myo-II accumulation at the straight cone cell : cone cell boundaries (Chan *et al*., 2017). Mechanisms that preferentially localise or activate Myo-II to establish the curved face of 1° cells are unclear but its importance is evident in mutant eyes where straight 1° cell : lattice cell interfaces are found: no accumulation of Myo-II is observed at these straight cell boundaries (DeAngelis *et al*., 2020).

Regulated changes in the actin cytoskeleton are also crucial for lattice cell intercalation. Here, it is the monomeric GTPase Arf6, which promotes Arp2/3 activity, that is thought to generate the pseudopodial-like projections that extend toward target 1°s as lattice cells intercalate (**Figure 3b**). The adaptor protein Cindr is considered key to restricting Arf6 activity to these projections. Cindr interacts with IgCAM complexes and would therefore be preferentially localised to lattice cell : 1° cell junctions where the IgCAMs accumulate. Since Cindr also binds Arf6-inactivators, Arf6 activity would be low at lattice : 1° cell junctions and higher elsewhere in the cell (Johnson *et al*., 2011). In addition, Cindr also promotes capping of F-actin which would stabilise the cytoskeleton at lattice: 1° cell junctions (Bruck *et al*., 2006; Tang and Brieher, 2012). Other mechanisms that contribute to lattice cell intercalation probably include polarised activity of Myo-II, but this has not yet been studied. Instead, Myo-II has been examined in lattice cells later, when they acquire their final stereotypical shapes and sizes (Del Signore *et al*., 2018). The lattice cell : lattice cell boundaries were observed to pulse in length, driven by alternating cycles of Myo-II and Arp2/3 activities, which extended and shorten the lengths of this cell-interface, respectively. This pulsing is suggested to allow lattice cells to sample different relative sizes and shapes until acquiring the most favourable arrangements.

A fifth factor that contributes to pupal eye morphogenesis is the removal of excess interommatidial cells via apoptosis (also termed programmed cell death) to leave only six 2°s and three 3°s about each ommatidium. Removal of an incorrect number of cells will compromise the final size and shape of individual lattice cells but, interestingly, unless apoptosis is severely altered, the hexagonal honeycomb lattice of the eye still mainly forms (DeAngelis *et al*., 2020; Larson *et al*., 2010). That is, if apoptosis is genetically enhanced so that too few cells occupy the interommatidial domain, they expand so that the honeycomb is still, for the most part, preserved (**Figure 3c**). Conversely, if apoptosis is inhibited so that there are too many lattice cells, these still mainly intercalate and pack together to form a near-perfect honeycomb lattice (**Figure 3d**). **See also**: **Apoptosis: Molecular Mechanisms**

Control of Apoptosis

Four core features indicate that the culling of excess interommatidial lattice cells is a highly regulated process. First, apoptosis occurs during a defined period and in two waves, the first from ~18 h to 24 h APF, and the second wave from ~27 h to 32 h APF, terminating as ecdysone levels decrease in the pupa. Second, with rare exceptions the correct number of lattice cells remain about each ommatidium, suggesting a cell-counting mechanism. Third, several signal transduction pathways function to promote or prevent lattice cell death, suggesting that spatial and/or temporal interactions between these pathways define the rate and amount of apoptosis. These death-inducing signals include Notch, Wingless and JNK signalling, whilst lattice cell survival is promoted

by DER signalling and activity of Yorkie, a transcription factor repressed by Hippo pathway activity. Fourth, the position of a cell within the interommatidial lattice influences whether it is more likely to live or die. Lattice cells in contact with more than one 1° are slightly less likely to die, consistent with the idea that survival signals (possibly activators of DER) emanate from the ommatidial core. Conversely, lattice cells close to bristle groups are more likely to undergo apoptosis (**Figure 3b**), an observation that leads to the idea of 'death zones' surrounding bristles although a specific apoptosis-inducing signal that emanates from bristles has not been identified and genetic removal of bristles does not perturb apoptosis. A comprehensive understanding of the relative amplitudes of Notch, Wingless, JNK, DER and Yki activities in lattice cells may one day allow us to unravel precisely how apoptosis is spatially regulated in the pupal eye.

Glossary

Chirality The asymmetry present in some structures or groups of cells; in this context, it refers to the asymmetric arrangement of photoreceptors on either side of the larval eye disc equator.

Morphogenetic furrow (MF) As cells arrest in a coordinated fashion in the mature larva, the constriction of their apical ends causes a downward movement of their nuclei, creating a groove in the neuroepithelium. The MF represents the point of cell fate initiation.

Ommatidia Each retina contains 700–750 of these unit eyes. Each consists of eight photoreceptor neurons plus six support cells; an additional 15 cells are shared with neighbouring ommatidia.

Orthologue One of the important advances in biology is the recognition that the molecules and molecular pathways important in development are highly conserved between species as distant as yeast, flies and humans. A protein or gene with similar sequence and structure to a protein or gene found in another species is said to be its 'orthologue'.

Rhabdomeres The light-gathering organs of *Drosophila* photoreceptors, they are the site of rhodopsin and phototransduction.

Translumenal processes Microtubule-based cell processes seen extending between the peripodial epithelium and the eye disc; they are thought to transport secreted ligands between the two tissues.

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