Developmental Biology 385 (2014) 168–178

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Review

Transcriptional regulation of tissue organization and cell morphogenesis: The fly retina as a case study

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ARTICLE INFO

Article history:
Received 17 May 2013
Received in revised form
27 September 2013
Accepted 29 September 2013
Available online 4 October 2013

Keywords:
Drosophila retina
Photoreceptor
Cell shape changes
Organogenesis
Myosin-II
Crumbs

ABSTRACT

Understanding how a functional organ can be produced from a small group of cells remains an outstanding question in cell and developmental biology. The developing compound eye of *Drosophila* has long been a model of choice for addressing this question by dissecting the cellular, genetic and molecular pathways that govern cell specification, differentiation, and multicellular patterning during organogenesis. In this review, the author focussed on cell and tissue morphogenesis during fly retinal development, including the regulated changes in cell shape and cell packing that ultimately determine the shape and architecture of the compound eye. In particular, the author reviewed recent studies that highlight the prominent roles of transcriptional and hormonal controls that orchestrate the cell shape changes, cell–cell junction remodeling and polarized membrane growth that underlie photoreceptor morphogenesis and retinal patterning.

Introduction

During animal development, specific genetic and molecular programs orchestrate the differentiation of the various cell types that assemble into organs. Cell differentiation can include the acquisition of specific morphological features that in turn enables the cell to perform specialized functions. Epithelial cells and neurons are two striking examples of cell types whose differentiation is based on the acquisition of a highly specialized polarized morphology. During development, epithelial cells acquire an apico-basal axis of polarity and discrete cell–cell contacts required for organogenesis, whereas neurons have to elaborate dendrites and an axon to participate in neural circuit formation. In both cases, the final shape and function of the cell emerges over developmental time from a combination of genetic and molecular programs.

Epithelial cells have the ability to adhere to each other to form coherent cellular sheets. This feature is crucial to generate organs such as the gut, kidney and lung, in which epithelia separate an internal luminal space from the outside world. The production of such epithelial structures in a reproducible manner requires stringent regulatory mechanisms to control the shape, packing, and positioning of cells within epithelia. Regulated epithelial cell shape changes and positioning depend on regulated remodeling of cell–cell contacts.

Invertebrate epithelial cells have two main junctional domains in their plasma membrane: the apical *zonula adherens* and the lateral paranodal-like septate junction (Fig. 1A). In the past few years, there has been especially intense study of apical junction remodeling during epithelial patterning. The *zonula adherens* not only mediates cell–cell adhesion; it also generates intracellular signals in response to mechanical tension within the epithelium (reviewed in (Guillot and Lecuit, 2013)). This is in part due to the ability of E-cadherin located in the junction to associate with the F-actin cytoskeleton and the motor protein non-muscle Myosin-II (Fig. 1B). Myosin-II is able to promote the formation of actin-Myosin-II foci and meshworks, which are associated with the *zonula adherens* or discrete *adherens junction* domains in developing epithelia (Blankenship et al., 2006a; Levayer and Lecuit, 2013; Martin et al., 2009; Rauzi et al., 2010; Robertson et al., 2012) (Fig. 1B). Myosin-II can generate contractile force to increase tension in the cell cortex (Fernandez-Gonzalez et al., 2009; Rauzi et al., 2008) and can also promote the endocytosis of *adherens junction* proteins, including E-cadherin (Levayer et al., 2011) (Fig. 1C). In developing epithelia, the actin-Myosin-II cortex can promote the suppression of *adherens junctions* between cells but also the creation of new *adherens junctions* (Bardet et al., 2013; Bertet et al., 2004; Blankenship et al., 2006a) (Fig. 1C). Thus, Myosin-II is a major regulator of cell shape, adhesion and packing within developing epithelia and is therefore at the core of much of organogenesis.

Organogenesis requires a high degree of coordination between epithelial cells to produce the folds or tubular structures within an organ tissue. It also requires temporal coordination of cell

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http://dx.doi.org/10.1016/j.ydbio.2013.09.031

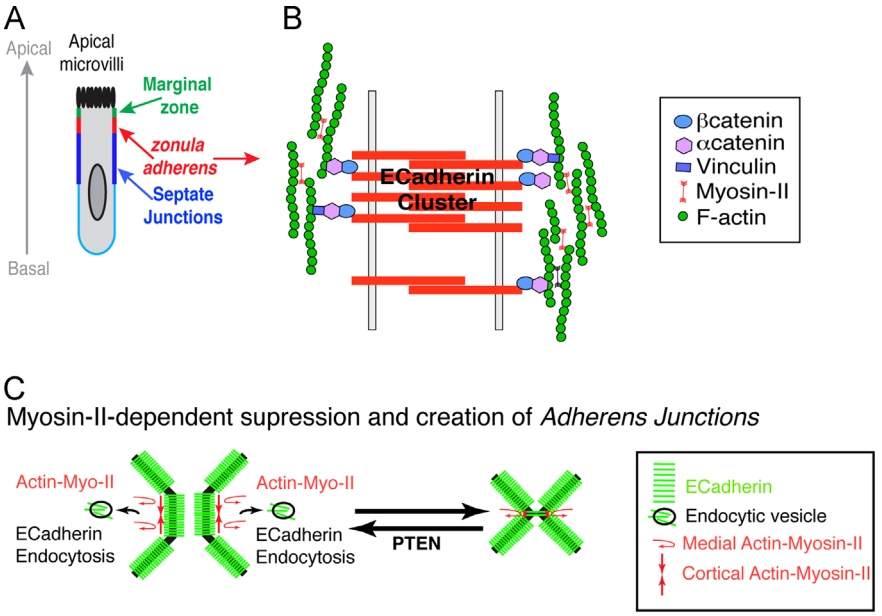

Fig. 1. *adherens junction* remodeling during organogenesis. (A) Depiction of an invertebrate epithelial cell. (B) Simplified schematic of the epithelial cell *zonula adherens*. (C) Depiction of the activity of Myosin-II (Medial meshwork and cortical; Red arrows) during *adherens junction* suppression and elongation. The lipid phosphatase PTEN is required for Myosin-II-dependent *adherens junction* elongation (Bardet et al., 2013).

differentiation. This coordination is governed by both long- and short-range signaling, which often operate in a tissue specific manner. To gain a fully integrated view of organogenesis remains a challenging task. The developing compound eye of *Drosophila melanogaster* is a particularly well-suited *in vivo* model system for attacking the problem. It consists of approximately 750 basic units called ommatidia (Fig. 2). Each ommatidium consists of eight photoreceptor neurons, four cone cells, two primary pigment cells, six shared secondary pigment cells, three shared tertiary pigment cells, and three shared mechanosensory bristles (Tomlinson, 1985a; Waddington and Perry, 1960) (Figs. 2A D). The compound eye originates from an unpatterned, pseudo-stratified, columnar epithelium, where all of the different cell types are induced from a common pool of equipotent epithelial precursors (Lawrence and Green, 1979; Ready et al., 1976).

Photoreceptor differentiation is initiated by the expression of the pro-neuronal basic helix-loop-helix transcription factor Atonal (Greenwood and Struhl, 1999; Jarman et al., 1994). The intracellular Ras/Raf/MAPK signaling pathway, acting reiteratively through the ETS transcription factor Pointed (Freeman, 1996; Xu and Rubin, 1993), is a major inducer of photoreceptor neurogenesis. This pathway is also subsequently required to generate the full complement of accessory cells (i.e., cone and pigment cells) that complete the ommatidium (Freeman, 1996). These steps of cell-fate commitment and early cell differentiation begin during the imaginal disc stage of eye development (reviewed in (Treisman, 2012)) and are completed during the first 10% of the pupal stage of fly development (where 0% is a newly formed white pupa, and 100% corresponds to the hatching of the adult fly from the pupal case) (Fig. 3).

Following this early phase of retinal cell differentiation, pupal eye development proceeds by an orderly sequence of morphogenetic events. As the ommatidial lattice is established, two consecutive transformations occur in the photoreceptors. First, at ~30% after puparium formation, the cells begin to establish their precise pattern of axon projections to the neuropil of the fly optic lobe (Fig. 3B). This process is genetically hard-wired and is referred to as *neural superposition* (Clandinin and Zipursky, 2000; Meinertzhagen and Hanson, 1993). Second, photoreceptors begin to undergo a striking remodeling of their plasma membrane to form a new *zonula adherens* domain, as well as the subapical stalk membrane (Fig. 3C E). This transformation begins at ~37% after puparium formation and leads to a 90 degree rotation of the cell's apico-basal axis (Fig. 3C–E). This step of polarity remodeling is required to align the future light-gathering organelle, the rhabdomere, with respect to the lens-to-brain axis of the retina. Finally, after the ommatidial lattice has been established, there is a further transformation at ~78% after puparium formation, when the photoreceptors express the visual pigment Rhodopsin (Earl and Britt, 2006; Kumar and Ready, 1995). This event culminates in the terminal differentiation of the rhabdomere (Fig. 3F), which includes the elaboration of a meshwork of F-actin called the rhabdomere terminal web that is required to support rhabdomere morphogenesis and maintenance (Chang and Ready, 2000; Kumar and Ready, 1995; Pinal and Pichaud, 2011).

Apical constriction initiates retinal patterning

Retinal differentiation begins in the fly larva at the morphogenetic furrow (Fig. 3A), a morphogenetic wave that sweeps across the retina from the posterior to the anterior margin of the epithelium (Ready et al., 1976) (Fig. 4A–B). Cells in the morphogenetic furrow undergo apical constriction, and, in its wake, one column of ommatidia is generated about every 2 h. Once the epithelium is fully patterned, a total of approximately 32 columns will have been laid down with exquisite reproducibility (Cagan and Ready, 1989; Campos-Ortega and Hofbauer, 1977).

Epithelial cell apical constriction occurs commonly when a developing epithelium invaginates to form a fold. The morphogenetic furrow is an example of transient apical constriction that does not lead to a permanent fold of the epithelium but instead promotes the onset of tissue patterning. Among the effector proteins that promote epithelial cell apical constriction are the small GTPase RhoA, its effector kinase ROCK, as well as Myosin-II and F-actin (for a recent review, see (Sawyer et al., 2010)) (Fig. 4C). Other factors that are also critical for promoting apical constriction in the morphogenetic furrow are the kinase DRak (Robertson et al., 2012), the F-actin polymerization factor Diaphanous and an increase in apical microtubule concentration (Corrigall et al., 2007) (Fig. 4C). This role for microtubule accumulation during apical constriction resembles that in neural tube closure in *Xenopus*, where parallel microtubule arrays accumulate at

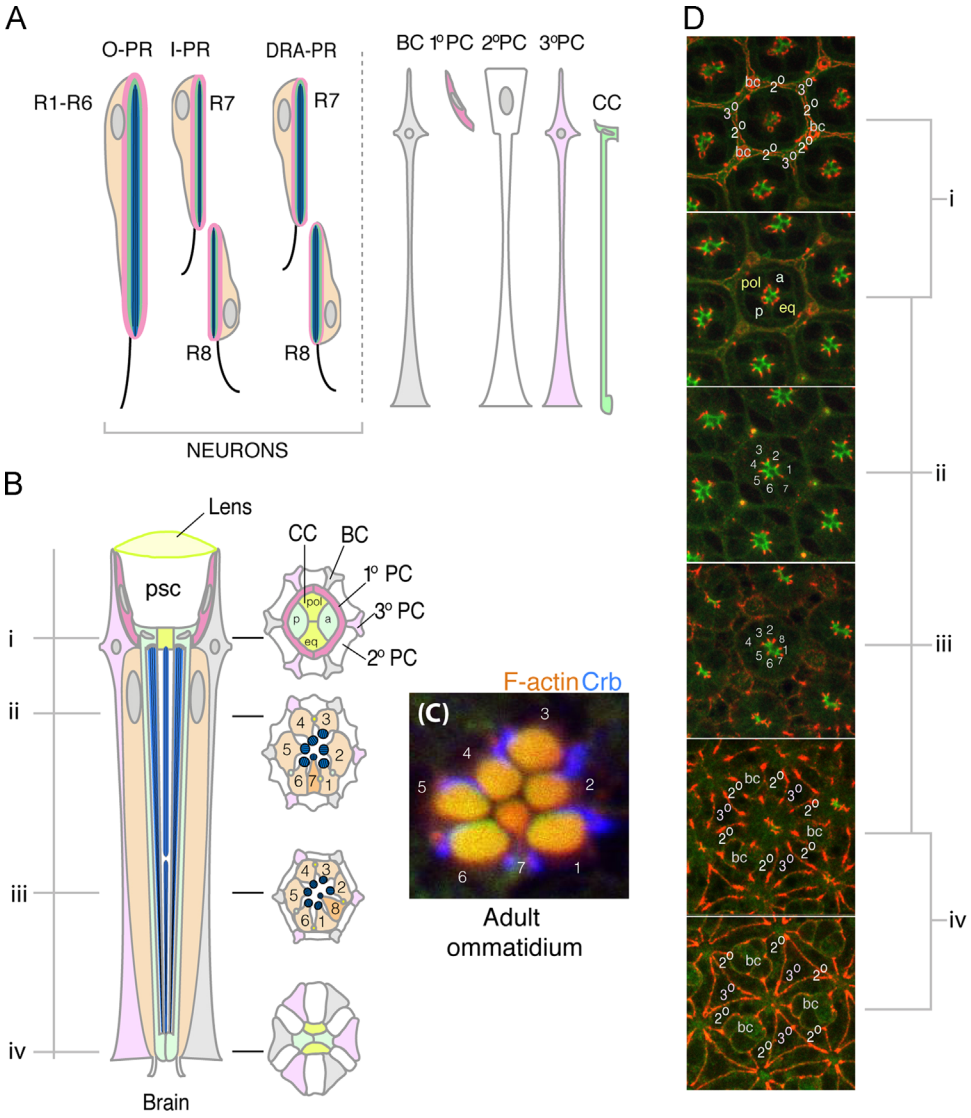

Fig. 2. Cellular diversity in the compound eye of *Drosophila*. (A) Depiction of the various cell types present in the adult retina. O-PR stands for outer photoreceptors; I-PR for inner (R7 and R8); DRA PR for dorsal rim area photoreceptors (R7 and R8); BC stands for bristle cell; 1° PC for primary pigment cell; 2° PC for secondary pigment cell; 3° PC for tertiary pigment cell; CC for cone cell. (B) An ommatidium with the full complement of cells (adapted from (Wolff and Ready, 1993). PSC stands for pseudocone cavity. (i) Cross section through the ommatidium at the level of the cone cells is depicted. The midline of the eye is called the equator. Accordingly, cells can be positioned with respect to both the polar–equatorial and antero-posterior axes. In the cross section, the equatorial (eq) and polar (pol) cone cells are yellow while the anterior (a) and posterior (p) cone cells are green. (ii) Cross section at the R7 level. (iii) Cross section at the R8 level. (iv) Cross section at the level of the cone cell feet. (C) Confocal image of one adult ommatidium stained for a marker of the photoreceptor stalk membrane, Crumbs (Crb, Blue) and F-actin (orange). (D) Confocal sections of one pupal ommatidium (~40% after puparium formation) from the lens (top panel) to brain (bottom panel) axis. Crumbs marks the stalk membranes (green) and Armadillo marks the *adherens junctions* (red). (i) Cross section through the ommatidium at the level of the cone and pigment cells. (ii) Cross section at the R7 level. (iii) Cross section at the R8 level. (iv) Cross section at the level of the cone and pigment cell feet.

the apical pole of the invaginating cells (Lee et al., 2007). Similarly, ectopic overexpression of Diaphanous in the fly epidermis is sufficient to promote cell constriction (Homem and Peifer, 2008). Interestingly, overexpression of an activated form of Myosin-II is sufficient to promote ectopic constriction in the anterior compartment of the developing retina (Corrigall et al., 2007; Escudero et al., 2007). Mechanisms and pathways promoting epithelial cell apical constriction might therefore govern Myosin-II and Diaphanous activation or promote the accumulation of the proteins at the apical pole of the cell, including at the *zonula adherens*. Among the possible local effectors of Myosin-II is RhoGEF2, a guanine nucleotide exchange factor and a known activator of the RhoA-ROCK pathway (Barrett et al., 1997; Grosshans et al., 2005; Hacker and Perrimon, 1998). RhoGEF2 promotes tissue invagination in fly ventral mesoderm (Barrett et al., 1997) and groove formation during embryonic segmentation (Mulinari et al., 2008). However, it is dispensable for the formation of the morphogenetic furrow (Corrigall et al., 2007; Escudero et al., 2007), suggesting that at least one other RhoGEF can regulate this process (Fig. 4C). Although apical constriction correlates with epithelial invagination, the relationship between these two intertwined features of tissue patterning is not fully understood. In the morphogenetic furrow, the two events are uncoupled, and apical constriction is only transient, which might explain some of the differences observed in the effectors involved in apical constriction during furrow formation compared to apical constriction during epithelial invagination in other developmental contexts.

Recent studies have shown that Hedgehog and Decapentaplegic signaling pathways govern apical constriction during morphogenetic furrow formation (Corrigall et al., 2007; Escudero et al., 2007). The alleviation of transcriptional repression by Cubitus interruptus (CiR) is sufficient to trigger this apical constriction provided that the transcription factor Mothers-Against-Dpp (Mad) is also activated

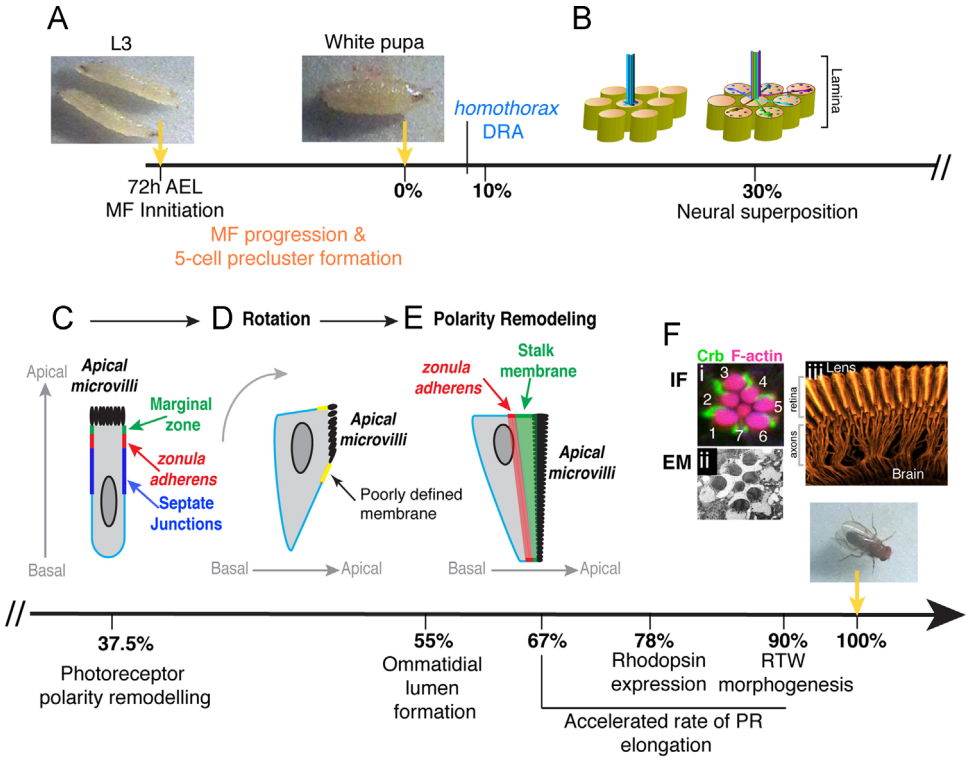

Fig. 3. Developmental time line of the fly retina. Representation of the third instar larvae and pupal retina developmental timeline based on (Cagan and Ready, 1989; Earl and Britt, 2006; Fichelson et al., 2012; Longley and Ready, 1995; Mikeladze-Dvali et al., 2005; Ready et al., 1976; Robertson et al., 2012; Tomlinson, 1985b; Wernet et al., 2003). (A) Developmental times are expressed in hours after egg laying (AEL) up to the white pupal stage that corresponds to 0%. All subsequent pupal times are expressed in percentages, with the eclosion of the animal at 100% after puparium formation (apf). (B) Representation of the genetically hard-wired synaptic pattern that is established at ~30% after puparium formation in the first neuropil (Lamina) of the optic lobe. The neuropils are represented as green cylinders. On the left, one bundle of axons originating from one ommatidium is represented in blue, before the onset of neural superposition. On the right, the same bundle is shown after neural superposition has taken place. In this drawing the axons corresponding to R1 to R6 have been color-coded and are shown innervating their respective neural cartridge according to the principle of neural superposition. (C–E) Photoreceptor polarity remodeling consists of a 90 degree rotation of the cell's apico-basal axis and the morphogenesis of a new *zonula adherens* (red) and a stalk membrane (Green). (F) Phase of increased photoreceptor elongation and rhodopsin expression. (i) Confocal section of an adult ommatidium stained for Crumbs (Crb, green) at the stalk membrane and F-actin (pink) for the rhabdomere. (ii) Electron micrograph of a fully differentiated ommatidium. (iii) Projection of confocal sections of an adult retina stained for the photoreceptor specific epitope 24B10. Abbreviations: MF=morphogenetic furrow, DRA=dorsal rim area, RTW=rhabdomere terminal web.

downstream of the Decapentaplegic signaling pathway (Fig. 4D). Together with previous reports showing that Mad is required to maintain apical microtubules in the developing eye disc (Shen and Dahmann, 2005), these studies highlight an important role for both the Hedgehog and Decapentaplegic signaling pathways in the transcriptional regulation of epithelial cell morphology in *Drosophila*. Further support for this view comes from the finding that expression of activated-Ci (CiA) in the embryonic dorsal epidermis is sufficient to induce apical cell constriction during groove formation (Mulinari and Hacker, 2009). Whether or not this function is conserved in vertebrates – for example, during neural tube closure, where sonic hedgehog plays a critical role – remains to be tested. More work will also be required to identify the genes that are regulated by Ci and Mad to promote apical constriction in the morphogenetic furrow.

Specific steps of cell intercalation in patterning the ommatidium

Ommatidial patterning can be first detected at the posterior margin of the morphogenetic furrow and correlates with the formation of a supra cellular cable of actin–Myosin-II that delineates the posterior margin of the morphogenetic furrow (Fig. 5A). This cable drives the early steps of ommatidia patterning (Escudero et al., 2007 Robertson et al., 2012) but it is not clear how it is generated. One possibility is that the combined action of apical constriction and *atonal* function that leads to a relative increase in the density of E-cadherin at the *zonula adherens* in the morphogenetic furrow (Brown et al., 2006; Corrigall et al., 2007; Robertson et al., 2012), contributes to the formation of such cable (Fig. 5A). In epithelia the apposition of E-cadherin-high and E-cadherin-low cells promotes the formation of an acto-Myosin-II cable at the interface (Batlle and Wilkinson, 2012; Wei et al., 2005). In addition, in the developing retina, the cells immediately flanking the posterior margin of the morphogenetic furrow relax from their apically constricted state, which might also contribute in promoting the formation of the acto-Myosin-II cable at the posterior margin of the furrow, as the cells might react to the corresponding tissue-stretching force (For a review on this topic see (Fernandez-Gonzalez and Zallen, 2009)) (Fig. 5B). In the retina, planar polarization of ROCK at this interface stimulates Myosin-II activation (Robertson et al., 2012)—a situation similar to that found in the fly embryo during germband extension (Simoes Sde et al., 2010). Regardless of the signals that stimulate the formation of acto-myosin-II cables in epithelia, the presence of such a supra-cellular structure correlates with cell alignment on one or both sides of the cable (Brodu and Casanova, 2006; Corrigall et al., 2007; Escudero et al., 2007; Robertson et al., 2012; Simone and DiNardo, 2010; Wei et al., 2005). This is likely because Myosin-II increases cortical tension at the cells' *adherens junctions* at the shared interface (Landsberg et al., 2009; Monier et al., 2010). Direct measurements and mechanical perturbation of this tension in the morphogenetic furrow will be required to test for a direct link between E-cadherin concentration at the *adherens junctions*, cortical tension and local cell alignment.

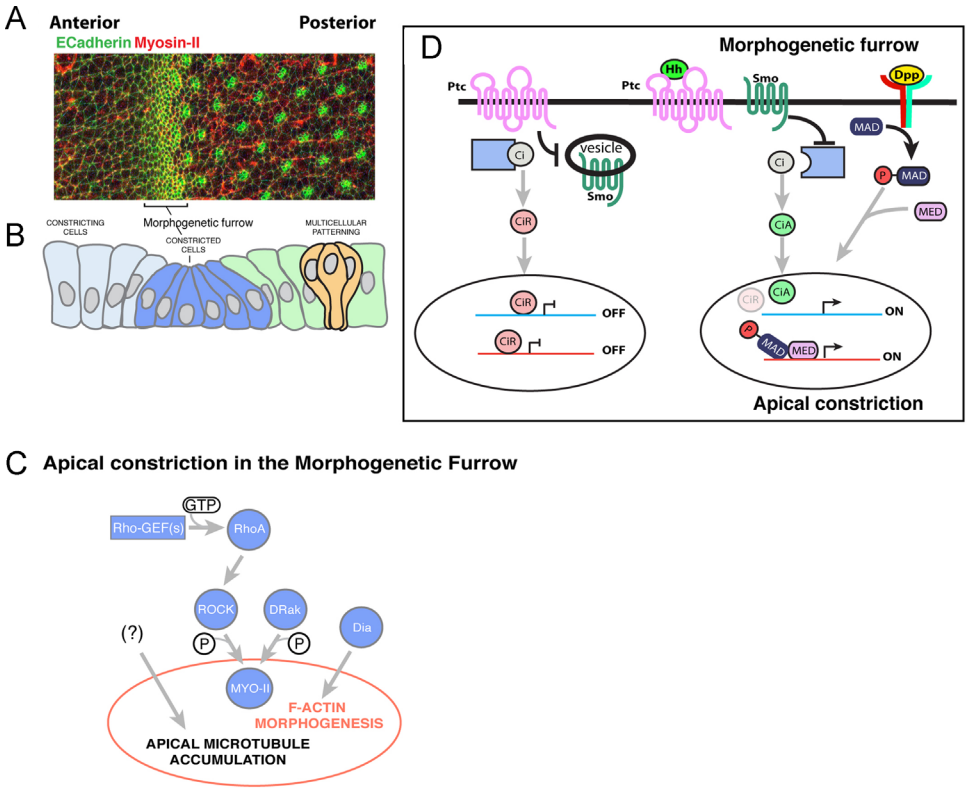

Fig. 4. Cubitus Interruptus and Mad regulate epithelial cell apical constriction. (A) Confocal image of a developing larval retina stained for the *zonula adherens* (Armadillo, green) and Myosin-II (red). (B) The region around the morphogenetic furrow, represented as a cross section through the developing epithelium. In the anterior compartment of the developing retina, cells begin to constrict (Cells in light blue). Apical constriction reaches it maximum in the morphogenetic furrow (Bottle-shape cells in dark blue). In the wake of the morphogenetic furrow, some cells relax their apical surfaces (green) while the newly differentiating photoreceptor neurons remain constricted and present apical nuclei (beige) (Ready et al., 1976; Tomlinson and Ready, 1987). These cells also undergo axonogenesis. (C) A simplified diagram of the gene-network that produces epithelial cell apical constriction in the developing retina. RhoA is upstream of Rho-Kinase (ROCK) and this kinase activates non-muscle Myosin-II (Myo-II). DRak acts redundantly with ROCK during this process (Robertson et al., 2012). The formin Diaphanous (Dia) regulates F-actin and is required for this cell response (Corrigall et al., 2007). The converging output of the Hh and Dpp-signaling pathways is to promote apical microtubule stabilization (D) The Hedgehog- (Hh) and Decapentaplegic (Dpp)-signaling pathways, which converge to govern apical constriction in the developing retina. For the Hh-signaling pathway, Hh stands for Hedgehog; Ptc for patched; Smo for smoothed; Ci for Cubitus interruptus; CiR for Ci Repressor; CiA for Ci Activator. For the Dpp pathway: Dpp stands for decapentaplegic; Mad for Mothers-Against-DPP; Med for Medea. By default the Hh-signaling pathway is off. Upon binding of Hh to Ptc, CiR-mediated inhibition of transcription is relieved and CiA-dependent transcriptional activation is enabled. In the scenario depicted here, apical constriction results from a combination of alleviating CiR-dependent transcriptional repression and activating gene transcription downstream of Dpp.

Following cell alignment, ommatidial morphogenesis consists of a stereotyped process of cell rearrangements, which is coupled to neurogenesis. The process was recently examined in detail using live retinal preparations (Chu et al., 2012; Escudero et al., 2007; Robertson et al., 2012). It is mainly driven by ROCK, Myosin-II and Bazooka (Fig. 1C). The evenly spaced lines of cells at the posterior margin of the morphogenetic furrow resolve into a series of multicellular structures, each resembling a closing cellular arc, which then forms a seven-to-six cell rosette. The rosette configuration then resolves into a five-cell pre-cluster, which contains the founder R8 photoreceptor, as well as the newly specified R2, R5, R3 and R4. The two remaining outer photoreceptors R1 and R6, as well as the inner photoreceptor R7, are subsequently recruited to the ommatidium (Wolff and Ready, 1993). During this process, the eight photoreceptors are recruited and occupy defined positions within the ommatidium. Cell rearrangement during ommatidial morphogenesis requires *adherens junction* suppression or elongation (Fig. 1C), both of which depend on Pointed-dependent transcription downstream of EGFR signaling (Fig. 5A and C) (Brown et al., 2006; Robertson et al., 2012). As EGFR also promotes neurogenesis within the developing ommatidium (Freeman, 1996), both the process of *adherens junction* remodeling and photoreceptor differentiation are linked. Because EGFR promotes both *adherens junction* suppression or elongation within the ommatidium, other factors must operate to determine which effect occurs, acting either downstream of the neurogenesis program, in parallel to it, or downstream of yet another signaling pathway.

Six-cell rosettes have been previously observed and characterized during germ-band extension in the fly embryo, a process that drives the elongation of the ectoderm along the antero-posterior axis. In that system, multicellular rosettes provide an efficient way to drive cell intercalation during the extension process (Blankenship et al., 2006b). In the developing retina, it is possible that the rosettes contribute to regulating the spacing between the nascent ommatidia. They could also be required to regulate some form of local cell–cell signaling. For instance, in the seven-to-six-cell rosette configuration, one or two cells are placed between the presumptive R3 and R4 photoreceptors (Fig. 5A). It is possible that this is a favorable transient configuration for these two cells, as they must interpret a positional cue within the plane of the epithelium. This positional cue comes in the form of a gradient of Frizzled activity, which is set up along the equatorial-to-polar axis of the retina. In this system, the equatorial R3 cell experiences a higher amount of Frizzled activity than the more polar R4 cell (Singh and Mlodzik, 2012). Frizzled activation in R3 results in the transcriptional activation of the gene encoding the Notch-ligand Delta in this cell, which leads to the activation of the Notch-signaling pathway in the R4 cell when it comes into contact with R3 in the five-cell precluster (Singh and Mlodzik, 2012). This differential activities of Frizzled and Notch in R3 and R4 govern the differentiation of these two cells and, together with the EGFR-signaling pathway

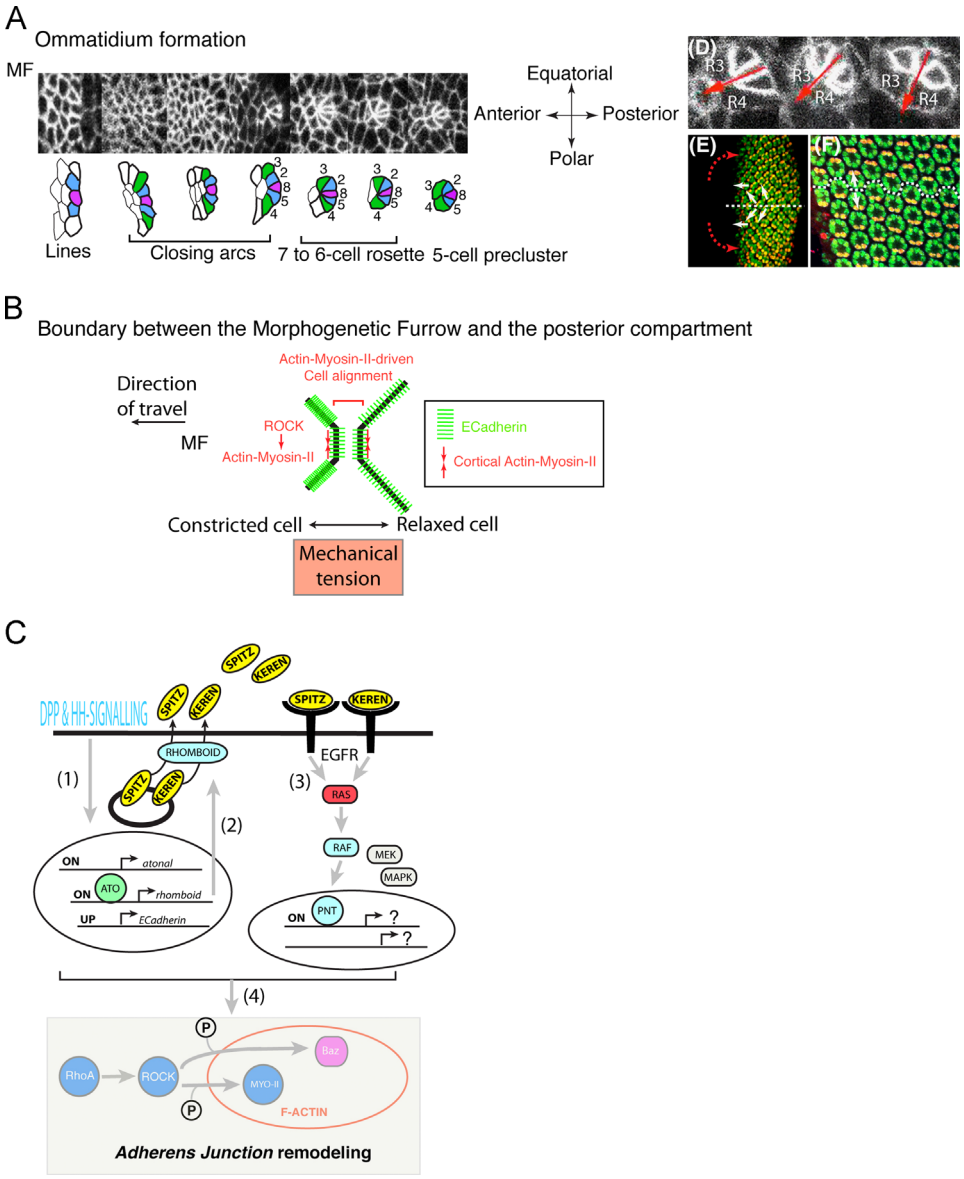

Fig. 5. *Adherens Junction* remodeling during ommatidia morphogenesis. (A) Series of *adherens junction* remodeling steps leading to the 5-cell precluster that contains the inner photoreceptor R8, as well as the outer photoreceptors R2/R5 and R3/R4. MF stands for morphogenetic furrow. (B) Tentative model of the mechanisms that lead to the formation of supracellular acto-myosin-II cable at the posterior margin of the morphogenetic furrow. Mechanical tension is imposed at the interface between the cells of the posterior compartment that are relaxing from their constricted state and the cells of the morphogenetic furrow that are constricted. This contributes to the commencement of an acto-myosin-II response that translates into a supra cellular cable for review see (Kasza and Zallen, 2010). The corresponding cortical tension could in turn contribute to promoting cell alignment at the posterior margin of the morphogenetic furrow. (C) The combination of Atonal-dependent cell alignment in the wake of the MF followed by EGFR-dependent *adherens junction* (*AJ*) remodeling to promote arc closing and the formation of the 5-cell precluster is depicted (Robertson et al., 2012). Atonal (Ato) is induced in the cells in the morphogenetic furrow and activates the transcription of both the intra-membrane protease Rhomboid and Rhomboid-3 (Baonza et al., 2001; Wasserman et al., 2000). In the retina, Rhomboid/Rhomboid-3 processes the EGFR ligands Spitz and Keren (Brown et al., 2007; Urban et al., 2001). These can then be secreted to activate EGFR signaling in the neighboring posterior cells. The RAS/MAPK pathway is activated downstream of EGFR and results in the transcriptional regulation of genes *via* Pointed (Pnt) (O'Neill et al., 1994). At the posterior margin of the morphogenetic furrow, Atonal upregulates the transcription of ECadherin (Brown et al., 2006). Both Atonal and the EGFR signaling pathway orchestrate *adherens junction* remodeling events *via* the RhoA, ROCK, Drak, Myo II pathway. Baz expression becomes clearly planar polarized and is required to promote *adherens junction* remodeling during ommatidia morphogenesis (Robertson et al., 2012). (D) Images extracted from a time-lapse recording to document the process of ommatidial rotation within the plane of the epithelium. The cells *adherens junctions* are labeled with ECadherin::GFP and the axis of symmetry the ommatidium is indicated with the red arrow. The time axis is from left to right. (E) Eye imaginal disc labeled for newly differentiating photoreceptors (Elav, green) as well as the R3/R4 (and to a lesser extend R1/R6) pair (Seven-up, Svp, red). This allows visualization of ommatidia rotation in a fixed preparation. The midline of the disc is indicated by a dashed line. White arrows indicate the axes of symmetry of several ommatidia. The red dashed arrows indicate the direction of ommatidia rotation with respect to the midline. (F) Shows a retina at a time point when all ommatidia have completed their rotation within the plane of the epithelium. White arrows indicate the axes of symmetry of two ommatidia and their polarity with respect to the midline of the eye (dashed white line).

(Brown and Freeman, 2003; Gaengel and Mlodzik, 2003; Strutt and Strutt, 2003), orchestrate the rotation of each ommatidium within the plane of the epithelium (Fig. 5D–E). Ommatidial rotation relies on *Adherens Junction* remodeling and this process is transcriptionally regulated downstream of Frizzled, Notch and EGFR (Weber et al., 2008). However, the connection between the transcriptional programs downstream of the Frizzled, Notch and EGFR pathways and *adherens junction* remodeling during ommatidial morphogenesis is not fully understood.

EGFR signaling also promotes *adherens junction* remodeling in the developing fly trachea (Brodu and Casanova, 2006) and is required for *adherens junction* remodeling and cell alignment

across cell boundaries in the embryonic denticle field (Simone and DiNardo, 2010). Thus, EGFR signaling is able to regulate specific *adherens junction* remodeling events in different developmental contexts. An important challenge in the future will be to link the EGFR pathway to the activity of the main effectors of *adherens junction* remodeling. In the retina, these includes ROCK, Myosin-II, the *zonula adherens* proteins Bazooka and E-cadherin, the F-actin effector Cofilin (Chu et al., 2012; Robertson et al., 2012), and the small GTPase Rap1 and its F-actin binding partner Canoe/AF6 (Gaengel and Mlodzik, 2003; Mirkovic and Mlodzik, 2006; O'Keefe et al., 2009). The developing retina is an ideal model system to take up this challenge, as it is relatively simple to manipulate experimentally. As EGFR activity has been associated with several types of epithelial cell motility, including in human carcinoma cells (Ciardiello and Tortora, 2008), the knowledge gained in the fly eye might once again prove relevant for medicine.

The polarity factor crumbs drives polarity remodeling

At the end of the third instar larval stage, the animal begins puparium formation, a developmental phase that will last approximately 160 h at 20 °C (Fig. 3). Within the developing retina, photoreceptors undergo a series of striking morphological changes. In particular, a phase of polarity remodeling is initiated at approximately 37% after puparium formation (Fig. 3C–E).

Epithelial cell polarity remodeling relies on the interplay between the apical factors Cdc42-Par6-DaPKC, Crumbs, Stardust and PATJ, as well as the *zonula adherens*-associated proteins Bazooka and E-cadherin (for recent review see St. Johnston and Ahringer. 2010) (Fig. 6A). In the photoreceptor, phosphorylation of Bazooka by DaPKC promotes the reorganization of the plasma membrane into a distinct stalk membrane and the *zonula adherens* (Fig. 6A–B). This partitioning also depends on the accumulation of Crumbs at the nascent stalk membrane, a process that is regulated by the small GTPase Cdc42, which functions as part of the apical aPKC-Par6 complex (Walther and Pichaud, 2010).

While we are beginning to understand the molecular pathway that drives photoreceptor polarity remodeling, it is still not clear what initiates the process. It is plausible that the upregulation of one or several of the polarity factors mentioned above might play a part. For example, the amount of Crumbs protein at the photoreceptor apical membrane is relatively low just before the onset of remodeling and increases dramatically during the remodeling process (Fig. 6B) (Walther and Pichaud, 2010). In addition, Crumbs is not expressed at the same levels (or at the same time) in the various developing outer pupal photoreceptors (Liu et al., 2009). This observation suggests that Crumbs expression might be upregulated (transcriptionally or post-transcriptionally), allowing apical membrane remodeling in the developing photoreceptor. This suggestion is supported by several studies indicating that *crumbs* can be transcriptionally regulated both *in vivo* and in epithelial cell culture (Campbell et al., 2011; Herranz et al., 2006; Lovegrove et al., 2006; Whiteman et al., 2008).

Collectively, these studies support the notion that, in the pupal photoreceptor, an upregulation of *crumbs* expression might trigger the remodeling of this cell. One possibility is that Crumbs protein could be upregulated by decreasing its rate of proteolytic degradation or endocytosis.

Transcriptional regulation of photoreceptor maturation

The unfolding of regulatory gene networks is likely to be one of the main ways that cells use to keep track of time as they step through their developmental programs. There is strong evidence for specific transcriptional pathways that operate during pupal photoreceptor morphogenesis but are not required for earlier steps of differentiation. A better understanding of these pathways and their temporal execution should help us to understand how these cells differentiate on a predictable schedule.

The *spalt* locus encodes transcriptional regulators involved in pupal photoreceptor morphogenesis. The locus encodes the conserved Spalt-major and Spalt-related C2H2 Zn-finger transcription factors, which are specifically required in the pupal R8 photoreceptor to prevent this cell from adopting an outer photoreceptor (R1–6) morphology (Domingos et al., 2004; Mollereau et al., 2001) (Fig. 2A and Fig. 7B). The difference in morphology between R1–R6 and the inner photoreceptors R7 and R8 is a striking example of an emergent phenotype that is acquired during terminal cell differentiation and for which much remains to be understood. The R8 and R7 inner photoreceptors are miniature versions of the outer photoreceptors, in that their length and apical diameter are approximately half that of R1–6 photoreceptors (Fig. 2A).

Whereas Spalt-major and Spalt-related are thought to act primarily as transcriptional repressors (de Celis and Barrio, 2009), in the pupal R8 photoreceptor, they might also function to promote the expression of the proneuronal gene *senseless* (Domingos et al., 2004; Xie et al., 2007), a gene that is in turn required to regulate rhodopsin expression and axon targeting in this subtype of photoreceptors

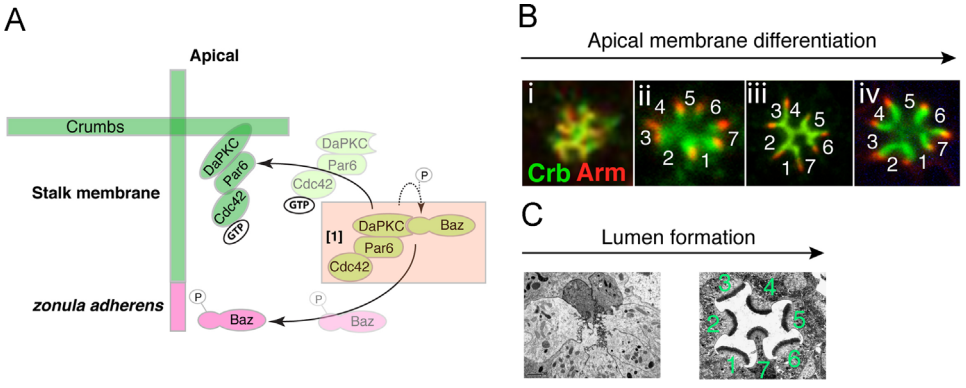

Fig. 6. Polarity remodeling in the pupal photoreceptor. (A) Bazooka (Baz) transiently associates with the Par6-DaPKC kinase (boxed in light red [1]). Bazooka is phosphorylated by DaPKC at Serine 980 and the corresponding enzymatic product (i.e. S980-P-Baz) is released from the catalytic pocket of DaPKC. The Cdc42-Par6-DaPKC complex is displaced toward the stalk membrane where it is captured by Crumbs (Crb). In this model, the release of S980-P-Baz from DaPKC is due to Crb, itself a DaPKC substrate, outcompeting S980-P-Baz for binding DaPKC (Morais-de-Sa et al., 2010). In addition, Bazooka phosphorylation at S980 releases the apical factor Stardust that can also bind to Crumbs (Krahn et al., 2010). (B) Photoreceptor membrane differentiation visualized by the stalk membrane marker Crumbs (Crb, Green) and the *zonula adherens* marker Armadillo, (Arm, Red). The arrow represents the time axis with (i: 30%, ii: 37%, iii: 45%, iv: 55% after puparium formation). (C) Electron micrograph of one ommatidium before (left panel) and after (right panel) lumen formation.

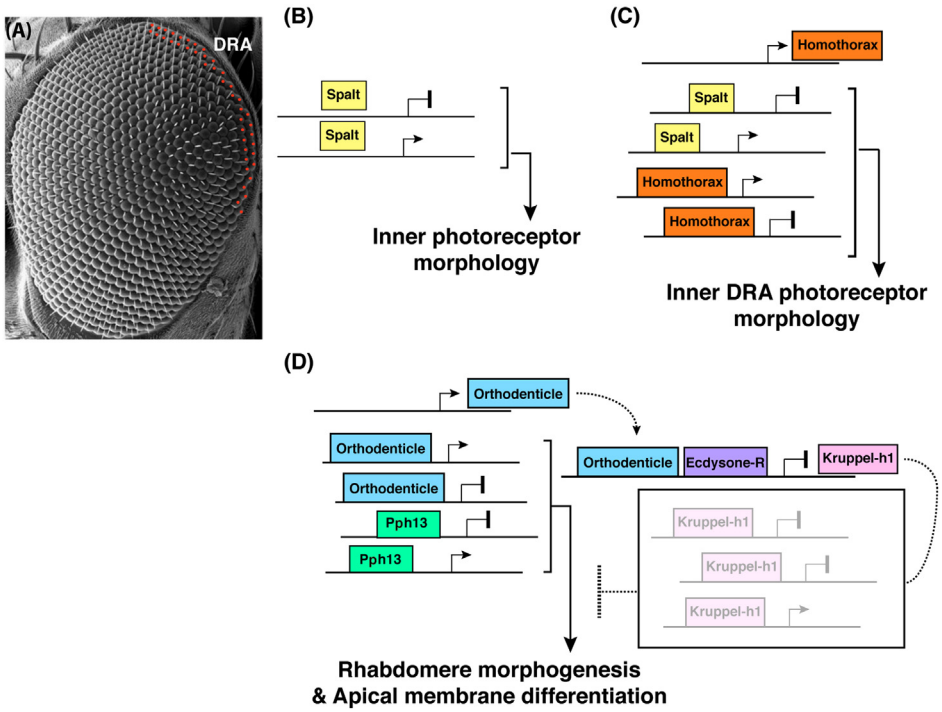

Fig. 7. Transcriptional regulation of photoreceptor morphology. (A) Scanning electron micrograph of a Drosophila eye. The Dorsal Rim Area (DRA) ommatidia are labeled with red dots. (B) Simplified transcriptional network governing R8 photoreceptor maturation in the pupal retina by Spalt. (C) Simplified transcriptional network governing Dorsal Rim Area (DRA) inner photoreceptor maturation in the pupal retina. *spalt* remains expressed in these cells and transcription downstream of both *splat* and *homothorax* governs DRA inner photoreceptor morphogenesis (Wernet et al., 2003; Xie et al., 2007). (D) Simplified transcriptional network governing photoreceptor maturation including polarity remodeling in the pupal retina. The transcription of the *kruppel-h*1 gene is transiently repressed by Orthodenticle and the Ecdysone Receptor (Ecdyson-R) at the onset of polarity remodeling in the photoreceptor (Fichelson et al., 2012). This enables photoreceptor polarity remodeling, a process that also dependent on *orthodenticle* and the related, partially redundant Pph13 transcription factor (Fichelson et al., 2012; Mishra et al., 2010).

(Morey et al., 2008). Very little is known about the function of the *spalt* locus in promoting R8 cell morphology, although it is likely that it may limit the expression of proteins that are required to build the rhabdomere and promote cell elongation. It is not known whether cell-size or protein-translation regulators such as Myc or the insulin/Tor signaling pathway are involved during this process. As the *spalt* locus seems to function as a master regulator of R8 maturation, it should be possible to identify its downstream targets, which would help us better understand how morphological diversity is generated during neurogenesis.

A second example of a master regulator of photoreceptor terminal maturation is found in the dorsal rim area of the developing fly retina (Fig. 7A). In this region, a row of ommatidia contains inner photoreceptors that possess enlarged rhabdomeres compared to the typical size of the R7 and R8 rhabdomere elsewhere in the retina (Hardie, 1984) (Fig. 1A). The Homeodomain transcription factor Homothorax governs both the enlargement of the rhabdomere and the expression of Rhodopsin 3 in both R7 and R8 (Wernet et al., 2003) (Fig. 7C). The expression of Homothorax in these R7/R8-like cells in the dorsal rim occurs at ~5–10% after puparium formation and therefore approximately 40 h after these cells have been specified at the posterior margin of the eye disc. Moreover, overexpression of *homothorax* in the retina is sufficient to convert all R7 and R8 into the dorsal-rim-type inner photoreceptors (Wernet et al., 2003). Interestingly, while the R8 cells of the dorsal rim fate still express the *spalt* locus, they lose the expression of the R8-specific transcription factor *senseless* (Wernet et al., 2003; Xie et al., 2007) (Fig. 7B–C). However, how the expression of Homothorax overrides Spalt's ability to limit rhabdomere size in dorsal-rim R8 cells is not clear. This example of the R8 photoreceptor highlights how much terminal cell differentiation can rely on the superimposition (i.e., Homothorax/Spalt) of genetic and transcriptional programs.

Systemic regulation during photoreceptor maturation

Alongside evidence for photoreceptor subtype-specific transcriptional programs are genetic and molecular pathways that seem to function in all photoreceptors to drive apical membrane specialization. This is the case for the paired-class homeodomain transcription factors Orthodenticle and Pph13 (Fichelson et al., 2012; Mishra et al., 2010; Vandendries et al., 1996). Orthodenticle is expressed in all newly differentiating photoreceptors in the developing eye disc. However, as for the *spalt* locus in the R8 photoreceptor neuron, so far Orthodenticle does not appear to play any role in early cell fate commitment (Fichelson et al., 2012; Vandendries et al., 1996). Instead, it is required later during photoreceptor pupal development to promote the timely remodeling of the apico-basal axis of the cell and rhabdomere elongation (Fichelson et al., 2012; Mishra et al., 2010; Ranade et al., 2008). Part of Orthodenticle's function here is to repress the expression of *kruppel-h*1, which encodes a member of the conserved Kruppel-like Zn-finger transcription factor family (Fichelson et al., 2012). The role of the *kruppel-h*1 locus in the developing retina is particularly interesting, as its transient repression depends on the integration by its promoter of both an intracellular protein (i.e., autonomous to the photoreceptor) and a systemic signal–a hormone. The intracellular protein is Orthodenticle, which binds directly to the *kruppel-h*1 promoter and the systemic signal is the steroid hormone 20-hydroxyecdysone, whose receptor binds to the promoter (Fichelson et al., 2012) (Fig. 7D). Because the levels of circulating 20-hydroxyecdysone fluctuate during development, it is possible that a threshold level of this hormone could regulate the onset of polarity remodeling in the photoreceptor.

Kruppel-h1 is known to orchestrate ecdysone-regulated pathways and has been shown to regulate axon morphogenesis in various species (Beck et al., 2004; Pecasse et al., 2000; Shi et al.,

2007). For instance, forced expression of Kruppel-h1 leads to reduced branching of the *Drosophila* mushroom body neurons, and evidence suggests that this protein represses neuronal morphogenesis in this part of the brain (Shi et al., 2007). The expression of the orthologue of Kruppel-h1 in the mushroom body of worker honeybees is also consistently decreased downstream of the queen mandibular pheromone. In this case, Kruppel-h1 is viewed as a major marker of a behavioral switch that correlates with axonal branch remodeling (Shpigler et al., 2010). In the *Drosophila* photoreceptor, the onset of polarity remodeling correlates with a transient downregulation of Kruppel-h1. Failure to downregulate Kruppel-h1 leads to strong defects in photoreceptor maturation (Fichelson et al., 2012). This result reinforces the idea that Kruppel-h1 might function as a broad facilitator of ecdysone-governed neuronal morphogenesis. It also raises the possibility that hormones regulate other cell morphogenesis events in the developing pupal retina. It will be interesting, for example, to examine the influence of hormones on the establishment of neural superposition at around 30% after puparium formation (Meinertzhagen, 2000) (Fig. 2B), lumen formation within the ommatidium at approximately 55% after puparium formation (Fig. 6C), and rhodopsin expression at around 78% (Ready, 2002) (Fig. 2E). All these events are precisely regulated in time and are synchronized with the overall development of the animal. It will also be interesting to test if epigenetic regulation, including histone modifications and remodeling, could play a part in modulating transcription at discrete loci governing timely differentiation. Striking examples of such regulation during cell differentiation have been recently found during gliogenesis (Popkova et al., 2012) and germ cell differentiation (Chen et al., 2011) in *Drosophila*. Hormonal control of developmental transitions can help ensure that developmental events occur at the right time and in the correct sequence (Fichelson et al., 2012). Understanding how the photoreceptor determines when to implement its specialized membrane differentiation, lumen formation and rhodopsin expression programs should continue to contribute to our broad understanding of the timing of cell differentiation and organogenesis during development.

Conclusion

The developing fly retina has long served as a powerful in vivo system to study cell fate commitment and differentiation and the patterning of neuroepithelia. It has the advantages of genetic tractability, suitability for live imaging and *in silico* modeling (Gemp et al., 2011; Lubensky et al., 2011), as well as a wealth of knowledge regarding retinal cell differentiation and pattern formation (Hilgenfeldt et al., 2008; Larson et al., 2010, 2008). Its repeating structure makes it easy to identify even subtle phenotypes and thereby uncover redundancies in gene function. Together, these features make it an ideal model for studying cell behavior during development and for analyzing the complex cell-cell interactions underlying organ development.

Acknowledgments

Work on eye development in the author's lab is supported by the Medical Research Council. The manuscript was improved by the critical comments of Nic Tapon, Martin Raff, Chiara Mencarelli, Rhian Walther as well as three anonymous reviewers.

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