Published in final edited form as:
Wiley Interdiscip Rev Dev Biol. 2013 July ; 2(4): 545–557. doi:10.1002/wdev.100.

Retinal differentiation in *Drosophila*

Jessica E. Treisman
Kimmel Center for Biology and Medicine of the Skirball Institute and Department of Cell Biology, NYU School of Medicine, 540 First Avenue, New York, NY 10016

Abstract

Drosophila eye development has been extensively studied, due to the ease of genetic screens for mutations disrupting this process. The eye imaginal disc is specified during embryonic and larval development by the Pax6 homolog Eyeless and a network of downstream transcription factors. Expression of these factors is regulated by signaling molecules and also indirectly by growth of the eye disc. Differentiation of photoreceptor clusters initiates in the third larval instar at the posterior of the eye disc and progresses anteriorly, driven by the secreted protein Hedgehog. Within each cluster, the combined activities of Hedgehog signaling and Notch-mediated lateral inhibition induce and refine the expression of the transcription factor Atonal, which specifies the founding R8 photoreceptor of each ommatidium. Seven additional photoreceptors, followed by cone and pigment cells, are successively recruited by the signaling molecules Spitz, Delta, and Bride of sevenless. Combinations of these signals and of intrinsic transcription factors give each ommatidial cell its specific identity. During the pupal stages, Rhodopsins are expressed, and the photoreceptors and accessory cells take on their final positions and morphologies to form the adult retina. Over the past few decades, the genetic analysis of this small number of cell types arranged in a repetitive structure has allowed a remarkably detailed understanding of the basic mechanisms controlling cell differentiation and morphological rearrangement.

Introduction

The adult *Drosophila* eye is a highly organized structure composed of approximately 800 ommatidial units arranged in a hexagonal lattice (Fig. 1A). Each ommatidium contains 8 photoreceptor cells, which extend their light-collecting rhabdomeres into the center of the ommatidium in a trapezoidal pattern (Fig. 1B). The outer photoreceptors R1-R6 have large rhabdomeres, express the rhodopsin Rh1, and project axons into the lamina, a region of the brain specialized for motion detection. The inner photoreceptors R7 and R8 have centrally located small rhabdomeres, with the R8 rhabdomere directly below R7, each express one of four rhodopsins (Rh3-Rh6), and project their axons into the medulla, the brain region responsible for color vision (Fig. 1C) [1]. The photoreceptors are surrounded by four cone cells, which secrete the lens, and by two primary pigment cells, which contribute to isolating each ommatidial light-sensing unit. These ommatidial clusters are separated from each other by a lattice of secondary and tertiary pigment cells and mechanosensory bristles (Fig. 1D) [2]. Because the eye has a highly repetitive structure and is not essential for survival of flies raised in the laboratory, it is well suited for genetic screens. The isolation of numerous mutations that affect the formation of the adult eye has led to a detailed mechanistic understanding of its development.

The eye develops from the eye imaginal disc, a bilayered epithelial tissue that invaginates from the embryonic epidermis, grows and differentiates inside the larva, and everts during metamorphosis. Retinal differentiation initiates at the posterior margin of the eye disc in the third larval instar and gradually progresses towards the anterior margin, reaching it after the first day of pupal development. The first overt sign of differentiation is a transient

invagination of the disc surface known as the morphogenetic furrow [3] (Fig. 2). Anterior to this moving furrow, cells divide in an unpatterned manner. On the posterior side of the furrow, their apical profiles become organized into evenly spaced arcs. These arcs close up and finally transform into 5-cell preclusters (Fig. 2B, C), within which the photoreceptors R8, R2 and R5, and R3 and R4 differentiate in sequence, as revealed by their expression of neuronal markers [4–6]. The cells that remain undifferentiated at this stage undergo a final round of division, the second mitotic wave, before differentiating as R1 and R6, R7, cone cells, and primary pigment cells [4, 5]. During pupal development, some of the remaining cells surrounding the ommatidial clusters die, and the rest reorganize to form a hexagonal lattice, in which the sides are formed by secondary pigment cells and the vertices by tertiary pigment cells alternating with bristles [7]. This review will describe the genetic and molecular mechanisms that underlie the process of retinal differentiation. Further information about all the genes discussed is available in Flybase (http://flybase.org/).

The eye field is specified by a network of transcription factors

The compound eye-antennal imaginal disc gives rise not only to the eye, but also to much of the head cuticle [8]. A cascade of transcription factors specifies first the entire disc and subsequently the eye field within it. During embryonic stages, the whole eye-antennal disc expresses Eyeless (Ey) and Twin of eyeless (Toy), two homologues of the Paired domain/homeodomain transcription factor Pax6 (Fig. 3A). Toy is also expressed earlier in a broad region of the embryonic head [9, 10]. Although *ey* and *toy* are required for head development, some mutant alleles of these genes result in a specific loss of eye tissue [11, 12]. Strikingly, misexpression of these genes in other imaginal discs can drive the development of ectopic eyes [9, 13], although this occurs only at positions where other factors necessary for eye development are present [14–18]. Interestingly, Pax6 appears to be at the top of a hierarchy of factors that regulate eye development not only in *Drosophila*, but in almost every species that has been examined, suggesting that cells important for vision came under the control of this transcription factor at a very primitive stage of their evolution [19].

During the second larval instar, *ey* expression is lost from the antennal disc and comes to define the eye field, while the transcription factor Cut specifies and maintains the antennal state [10, 20, 21]. Ey is coexpressed with two other transcription factors, Homothorax (Hth) and Teashirt (Tsh), and acts in combination with them to promote the growth of the early eye disc [17, 22]. Ey also induces the expression of *eyes absent* (*eya*) and *sine oculis* (*so*) at the posterior of the eye disc, acting directly through binding sites in the regulatory regions of these genes (Fig. 3B, D) [10, 23, 24]. Eya and So can form a compound transcription factor that is targeted to specific sequences by the DNA-binding domain of So [25, 26]. Eya and So are in turn required for the expression of Dachshund (Dac) [27], another protein that can both interact with Eya and bind to specific DNA sequences (Fig. 3D) [28, 29]. Eya, So and Dac are all essential for eye differentiation [25, 30–33]. When ectopically expressed, these downstream factors have a more limited ability to induce ectopic eye development, and this is accompanied by induction of *ey* expression, indicating the existence of feedback loops within the retinal determination network [25, 29, 34, 35]. In addition to its role as a transcription factor, Eya has a second function in the cytoplasm as a tyrosine phosphatase enzyme. Although its phosphatase activity seems to contribute to eye specification, the mechanism of this effect is not fully understood [36–38].

eya expression is confined to the posterior part of the eye disc due to its regulation by localized signaling molecules. Posteriorly expressed Hedgehog (Hh) and Decapentaplegic (Dpp), a Bone Morphogenetic Protein (BMP) homologue, act as positive regulators of *eya*, and anteriorly expressed Wingless (Wg) as a negative regulator [18, 20, 39, 40]. The lateral regions that express Wg do not form part of the eye field, but will instead give rise to head

cuticle [41]. Wg is both necessary and sufficient to promote the head cuticle fate; loss of Wg activity expands the eye field into the dorsal head, while ectopic activation of the Wg pathway within the eye field produces cuticular outgrowths [42–45]. Because Wg acts at a long range, the disc must reach a certain size before Wg levels are low enough in its posterior region for *eya* expression to be initiated [20]. Growth of the disc thus controls the timing of *eya* expression, and mutations that disrupt growth will secondarily block differentiation.

Growth of the eye disc depends on the subdivision of the disc into dorsal and ventral compartments, which allows activation of Notch signaling specifically at the dorsoventral boundary. This is achieved by partitioning the glycosyltransferase enzyme Fringe (Fng) specifically into the ventral compartment. Modification of Notch by Fng renders it insensitive to the ventrally expressed ligand Serrate (Ser), but sensitive to the dorsally expressed ligand Delta (Dl), ensuring that it is active only where the two domains meet [46–48] (Fig. 3E). The upstream regulators of *Dl*, *Ser* and *fng* expression are three homeodomain transcription factors known as the Iroquois complex (Iro-C) in the dorsal compartment, and two Sloppy-paired transcription factors, members of the Forkhead family, in the ventral compartment; mutual repression maintains this complementary arrangement [49, 50]. Expression of the *Iro-C* genes is initiated by Wg, which is predominantly dorsal in the early eye disc, due to its activation by Pannier (Pnr), a transcription factor expressed at the dorsal margin of the eye disc, and ventral repression by the JAK/STAT pathway ligand Unpaired (Upd) [51–54]. Interestingly, Upd expression is also induced by Notch activation at the midline of the posterior margin, a point from which it acts at a long range to stimulate growth throughout the eye disc [55–58]. In addition, Notch signaling promotes growth autonomously through the downstream effectors Eyegone (Eyg) and Four-jointed [53, 55, 58].

Progressive photoreceptor differentiation is driven by autoregulatory loops

In the middle of the third larval instar, photoreceptor clusters begin to form at the posterior of the eye disc. As the morphogenetic furrow progresses anteriorly, successive rows of ommatidia differentiate approximately every two hours until the eye field is complete 24 hours after pupariation [3]. The driving force behind this differentiation wave is the signaling molecule Hh, which is essential for both the initiation and progression of photoreceptor differentiation [59–61]. Hh expression at the posterior margin of the early eye disc is established by three zinc finger transcription factors in the Odd-skipped family [62]. Subsequently, Hh itself induces additional Hh expression in the photoreceptors as they differentiate, through an indirect autoregulatory loop. This gradual spread of Hh expression drives the progression of differentiation from posterior to anterior across the disc [59, 60]. In the eye disc, Hh acts primarily to inactivate the repressor form of the downstream transcription factor Cubitus interruptus (Ci), allowing the expression of *dpp* [18, 63]. *dpp* is expressed in a stripe in the morphogenetic furrow, immediately anterior to the zone of *hh* expression (Fig. 4A, B) [60]. Posterior to the morphogenetic furrow, cells can no longer respond to Hh because Ci is degraded by a ubiquitin ligase complex containing Cullin3 and the adaptor protein Roadkill [64, 65]. The morphogen Dpp acts at a long range to restrict the expression of the transcription factor Hth to the anterior of the eye disc, where it represses *eya* in combination with Ey and Tsh [17]. Dpp signaling thus creates a preproneural zone in which cells express both *ey* and *eya* and are primed to respond to the shorter-range Hh signal (Fig. 4A, B). Hh and Dpp signaling, in combination with additional inputs, lead to the down-regulation of *ey* and *tsh* in differentiating cells and the up-regulation of *so* and *dac* as well as *eya* in the preproneural zone and more posteriorly [40].

Another critical target gene that is activated redundantly by Hh and Dpp signaling is *atonal* (*ato*), which encodes a proneural basic helix-loop-helix transcription factor [66, 67]. *ato* is first expressed in all cells in a stripe just anterior to the morphogenetic furrow, but it rapidly

resolves more posteriorly into regularly spaced groups of cells and then into single cells, the future R8 photoreceptors (Fig. 4B) [68]. Its initial expression is driven by a 3' enhancer region that contains essential binding sites for Ey and So [69–71], suggesting that the input from Hh and Dpp signaling may be mediated by these eye determination factors [72]. A 5' enhancer that responds to Ato autoregulation and to the zinc finger transcription factor Roughened eye mediates its later expression [69, 73]. This expression is restricted to R8 precursors by lateral inhibition, which is mediated by the Notch pathway through the transcription factors Enhancer of split and Daughterless (Da) [74, 75]. Notch signaling and the spacing of *ato*-expressing groups of cells are also regulated by the secreted protein Scabrous [76–78]. Downstream of Ato, which is only transiently expressed, permanent expression of the zinc finger transcription factor Senseless (Sens) seals the fate of the R8 cell [79]. Ato is nevertheless predicted to directly regulate numerous downstream genes, only a subset of which are shared with Sens [80].

Photoreceptor differentiation must be precisely coordinated in space and time. Anterior to the morphogenetic furrow, two repressors and antagonists of Ato, Extramacrochaetae (Emc) and Hairy, prevent it from functioning prematurely [81]. Emc acts primarily by inhibiting the expression of Da, an obligate partner for the Ato protein [82]. These antagonists are themselves induced by Hh and Dpp, and are subsequently repressed by Dl, a very short-range signal also emanating from the morphogenetic furrow [17, 67, 82, 83]. Posterior to the morphogenetic furrow, Ato expression in undifferentiated cells is terminated by the homeodomain proteins BarH1 and BarH2 [84].

The primary role of Hh signaling is to activate Ato and thus promote the specification of the R8 precursor in each ommatidium. This is sufficient to set in motion the construction of the entire ommatidium, not because the remaining cells are descendants of R8, but because they are recruited by R8 from the surrounding pool of undifferentiated cells (Fig. 4C) [3]. Under the control of Ato, R8 expresses Rhomboid (Rho) and Rhomboid-3/Roughoid, two proteases that cleave and activate the transmembrane precursor form of Spitz (Spi), a ligand for the Epidermal growth factor receptor (EGFR) [85–87]. Spi signaling promotes the stepwise differentiation of R2 and R5, R3 and R4, R1 and R6, R7, and the cone and primary pigment cells [88–90]. Rho is also expressed in R2 and R5, and Spi produced by these cells contributes to the differentiation of other photoreceptors [88, 90, 91]. The short range of Spi action [92] and the geometry of the initial arc may explain why R3 and R4, at the tips of the arc, differentiate later than R2 and R5, which are immediately adjacent to R8. The precursors of R1, R6 and R7 divide in the second mitotic wave, which may delay the response to Spi signaling in these cells. Activation of the EGFR pathway in more distant cells is also prevented by Argos, a secreted feedback inhibitor that prevents Spi from binding to the EGFR [93–95]. Later differentiating cells require input from the Notch ligand Dl in addition to Spi. Dl is itself transcribed in response to Spi signaling, creating a feedforward loop [96]. Dl produced by R1-R6 helps to recruit R7 and cone cells [96–98], while Dl produced by cone cells in response to EGFR signaling can recruit primary pigment cells [99].

In addition to promoting the differentiation of R2 and R5, Spi signaling also induces these cells to express Hh. An eye-specific enhancer of the *hh* gene integrates input from Pointed P2 (PntP2), the Ets transcription factor responsive to EGFR signaling, and the retinal determination transcription factor So [100]. Hh secreted by more posterior photoreceptors thus promotes Ato expression, R8 differentiation, Spi-dependent recruitment of R2 and R5, and eventually its own expression in response to EGFR signaling in these cells. This indirect autoregulatory loop is the basis for the gradual anterior progression of differentiation.

Combinatorial signals control the differentiation of specific cell types

All the photoreceptors other than R8 require Spi for their induction. Nevertheless, they differ in other properties, such as their position, timing of differentiation, and gene expression. Mutations that transform one subtype into another have given some insight into how individual subtypes are specified. A classic example is the *sevenless* (*sev*) mutation, in which R7 photoreceptors are absent and the R7 precursor instead differentiates into a non-neuronal cone cell [101]. A second mutation, *bride of sevenless* (*boss*) was later shown to have the same phenotype [102]. Since only R7 cells express rhodopsins that detect ultraviolet light, both mutations were initially isolated based on the failure of the adult flies to choose ultraviolet light over visible light [102, 103]. *sev* was subsequently shown to encode a receptor tyrosine kinase required in the R7 cell [104–106], while *boss* encodes a transmembrane ligand required in the R8 cell [102, 107]. The pathway downstream of Sev was elucidated using a genetic screen for dominant modifiers of a temperature-sensitive *sev* allele [108]. Interestingly, Sev signals through the Ras/Mitogen-activated protein kinase (MAPK) cassette, the same pathway that is downstream of the EGFR and other receptor tyrosine kinases [109, 110]. Analysis of the promoter of *prospero* (*pros*), a gene expressed strongly in R7 and weakly in cone cells that encodes a transcription factor important for their differentiation, suggested that combined signaling through both receptors is necessary for high-level *pros* activation [111] (Fig. 5). Degradation of Tramtrack88 (Ttk88), a transcription factor that represses neuronal differentiation and *pros* expression, may integrate the two pathways. Ttk88 degradation is mediated by a ubiquitin ligase complex that contains both the adaptor protein Phyllopod, which is regulated by Sev, and the F-box protein Ebi, which is regulated by EGFR, as well as the RING domain protein Seven in absentia [112–114].

R7 differentiation also requires input from R1 and R6; these cells express Dl, which activates the Notch pathway in the R7 precursor [97, 115]. Notch signaling exerts antagonistic activities. It appears to increase Ttk88 levels to oppose photoreceptor differentiation. However, it also induces expression of *sev*, allowing R7 to receive the Boss signal that overrides the effect of Ttk88 [116]. In addition, Notch signaling represses *seven-up* (*svp*), which encodes a direct repressor of *pros* expression [117]. Differentiation of R7 and transcription of *pros* also require input from the Runt domain transcription factor Lozenge (Lz) [118 111]. Lz is expressed in undifferentiated cells posterior to the furrow and is maintained in the descendants of cells that divide in the second mitotic wave [119]. Its expression is activated by So and another retinal-specific transcription factor, Glass [120, 121], both of which also directly regulate *pros* and other cell type-specific genes [80, 117]. The intricacy of *pros* regulation illustrates the complex combinatorial mechanisms necessary to render a single cell distinct from its immediate neighbors (Fig. 5).

While the R7 precursor becomes a cone cell if it does not receive the appropriate signals, the R7 fate can itself be a default choice for earlier differentiating cells. Svp is an orphan nuclear receptor required in R1, R3, R4 and R6; in its absence all these cells were initially thought to become R7 cells [122]. However, it was recently shown that *svp* mutant cells are equally likely to differentiate as either R7 or R8 cells. Interestingly, in this case the cells choose one of these two fates only quite late in development, and Notch signaling promotes the R7 fate by repressing the R8-specific transcription factor Sens [123]. Additional transcription factors specify the other cell fates within the ommatidium. Sens and the homeodomain protein Rough (Ro), which is expressed in the R2 and R5 cells, mutually repress each other's expression to prevent R8, R2 and R5 from switching their fates [124, 125]. In *rough* mutants, Sens is misexpressed in the R2 and R5 precursors, and these cells differentiate as R8 cells [125]. The transcription factors Spalt, expressed in R3 and R4, and Bar, expressed in R1 and R6, differentiate these photoreceptor pairs from each other [126, 127]. However, the upstream regulators of these factors are largely unknown. Finally, R3 and R4

are differently specified under the influence of planar polarity signaling, a topic covered by another review in this series (Singh and Mlodzik, 2012).

Terminal differentiation is regulated independently from cell type specification

Photoreceptors establish specific patterns of gene expression during the larval and early pupal stages, but do not take on their characteristic morphologies until later in pupal development. Analysis of mutants lacking the two adjacent *spalt* genes revealed that morphogenesis and cell fate are under separate control. In these mutants, the adult R7 and R8 cells resemble outer photoreceptors in that they form large rhabdomeres and express the rhodopsin Rh1 [128]. However, during larval development R8 differentiates normally, and although R7 fails to express genes such as *pros* and *runt*, it does not take on an outer photoreceptor fate at this stage [129]. Thus Spalt transcription factors are specifically required for late steps in inner photoreceptor differentiation.

Terminal differentiation of photoreceptors involves modification of the apical membrane to create the rhabdomere, a stack of membranes packed with rhodopsin that functions as a photon detector. The apical membrane rotates 90° to face the center of the ommatidium rather than the apical surface of the eye, becomes folded into numerous microvilli, and is connected to the cell body by a proximal stalk region (Fig. 6A) [130]. The transmembrane protein Crumbs and other protein complexes that regulate apical-basal polarity carry out this reorganization of the apical surface [131, 132]. In addition, rhodopsin and proteins that transport it into the rhabdomere are essential for rhabdomere morphogenesis [133, 134]. At the transcriptional level, rhabdomere formation is redundantly controlled by Orthodenticle (Otd) and Pph13, two homeodomain proteins [135]. Together with the steroid hormone ecdysone, Otd directs the timing of photoreceptor maturation [136].

During pupal development, each photoreceptor must also choose only one specific Rhodopsin (Rh) molecule to express; Rh1 and Rh5 detect blue light, while Rh6 detects green light and Rh3 and Rh4 detect ultraviolet light [137]. All R1-R6 cells express Rh1, but inner photoreceptors are divided into several distinct groups. In a dorsal region of the eye specialized for polarized light detection, R7 and R8 both express Rh3, a fate determined by the transcription factor Hth [138]. In the remainder of the retina, about 30% of ommatidia have R7 cells that express Rh3 and R8 cells that express Rh5, and 70% have R7 cells that express Rh4 and R8 cells that express Rh6 [1]. This distinction is controlled by transient stochastic expression of the transcription factor Spineless (Ss) in the subset of R7 cells that will later express Rh4 [139]. The Rhodopsin expressed by an R8 cell depends on signaling from the R7 cell within the same ommatidium. Rh3-expressing R7 cells induce neighboring R8 cells to express Rh5; in the absence of this signal, R8 cells express Rh6. Although the nature of the signal is still unknown, it is transduced by molecules better known for their role in growth control in proliferating tissues: components of the Hippo (Hpo) pathway and the pleckstrin homology domain protein Melted (Melt) (Fig. 6B) [140, 141].

During the pupal period, the non-photoreceptor cells also take on their final positions and morphologies. The second mitotic wave generates an excess of undifferentiated cells, and some of these must be eliminated by cell death to create a hexagonal lattice with only a single secondary pigment cell at each edge and a single tertiary pigment cell or bristle group at each vertex [142]. Reorganization of these lattice cells is driven by differential adhesion between two proteins of the immunoglobulin superfamily: Roughest (Rst) on the membrane of secondary and tertiary pigment cells binds to Hibris on the membrane of primary pigment cells. Those cells with the highest levels of Rst form the largest contact surfaces with primary pigment cells, enabling them to survive and adopt an extended morphology, while

other cells lose contact and ultimately die [143] [144]. Survival appears to depend on Spi produced by cone and primary pigment cells, which activates the EGFR in lattice cells to downregulate Head involution defective, an inducer of apoptosis [145]. Computational modeling suggests that apical expansion of the cone and primary pigment cell profiles also plays an important role in generating the normal pattern of secondary and tertiary pigment cells [143].

Conclusions

Clearly, the repetitive structure and accessibility of the eye and the power of *Drosophila* genetics have led to a wealth of knowledge about how this organ develops. The identification of numerous genes required for normal development of the eye and the characterization of enhancer elements that control the expression of many of these genes have been particularly informative. Autoregulation and feed-forward loops play a role at several developmental stages. However, we still do not fully understand how a combination of external signals and intrinsic factors leads to the production of specific cell types in a precise temporal and spatial pattern. Further study of eye development will doubtless provide us with additional insight into fate specification, combinatorial signaling networks that generate complex patterns, and cell biological aspects of differentiation.

Acknowledgments

Work on eye development in the author's lab is supported by the National Institutes of Health (grant EY013777). The manuscript was improved by the critical comments of Kevin Legent and Justine Oyallon.

References

1. Morante J, Desplan C, Celik A. Generating patterned arrays of photoreceptors. Curr Opin Genet Dev. 2007; 17:314–319. [PubMed: 17616388]
2. Wolff, T.; Ready, DF. Pattern formation in the *Drosophila* retina. In: Bate, M.; Martinez-Arias, A., editors. The development of Drosophila melanogaster. Vol. Vol. II. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1993. p. 1277-1316.
3. Ready DF, Hanson TE, Benzer S. Development of the *Drosophil* a retina, a neurocrystalline lattice. Dev Biol. 1976; 53:217–240. [PubMed: 825400]
4. Wolff T, Ready DF. The beginning of pattern formation in the *Drosophila* compound eye: the morphogenetic furrow and the second mitotic wave. Development. 1991; 113:841–850. [PubMed: 1726564]
5. Tomlinson A, Ready DF. Neuronal differentiation in the *Drosophila* ommatidium. Dev. Biol. 1987; 120:366–376. [PubMed: 17985475]
6. Robertson F, Pinal N, Fichelson P, Pichaud F. Atonal and EGFR signalling orchestrate rok- and Drak-dependent adherens junction remodelling during ommatidia morphogenesis. Development. 2012; 139:3432–3441. [PubMed: 22874916]
7. Cagan RL, Ready DF. The emergence of order in the *Drosophila* pupal retina. Dev Biol. 1989; 136:346–362. [PubMed: 2511048]
8. Dominguez M, Casares F. Organ specification-growth control connection: new in-sights from the *Drosophila* eye-antennal disc. Dev Dyn. 2005; 232:673–684. [PubMed: 15704149]
9. Czerny T, Halder G, Kloter U, Souabni A, Gehring WJ, Busslinger M. *twin of eyeless* a second *Pax-6* gene of *Drosophila* acts upstream of *eyeless* in the control of eye development. Mol Cell. 1999; 3:297–307. [PubMed: 10198632]
10. Halder G, Callaerts P, Flister S, Walldorf U, Kloter U, Gehring WJ. Eyeless initiates the expression of both *sine oculis* and *eyes absent* during *Drosophila* compound eye development. Development. 1998; 125:2181–2191. [PubMed: 9584118]
11. Quiring R, Walldorf U, Kloter U, Gehring WJ. Homology of the *eyeless* gene of *Drosophila* to the *Small eye* gene in mice and *Aniridia* in humans. Science. 1994; 265:785–789. [PubMed: 7914031]

12. Kronhamn J, Frei E, Daube M, Jiao R, Shi Y, Noll M, Rasmuson-Lestander A. Headless flies produced by mutations in the paralogous *Pax6* genes *eyeless* and *twin of eyeless*. Development. 2002; 129:1015–1026. [PubMed: 11861484]
13. Halder G, Callaerts P, Gehring WJ. Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*. Science. 1995; 267:1788–1792. [PubMed: 7892602]
14. Chen R, Halder G, Zhang Z, Mardon G. Signaling by the TGF-beta homolog Decapentaplegic functions reiteratively within the network of genes controlling retinal cell fate determination in *Drosophila*. Development. 1999; 126:935–943. [PubMed: 9927595]
15. Niwa N, Hiromi Y, Okabe M. A conserved developmental program for sensory organ formation in *Drosophila melanogaster*. Nat Genet. 2004; 36:293–297. [PubMed: 14981517]
16. Kango-Singh M, Singh A, Henry Sun Y. Eyeless collaborates with Hedgehog and Decapentaplegic signaling in *Drosophila* eye induction. Dev Biol. 2003; 256:49–60. [PubMed: 12654291]
17. Bessa J, Gebelein B, Pichaud F, Casares F, Mann RS. Combinatorial control of *Drosophila* eye development by *eyeless*, *homothorax* and *teashirt*. Genes Dev. 2002; 16:2415–2427. [PubMed: 12231630]
18. Pappu KS, Chen R, Middlebrooks BW, Woo C, Heberlein U, Mardon G. Mechanism of Hedgehog signaling during *Drosophila* eye development. Development. 2003; 130:3053–3062. [PubMed: 12756186]
19. Gehring WJ, Ikeo K. Pax 6: mastering eye morphogenesis and eye evolution. Trends Genet. 1999; 15:371–377. [PubMed: 10461206]
20. Kenyon KL, Ranade SS, Curtiss J, Mlodzik M, Pignoni F. Coordinating proliferation and tissue specification to promote regional identity in the *Drosophila* head. Dev Cell. 2003; 5:403–414. [PubMed: 12967560]
21. Wang CW, Sun YH. Segregation of eye and antenna fates maintained by mutual antagonism in *Drosophila*. Development. 2012; 139:3413–3421. [PubMed: 22912416]
22. Peng HW, Slattery M, Mann RS. Transcription factor choice in the Hippo signaling pathway: Homothorax and Yorkie regulation of the microRNA *bantam* in the progenitor domain of the *Drosophila* eye imaginal disc. Genes Dev. 2009; 23:2307–2319. [PubMed: 19762509]
23. Ostrin EJ, Li Y, Hoffman K, Liu J, Wang K, Zhang L, Mardon G, Chen R. Genome-wide identification of direct targets of the *Drosophila* retinal determination protein Eyeless. Genome Res. 2006; 16:466–476. [PubMed: 16533912]
24. Niimi T, Seimiya M, Kloter U, Flister S, Gehring WJ. Direct regulatory interaction of the Eyeless protein with an eye-specific enhancer in the *sine oculis* gene during eye induction in *Drosophila.* Development. 1999; 126:2253–2260. [PubMed: 10207149]
25. Pignoni F, Hu B, Zavitz KH, Xiao J, Garrity PA, Zipursky SL. The eye-specification proteins So and Eya form a complex and regulate multiple steps in *Drosophila* eye development. Cell. 1997; 91:881–891. [PubMed: 9428512]
26. Pauli T, Seimiya M, Blanco J, Gehring WJ. Identification of functional Sine oculis motifs in the autoregulatory element of its own gene, in the *eyeless* enhancer and in the signalling gene *hedgehog.* Development. 2005; 132:2771–2782. [PubMed: 15901665]
27. Pappu KS, Ostrin EJ, Middlebrooks BW, Sili BT, Chen R, Atkins MR, Gibbs R, Mardon G. Dual regulation and redundant function of two eye-specific enhancers of the *Drosophila* retinal determination gene *dachshund*. Development. 2005; 132:2895–2905. [PubMed: 15930118]
28. Kim SS, Zhang RG, Braunstein SE, Joachimiak A, Cvekl A, Hegde RS. Structure of the retinal determination protein Dachshund reveals a DNA binding motif. Structure. 2002; 10:787–795. [PubMed: 12057194]
29. Chen R, Amoui M, Zhang Z, Mardon G. Dachshund and Eyes absent proteins form a complex and function synergistically to induce ectopic eye development in *Drosophila*. Cell. 1997; 91:893–903. [PubMed: 9428513]
30. Mardon G, Solomon NM, Rubin GM. *dachshund* encodes a nuclear protein required for normal eye and leg development in *Drosophila*. Development. 1994; 120:3473–3486. [PubMed: 7821215]
31. Bonini NM, Leiserson WM, Benzer S. The *eyes absent* gene: genetic control of cell survival and differentiation in the developing *Drosophila* eye. Cell. 1993; 72:379–395. [PubMed: 8431945]

32. Cheyette BN, Green PJ, Martin K, Garren H, Hartenstein V, Zipursky SL. The *Drosophila sine oculis* locus encodes a homeodomain-containing protein required for the development of the entire visual system. Neuron. 1994; 12:977–996. [PubMed: 7910468]
33. Serikaku MA, O'Tousa JE. *sine oculis* is a homeobox gene required for *Drosophila* visual system development. Genetics. 1994; 138:1137–1150. [PubMed: 7896096]
34. Bonini NM, Bui QT, Gray-Board GL, Warrick JM. The *Drosophila eyes absent* gene directs ectopic eye formation in a pathway conserved between flies and vertebrates. Development. 1997; 124:4819–4826. [PubMed: 9428418]
35. Weasner B, Salzer C, Kumar JP. Sine oculis, a member of the SIX family of transcription factors, directs eye formation. Dev Biol. 2007; 303:756–771. [PubMed: 17137572]
36. Xiong W, Dabbouseh NM, Rebay I. Interactions with the Abelson tyrosine kinase reveal compartmentalization of Eyes absent function between nucleus and cytoplasm. Dev Cell. 2009; 16:271–279. [PubMed: 19217428]
37. Li X, Oghi KA, Zhang J, Krones A, Bush KT, Glass CK, Nigam SK, Aggarwal AK, Maas R, Rose DW, et al. Eya protein phosphatase activity regulates Six1-Dach-Eya transcriptional effects in mammalian organogenesis. Nature. 2003; 426:247–254. [PubMed: 14628042]
38. Tootle TL, Silver SJ, Davies EL, Newman V, Latek RR, Mills IA, Selengut JD, Parlikar BE, Rebay I. The transcription factor Eyes absent is a protein tyrosine phosphatase. Nature. 2003; 426:299–302. [PubMed: 14628053]
39. Curtiss J, Mlodzik M. Morphogenetic furrow initiation and progression during eye development in *Drosophila* : the roles of *decapentaplegic*, *hedgehog* and *eyes absent*. Development. 2000; 127:1325–1336. [PubMed: 10683184]
40. Firth LC, Baker NE. Retinal determination genes as targets and possible effectors of extracellular signals. Dev Biol. 2009; 327:366–375. [PubMed: 19135045]
41. Baker NE. Transcription of the segment-polarity gene *wingless* in the imaginal discs of *Drosophila* and the phenotype of a pupal-lethal *wg* mutation. Development. 1988; 102:489–497. [PubMed: 3181031]
42. 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. 1995; 121:2279–2289. [PubMed: 7671795]
43. Treisman JE, Rubin GM. *wingless* inhibits morphogenetic furrow movement in the *Drosophila* eye disc. Development. 1995; 121:3519–3527. [PubMed: 8582266]
44. Heslip TR, Theisen H, Walker H, Marsh JL. Shaggy and Dishevelled exert opposite effects on Wingless and Decapentaplegic expression and on positional identity in imaginal discs. Development. 1997; 124:1069–1078. [PubMed: 9056781]
45. Legent K, Treisman JE. Wingless signaling in *Drosophila* eye development. Methods Mol Biol. 2008; 469:141–161. [PubMed: 19109709]
46. Cho KO, Choi KW. Fringe is essential for mirror symmetry and morphogenesis in the *Drosophila* eye. Nature. 1998; 396:272–276. [PubMed: 9834034]
47. Papayannopoulos V, Tomlinson A, Panin VM, Rauskolb C, Irvine KD. Dorsal-ventral signaling in the *Drosophila* eye. Science. 1998; 281:2031–2034. [PubMed: 9748163]
48. Dominguez M, de Celis JF. A dorsal/ventral boundary established by Notch controls growth and polarity in the *Drosophila* eye. Nature. 1998; 396:276–278. [PubMed: 9834035]
49. Cavodeassi F, Diez Del Corral R, Campuzano S, Dominguez M. Compartments and organising boundaries in the *Drosophila* eye: the role of the homeodomain Iroquois proteins. Development. 1999; 126:4933–4942. [PubMed: 10529412]
50. Sato A, Tomlinson A. Dorsal-ventral midline signaling in the developing *Drosophila* eye. Development. 2007; 134:659–667. [PubMed: 17215299]
51. Maurel-Zaffran C, Treisman JE. *pannier* acts upstream of *wingless* to direct dorsal eye disc development in *Drosophila*. Development. 2000; 127:1007–1016. [PubMed: 10662640]
52. Pereira PS, Pinho S, Johnson K, Couso JP, Casares F. A 3' cis-regulatory region controls *wingless* expression in the Drosophila eye and leg primordia. Dev Dyn. 2006; 235:225–234. [PubMed: 16261625]

53. Gutierrez-Avino FJ, Ferres-Marco D, Dominguez M. The position and function of the Notch-mediated eye growth organizer: the roles of JAK/STAT and Four-jointed. EMBO Rep. 2009; 10:1051–1058. [PubMed: 19662079]
54. Ekas LA, Baeg GH, Flaherty MS, Ayala-Camargo A, Bach EA. JAK/STAT signaling promotes regional specification by negatively regulating *wingless* expression in *Drosophila*. Development. 2006; 133:4721–4729. [PubMed: 17079268]
55. Chao JL, Tsai YC, Chiu SJ, Sun YH. Localized Notch signal acts through *eyg* and *upd* to promote global growth in *Drosophila* eye. Development. 2004; 131:3839–3847. [PubMed: 15253935]
56. Bach EA, Vincent S, Zeidler MP, Perrimon N. A sensitized genetic screen to identify novel regulators and components of the *Drosophila* janus kinase/signal transducer and activator of transcription pathway. Genetics. 2003; 165:1149–1166. [PubMed: 14668372]
57. Tsai YC, Sun YH. Long-range effect of Upd, a ligand for Jak/STAT pathway, on cell cycle in *Drosophila* eye development. Genesis. 2004; 39:141–153. [PubMed: 15170700]
58. Reynolds-Kenneally J, Mlodzik M. Notch signaling controls proliferation through cell-autonomous and non-autonomous mechanisms in the *Drosophila* eye. Dev Biol. 2005; 285:38–48. [PubMed: 16039641]
59. Ma C, Zhou Y, Beachy PA, Moses K. The segment polarity gene *hedgehog* is required for progression of the morphogenetic furrow in the developing *Drosophila* eye. Cell. 1993; 75:927–938. [PubMed: 8252628]
60. Heberlein U, Wolff T, Rubin GM. The TGF beta homolog Dpp and the segment polarity gene *hedgehog* are required for propagation of a morphogenetic wave in the *Drosophila* retina. Cell. 1993; 75:913–926. [PubMed: 8252627]
61. Borod ER, Heberlein U. Mutual regulation of *decapentaplegic* and *hedgehog* during the initiation of differentiation in the *Drosophila* retina. Dev Biol. 1998; 197:187–197. [PubMed: 9630745]
62. Bras-Pereira C, Bessa J, Casares F. *odd-skipped* genes specify the signaling center that triggers retinogenesis in *Drosophila*. Development. 2006; 133:4145–4149. [PubMed: 17021046]
63. Fu W, Baker NE. Deciphering synergistic and redundant roles of Hedgehog, Decapentaplegic and Delta that drive the wave of differentiation in *Drosophila* eye development. Development. 2003; 130:5229–5239. [PubMed: 12954721]
64. Ou CY, Lin YF, Chen YJ, Chien CT. Distinct protein degradation mechanisms mediated by Cul1 and Cul3 controlling Ci stability in *Drosophila* eye development. Genes Dev. 2002; 16:2403–2414. [PubMed: 12231629]
65. Baker NE, Bhattacharya A, Firth LC. Regulation of Hh signal transduction as *Drosophila* eye differentiation progresses. Dev Biol. 2009; 335:356–366. [PubMed: 19761763]
66. Jarman AP, Grell EH, Ackerman L, Jan LY, Jan YN. *atonal* is the proneural gene for *Drosophila* photoreceptors. Nature. 1994; 369:398–400. [PubMed: 8196767]
67. Greenwood S, Struhl G. Progression of the morphogenetic furrow in the *Drosophila* eye: the roles of Hedgehog, Decapentaplegic and the Raf pathway. Development. 1999; 126:5795–5808. [PubMed: 10572054]
68. Jarman AP, Sun Y, Jan LY, Jan YN. Role of the proneural gene, *atonal* in formation of *Drosophila* chordotonal organs and photoreceptors. Development. 1995; 121:2019–2030. [PubMed: 7635049]
69. Sun Y, Jan LY, Jan YN. Transcriptional regulation of *atonal* during development of the *Drosophila* peripheral nervous system. Development. 1998; 125:3731–3740. [PubMed: 9716538]
70. Zhang T, Ranade S, Cai CQ, Clouser C, Pignoni F. Direct control of neurogenesis by selector factors in the fly eye: regulation of *atonal* by Ey and So. Development. 2006; 133:4881–4889. [PubMed: 17108002]
71. Tanaka-Matakatsu M, Du W. Direct control of the proneural gene *atonal* by retinal determination factors during *Drosophila* eye development. Dev Biol. 2008; 313:787–801. [PubMed: 18083159]
72. Baker NE, Firth LC. Retinal determination genes function along with cell-cell signals to regulate *Drosophila* eye development: examples of multi-layered regulation by master regulators. BioEssays. 2011; 33:538–546. [PubMed: 21607995]
73. Melicharek D, Shah A, DiStefano G, Gangemi AJ, Orapallo A, Vrailas-Mortimer AD, Marenda DR. Identification of novel regulators of *atonal* expression in the developing *Drosophila* retina. Genetics. 2008; 180:2095–2110. [PubMed: 18832354]

74. Baker NE, Zitron AE. *Drosophila* eye development: Notch and Delta amplify a neurogenic pattern conferred on the morphogenetic furrow by Scabrous. Mech Dev. 1995; 49:173–189. [PubMed: 7734391]
75. Lim J, Jafar-Nejad H, Hsu YC, Choi KW. Novel function of the class I bHLH protein Daughterless in the negative regulation of proneural gene expression in the *Drosophila* eye. EMBO Rep. 2008; 9:1128–1133. [PubMed: 18758436]
76. Lee EC, Hu X, Yu SY, Baker NE. The *scabrous* gene encodes a secreted glycoprotein dimer and regulates proneural development in *Drosophila* eyes. Mol Cell Biol. 1996; 16:1179–1188. [PubMed: 8622662]
77. Powell PA, Wesley C, Spencer S, Cagan RL. Scabrous complexes with Notch to mediate boundary formation. Nature. 2001; 409:626–630. [PubMed: 11214322]
78. Baker NE, Yu S, Han D. Evolution of proneural *atonal* expression during distinct regulatory phases in the developing *Drosophila* eye. Curr Biol. 1996; 6:1290–1301. [PubMed: 8939576]
79. Frankfort BJ, Nolo R, Zhang Z, Bellen H, Mardon G. Senseless repression of *rough* is required for R8 photoreceptor differentiation in the developing *Drosophila* eye. Neuron. 2001; 32:403–414. [PubMed: 11709152]
80. Aerts S, Quan XJ, Claeys A, Naval Sanchez M, Tate P, Yan J, Hassan BA. Robust target gene discovery through transcriptome perturbations and genome-wide enhancer predictions in *Drosophila* uncovers a regulatory basis for sensory specification. PLoS Biol. 2010; 8:e1000435. [PubMed: 20668662]
81. Brown NL, Sattler CA, Paddock SW, Carroll SB. Hairy and Emc negatively regulate morphogenetic furrow progression in the *Drosophila* eye. Cell. 1995; 80:879–887. [PubMed: 7697718]
82. Bhattacharya A, Baker NE. A network of broadly expressed HLH genes regulates tissue-specific cell fates. Cell. 2011; 147:881–892. [PubMed: 22078884]
83. Baonza A, Freeman M. Notch signalling and the initiation of neural development in the *Drosophila* eye. Development. 2001; 128:3889–3898. [PubMed: 11641214]
84. Lim J, Choi KW. Bar homeodomain proteins are anti-proneural in the *Drosophila* eye: transcriptional repression of *atonal* by Bar prevents ectopic retinal neurogenesis. Development. 2003; 130:5965–5974. [PubMed: 14573515]
85. Wasserman JD, Urban S, Freeman M. A family of *rhomboid* -like genes: *Drosophila rhomboid-1* and *roughoid/rhomboid-3* cooperate to activate EGF receptor signaling. Genes Dev. 2000; 14:1651–1663. [PubMed: 10887159]
86. Urban S, Lee JR, Freeman M. *Drosophila* Rhomboid-1 defines a family of putative intramembrane serine proteases. Cell. 2001; 107:173–182. [PubMed: 11672525]
87. Baonza A, Casci T, Freeman M. A primary role for the epidermal growth factor receptor in ommatidial spacing in the *Drosophila* eye. Curr Biol. 2001; 11:396–404. [PubMed: 11301250]
88. Freeman M. The *spitz* gene is required for photoreceptor determination in the *Drosophila* eye where it interacts with the EGF receptor. Mech Dev. 1994; 48:25–33. [PubMed: 7833286]
89. Freeman M. Reiterative use of the EGF receptor triggers differentiation of all cell types in the *Drosophila* eye. Cell. 1996; 87:651–660. [PubMed: 8929534]
90. Tio M, Ma C, Moses K. *spitz* a *Drosophila* homolog of *transforming growth factor-alpha* is required in the founding photoreceptor cells of the compound eye facets. Mech Dev. 1994; 48:13–23. [PubMed: 7833285]
91. Freeman M, Kimmel BE, Rubin GM. Identifying targets of the Rough homeobox gene of *Drosophila* : evidence that *rhomboid* functions in eye development. Development. 1992; 116:335–346. [PubMed: 1363086]
92. Miura GI, Buglino J, Alvarado D, Lemmon MA, Resh MD, Treisman JE. Palmitoylation of the EGFR ligand Spitz by Rasp increases Spitz activity by restricting its diffusion. Dev Cell. 2006; 10:167–176. [PubMed: 16459296]
93. Golembo M, Schweitzer R, Freeman M, Shilo BZ. *argos* transcription is induced by the *Drosophila* EGF receptor pathway to form an inhibitory feedback loop. Development. 1996; 122:223–230. [PubMed: 8565833]

94. Freeman M. Cell determination strategies in the *Drosophila* eye. Development. 1997; 124:261–270. [PubMed: 9053303]
95. Klein DE, Nappi VM, Reeves GT, Shvartsman SY, Lemmon MA. Argos inhibits epidermal growth factor receptor signalling by ligand sequestration. Nature. 2004; 430:1040–1044. [PubMed: 15329724]
96. Tsuda L, Nagaraj R, Zipursky SL, Banerjee U. An EGFR/Ebi/Sno pathway promotes Delta expression by inactivating Su(H)/SMRTER repression during inductive Notch signaling. Cell. 2002; 110:625–637. [PubMed: 12230979]
97. Tomlinson A, Struhl G. Delta/Notch and Boss/Sevenless signals act combinatorially to specify the *Drosophila* R7 photoreceptor. Mol Cell. 2001; 7:487–495. [PubMed: 11463374]
98. Flores GV, Duan H, Yan H, Nagaraj R, Fu W, Zou Y, Noll M, Banerjee U. Combinatorial signaling in the specification of unique cell fates. Cell. 2000; 103:75–85. [PubMed: 11051549]
99. Nagaraj R, Banerjee U. Combinatorial signaling in the specification of primary pigment cells in the *Drosophila* eye. Development. 2007; 134:825–831. [PubMed: 17251265]
100. Rogers EM, Brennan CA, Mortimer NT, Cook S, Morris AR, Moses K. Pointed regulates an eye-specific transcriptional enhancer in the *Drosophila hedgehog* gene, which is required for the movement of the morphogenetic furrow. Development. 2005; 132:4833–4843. [PubMed: 16207753]
101. Tomlinson A, Ready DF. Sevenless: a cell-specific homeotic mutation of the *Drosophila* eye. Science. 1986; 231:400–402. [PubMed: 17735014]
102. Reinke R, Zipursky SL. Cell-cell interaction in the *Drosophila* retina: the *bride of sevenless* gene is required in photoreceptor cell R8 for R7 cell development. Cell. 1988; 55:321–330. [PubMed: 3167983]
103. Harris WA, Stark WS, Walker JA. Genetic dissection of the photoreceptor system in the compound eye of *Drosophila melanogaster*. J Physiol. 1976; 256:415–439. [PubMed: 16992509]
104. Banerjee U, Renfranz PJ, Pollock JA, Benzer S. Molecular characterization and expression of *sevenless* a gene involved in neuronal pattern formation in the *Drosophila* eye. Cell. 1987; 49:281–291. [PubMed: 2882857]
105. Hafen E, Basler K, Edstroem JE, Rubin GM. *sevenless* a cell-specific homeotic gene of *Drosophila* encodes a putative transmembrane receptor with a tyrosine kinase domain. Science. 1987; 236:55–63. [PubMed: 2882603]
106. Tomlinson A, Ready DF. Cell fate in the *Drosophila* ommatidium. Dev Biol. 1987; 123:264–275. [PubMed: 17985474]
107. Kramer H, Cagan RL, Zipursky SL. Interaction of Bride of sevenless membrane-bound ligand and the Sevenless tyrosine-kinase receptor. Nature. 1991; 352:207–212. [PubMed: 1857416]
108. Simon MA, Bowtell DD, Dodson GS, Laverty TR, Rubin GM. Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the Sevenless protein tyrosine kinase. Cell. 1991; 67:701–716. [PubMed: 1934068]
109. Simon MA, Carthew RW, Fortini ME, Gaul U, Mardon G, Rubin GM. Signal transduction pathway initiated by activation of the Sevenless tyrosine kinase receptor. Cold Spring Harbor Symp Quant Biol. 1992; 57:375–380. [PubMed: 1339672]
110. McKay MM, Morrison DK. Integrating signals from RTKs to ERK/MAPK. Oncogene. 2007; 26:3113–3121. [PubMed: 17496910]
111. Xu C, Kauffmann RC, Zhang J, Kladny S, Carthew RW. Overlapping activators and repressors delimit transcriptional response to receptor tyrosine kinase signals in the *Drosophila* eye. Cell. 2000; 103:87–97. [PubMed: 11051550]
112. Tang AH, Neufeld TP, Kwan E, Rubin GM. PHYL acts to down-regulate TTK88, a transcriptional repressor of neuronal cell fates, by a SINA-dependent mechanism. Cell. 1997; 90:459–467. [PubMed: 9267026]
113. Li S, Li Y, Carthew RW, Lai ZC. Photoreceptor cell differentiation requires regulated proteolysis of the transcriptional repressor Tramtrack. Cell. 1997; 90:469–478. [PubMed: 9267027]
114. Dong X, Tsuda L, Zavitz KH, Lin M, Li S, Carthew RW, Zipursky SL. *ebi* regulates epidermal growth factor receptor signaling pathways in *Drosophila*. Genes Dev. 1999; 13:954–965. [PubMed: 10215623]

115. Cooper MT, Bray SJ. R7 photoreceptor specification requires Notch activity. Curr Biol. 2000; 10:1507–1510. [PubMed: 11114517]
116. Tomlinson A, Mavromatakis YE, Struhl G. Three distinct roles for Notch in *Drosophila* R7 photoreceptor specification. PLoS Biol. 2011; 9:e1001132. [PubMed: 21886484]
117. Hayashi T, Xu C, Carthew RW. Cell-type-specific transcription of *prospero* is controlled by combinatorial signaling in the *Drosophila* eye. Development. 2008; 135:2787–2796. [PubMed: 18635611]
118. Daga A, Karlovich CA, Dumstrei K, Banerjee U. Patterning of cells in the *Drosophila* eye by Lozenge, which shares homologous domains with AML1. Genes Dev. 1996; 10:1194–1205. [PubMed: 8675007]
119. Flores GV, Daga A, Kalhor HR, Banerjee U. Lozenge is expressed in pluripotent precursor cells and patterns multiple cell types in the *Drosophila* eye through the control of cell-specific transcription factors. Development. 1998; 125:3681–3687. [PubMed: 9716533]
120. Yan H, Canon J, Banerjee U. A transcriptional chain linking eye specification to terminal determination of cone cells in the *Drosophila* eye. Dev Biol. 2003; 263:323–329. [PubMed: 14597205]
121. Moses K, Rubin GM. *glass* encodes a site-specific DNA-binding protein that is regulated in response to positional signals in the developing *Drosophila* eye. Genes Dev. 1991; 5:583–593. [PubMed: 2010085]
122. Mlodzik M, Hiromi Y, Weber U, Goodman CS, Rubin GM. The *Drosophila seven-up* gene, a member of the steroid receptor gene superfamily, controls photoreceptor cell fates. Cell. 1990; 60:211–224. [PubMed: 2105166]
123. Miller AC, Seymour H, King C, Herman TG. Loss of *seven-up* from *Drosophila* R1/R6 photoreceptors reveals a stochastic fate choice that is normally biased by Notch. Development. 2008; 135:707–715. [PubMed: 18199577]
124. Dokucu ME, Zipursky SL, Cagan RL. Atonal, Rough and the resolution of proneural clusters in the developing *Drosophila* retina. Development. 1996; 122:4139–4147. [PubMed: 9012533]
125. Pepple KL, Atkins M, Venken K, Wellnitz K, Harding M, Frankfort B, Mardon G. Two-step selection of a single R8 photoreceptor: a bistable loop between Senseless and Rough locks in R8 fate. Development. 2008; 135:4071–4079. [PubMed: 19004852]
126. Domingos PM, Mlodzik M, Mendes CS, Brown S, Steller H, Mollereau B. Spalt transcription factors are required for R3/R4 specification and establishment of planar cell polarity in the *Drosophila* eye. Development. 2004; 131:5695–5702. [PubMed: 15509769]
127. Higashijima S, Kojima T, Michiue T, Ishimaru S, Emori Y, Saigo K. Dual *Bar* homeo box genes of *Drosophila* required in two photoreceptor cells, R1 and R6, and primary pigment cells for normal eye development. Genes Dev. 1992; 6:50–60. [PubMed: 1346120]
128. Mollereau B, Dominguez M, Webel R, Colley NJ, Keung B, de Celis JF, Desplan C. Two-step process for photoreceptor formation in *Drosophila*. Nature. 2001; 412:911–913. [PubMed: 11528479]
129. Domingos PM, Brown S, Barrio R, Ratnakumar K, Frankfort BJ, Mardon G, Steller H, Mollereau B. Regulation of R7 and R8 differentiation by the *spalt* genes. Dev Biol. 2004; 273:121–133. [PubMed: 15302602]
130. Longley RL Jr, Ready DF. Integrins and the development of three-dimensional structure in the *Drosophila* compound eye. Dev Biol. 1995; 171:415–433. [PubMed: 7556924]
131. Hong Y, Ackerman L, Jan LY, Jan YN. Distinct roles of Bazooka and Stardust in the specification of *Drosophila* photoreceptor membrane architecture. Proc Natl Acad Sci USA. 2003; 100:12712–12717. [PubMed: 14569003]
132. Izaddoost S, Nam SC, Bhat MA, Bellen HJ, Choi KW. *Drosophila* Crumbs is a positional cue in photoreceptor adherens junctions and rhabdomeres. Nature. 2002; 416:178–183. [PubMed: 11850624]
133. Kumar JP, Ready DF. Rhodopsin plays an essential structural role in *Drosophila* photoreceptor development. Development. 1995; 121:4359–4370. [PubMed: 8575336]

134. Li BX, Satoh AK, Ready DF. Myosin V, Rab11, and dRip11 direct apical secretion and cellular morphogenesis in developing *Drosophila* photoreceptors. J Cell Biol. 2007; 177:659–669. [PubMed: 17517962]
135. Mishra M, Oke A, Lebel C, McDonald EC, Plummer Z, Cook TA, Zelhof AC. Pph13 and Orthodenticle define a dual regulatory pathway for photoreceptor cell morphogenesis and function. Development. 2010; 137:2895–2904. [PubMed: 20667913]
136. Fichelson P, Brigui A, Pichaud F. Orthodenticle and Kruppel homolog 1 regulate *Drosophila* photoreceptor maturation. Proc Natl Acad Sci USA. 2012
137. Yamaguchi S, Desplan C, Heisenberg M. Contribution of photoreceptor subtypes to spectral wavelength preference in *Drosophila*. Proc Natl Acad Sci USA. 2010; 107:5634–5639. [PubMed: 20212139]
138. Wernet MF, Labhart T, Baumann F, Mazzoni EO, Pichaud F, Desplan C. Homothorax switches function of *Drosophila* photoreceptors from color to polarized light sensors. Cell. 2003; 115:267–279. [PubMed: 14636555]
139. Wernet MF, Mazzoni EO, Celik A, Duncan DM, Duncan I, Desplan C. Stochastic *spineless* expression creates the retinal mosaic for colour vision. Nature. 2006; 440:174–180. [PubMed: 16525464]
140. Mikeladze-Dvali T, Wernet MF, Pistillo D, Mazzoni EO, Teleman AA, Chen YW, Cohen S, Desplan C. The growth regulators Warts/Lats and Melted interact in a bistable loop to specify opposite fates in *Drosophila* R8 photoreceptors. Cell. 2005; 122:775–787. [PubMed: 16143107]
141. Jukam D, Desplan C. Binary regulation of Hippo pathway by Merlin/NF2, Kibra, Lgl, and Melted specifies and maintains postmitotic neuronal fate. Dev Cell. 2011; 21:874–887. [PubMed: 22055343]
142. Wolff T, Ready DF. Cell death in normal and rough eye mutants of *Drosophila*. Development. 1991; 113:825–839. [PubMed: 1821853]
143. Larson DE, Johnson RI, Swat M, Cordero JB, Glazier JA, Cagan RL. Computer simulation of cellular patterning within the *Drosophila* pupal eye. PLoS Comp Biol. 2010; 6:e1000841.
144. Bao S, Cagan R. Preferential adhesion mediated by Hibris and Roughest regulates morphogenesis and patterning in the *Drosophila* eye. Dev Cell. 2005; 8:925–935. [PubMed: 15935781]
145. Brachmann CB, Cagan RL. Patterning the fly eye: the role of apoptosis. Trends Genet. 2003; 19:91–96. [PubMed: 12547518]

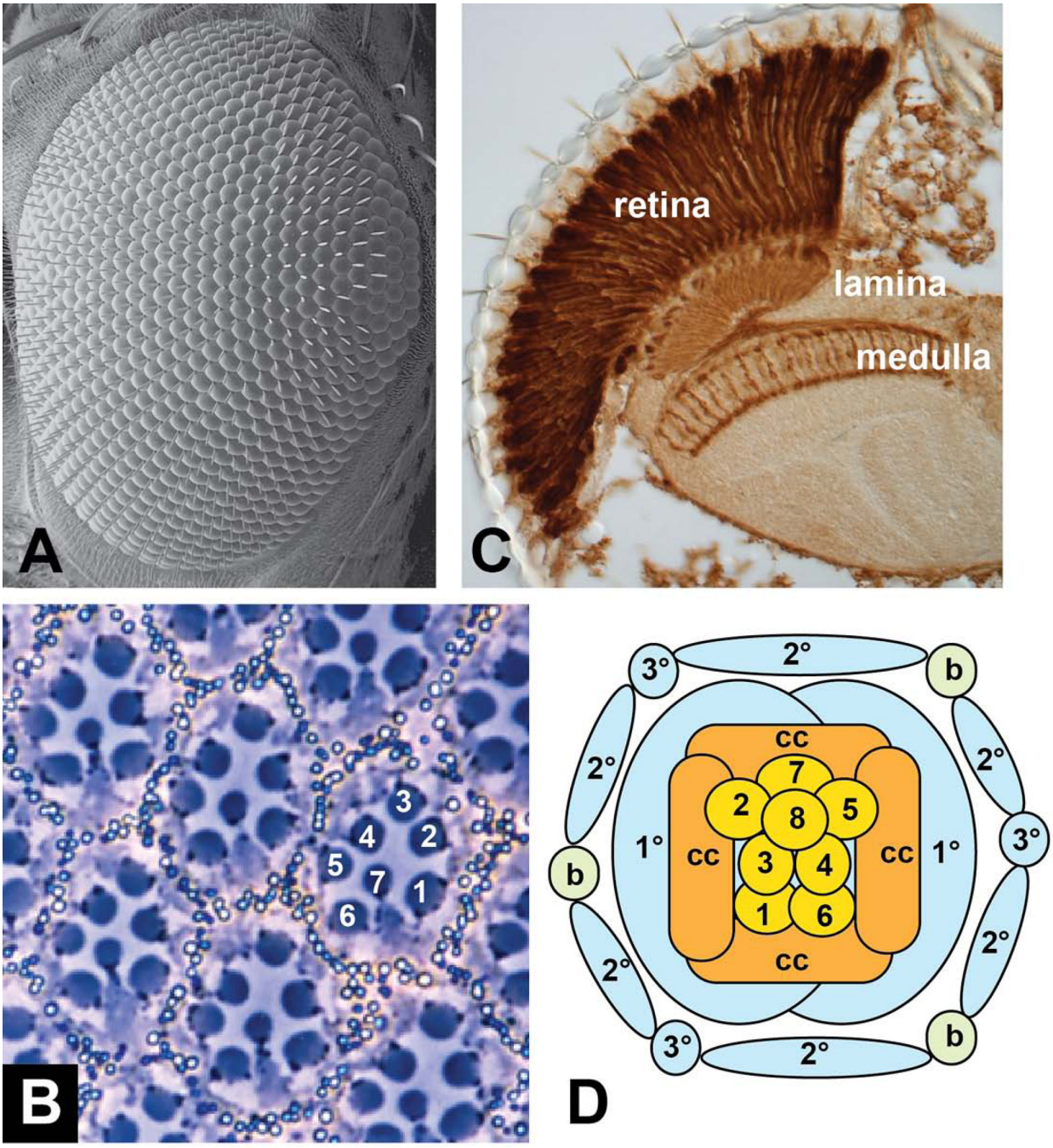

Figure 1. Structure of the adult *Drosophila* eye

(A) shows a scanning electron micrograph of the surface of the eye, demonstrating the hexagonal packing of the ommatidia. (B) shows a tangential section through the eye, illustrating the characteristic trapezoidal arrangement of the rhabdomeres of photoreceptors R1-R7. The rhabdomere of R8 lies below that of R7. (C) shows a coronal section through the adult head of a fly expressing *lacZ* in all photoreceptors, stained with anti-β-galactosidase. This section shows the elongated shape of the photoreceptor cells in the retina and their axons extending to the lamina and medulla. (D) is a diagram of the arrangement of

cell types found in each ommatidium. 1–8, photoreceptors R1-R8; cc, cone cells; 1°, 2°, 3°, pigment cells; b, mechanosensory bristle.

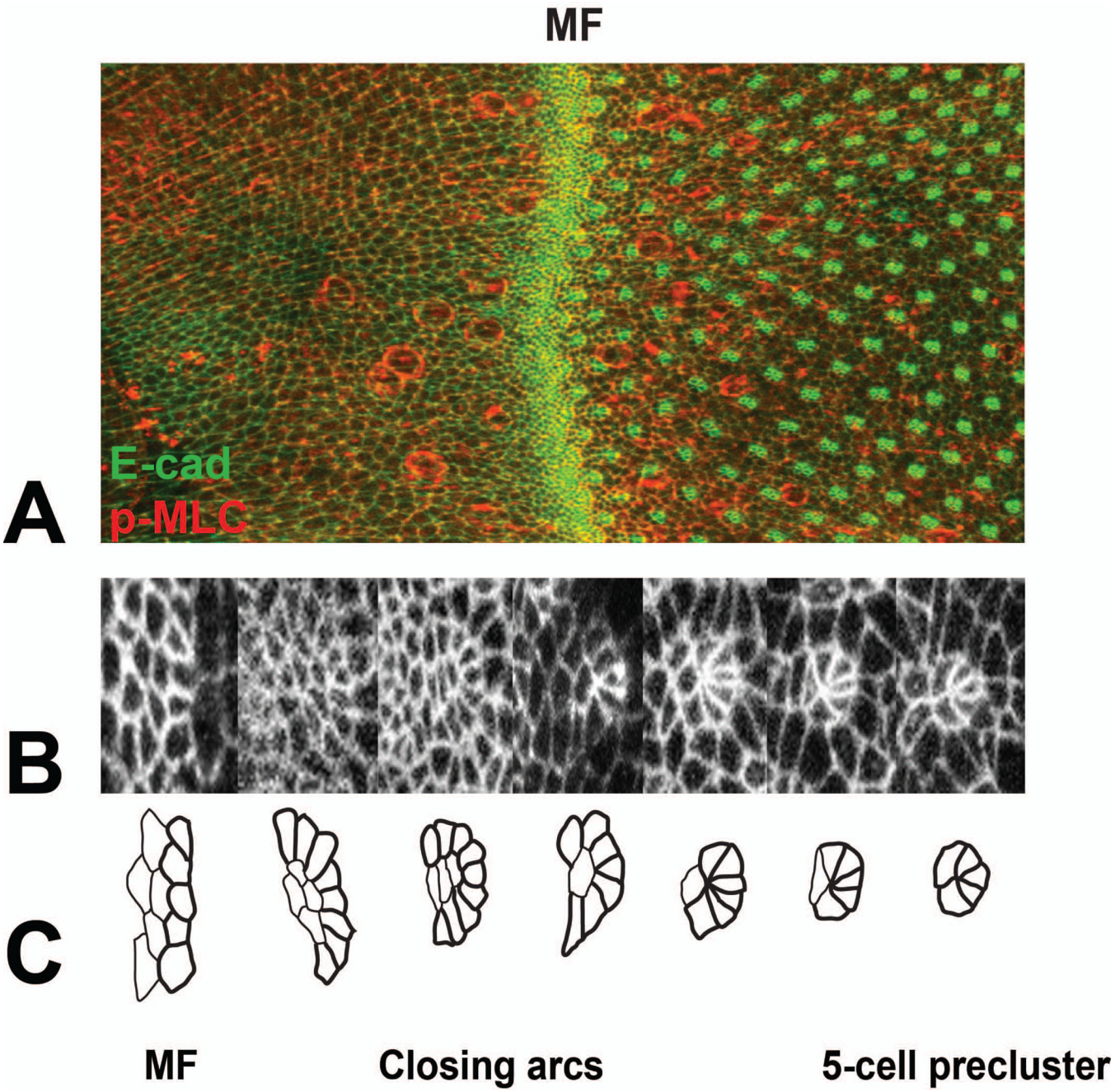

Figure 2. Pattern formation in the developing eye disc

(A) shows part of an eye disc labeled with E-cadherin-GFP (E-cad-GFP, green) and anti-phosphorylated myosin light chain (p-MLC, red). Anterior is to the left. The morphogenetic furrow (MF) is indicated. (B) shows a series of E-cad-GFP-labeled cell clusters increasing in age from left to right, and (C) shows tracings of the same clusters. Unpatterned cells in the morphogenetic furrow transform into arcs, which close by removal of the central cells and ultimately become 5-cell preclusters. Figure kindly provided by Franck Pichaud.

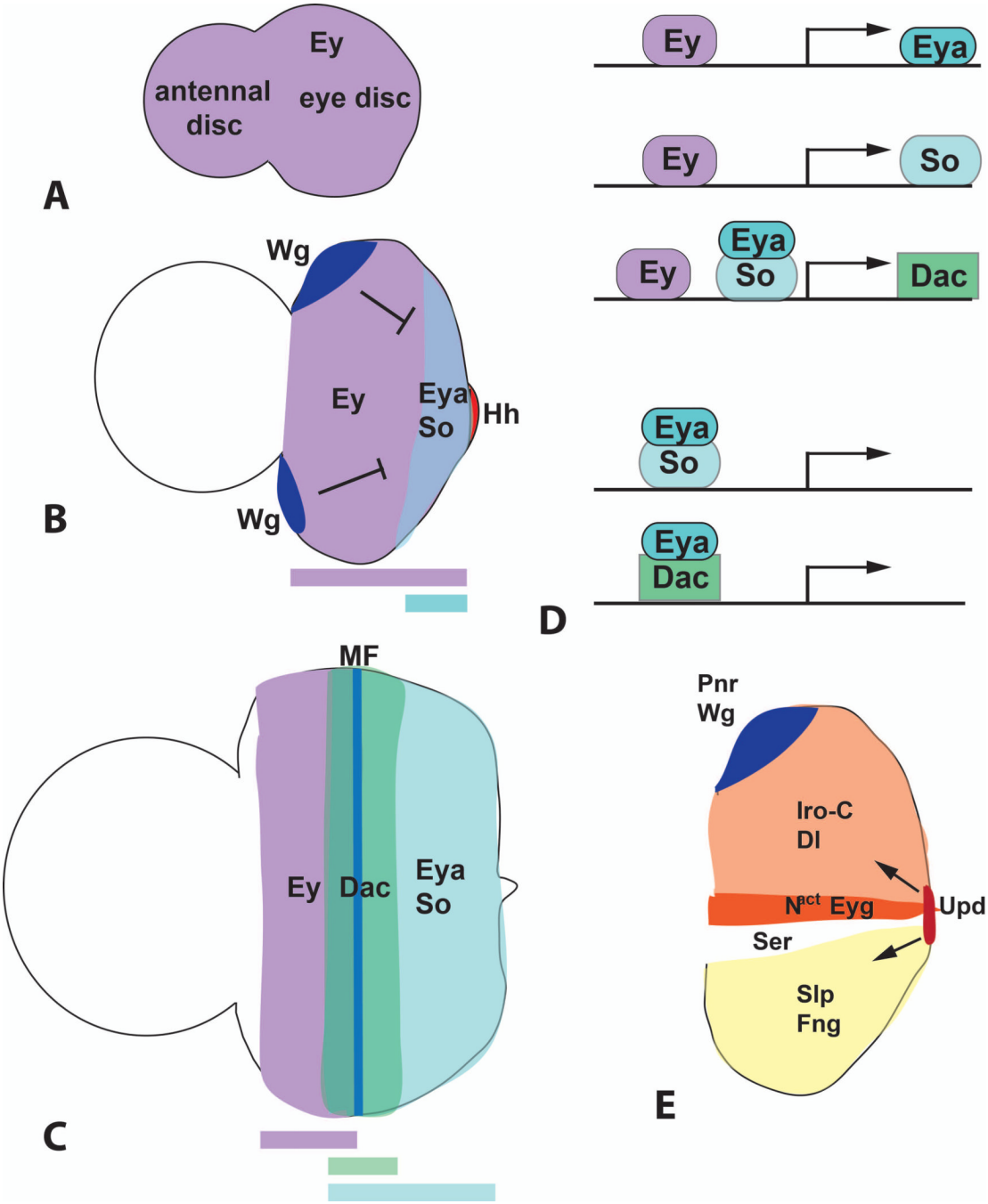

Figure 3. Retinal determination genes

(A–C) are diagrams showing the expression pattern of Ey, Eya, So and Dac in first instar (A), second instar (B) and third instar (C) eye-antennal discs. Colored bars below the diagrams indicate the regions in which these expression domains overlap. Repression of *eya* by anterior Wg and its activation by posterior Hh is indicated in (B). MF, morphogenetic furrow. (D) represents the functional relationships between these transcription factors. Ey directly activates *eya* and *so* transcription, and Ey, Eya and So all contribute to *dac* activation. Eya can interact with the DNA-binding protein So to form a compound transcription factor that regulates downstream genes, and may also regulate gene expression in a complex with Dac. (E) shows the expression domains of some of the factors that drive

dorsal-ventral compartmentalization and growth of the early eye disc. Dorsally expressed Pnr activates *wg* expression, and Wg then establishes the expression domains of the Iro-C and Slp transcription factors. These control the compartmentalized distribution of Notch ligands and modifying enzymes that lead to Notch activation at the dorsoventral midline. Downstream targets of Notch that regulate growth include the transcription factor Eyg and the long-range signaling molecule Upd.

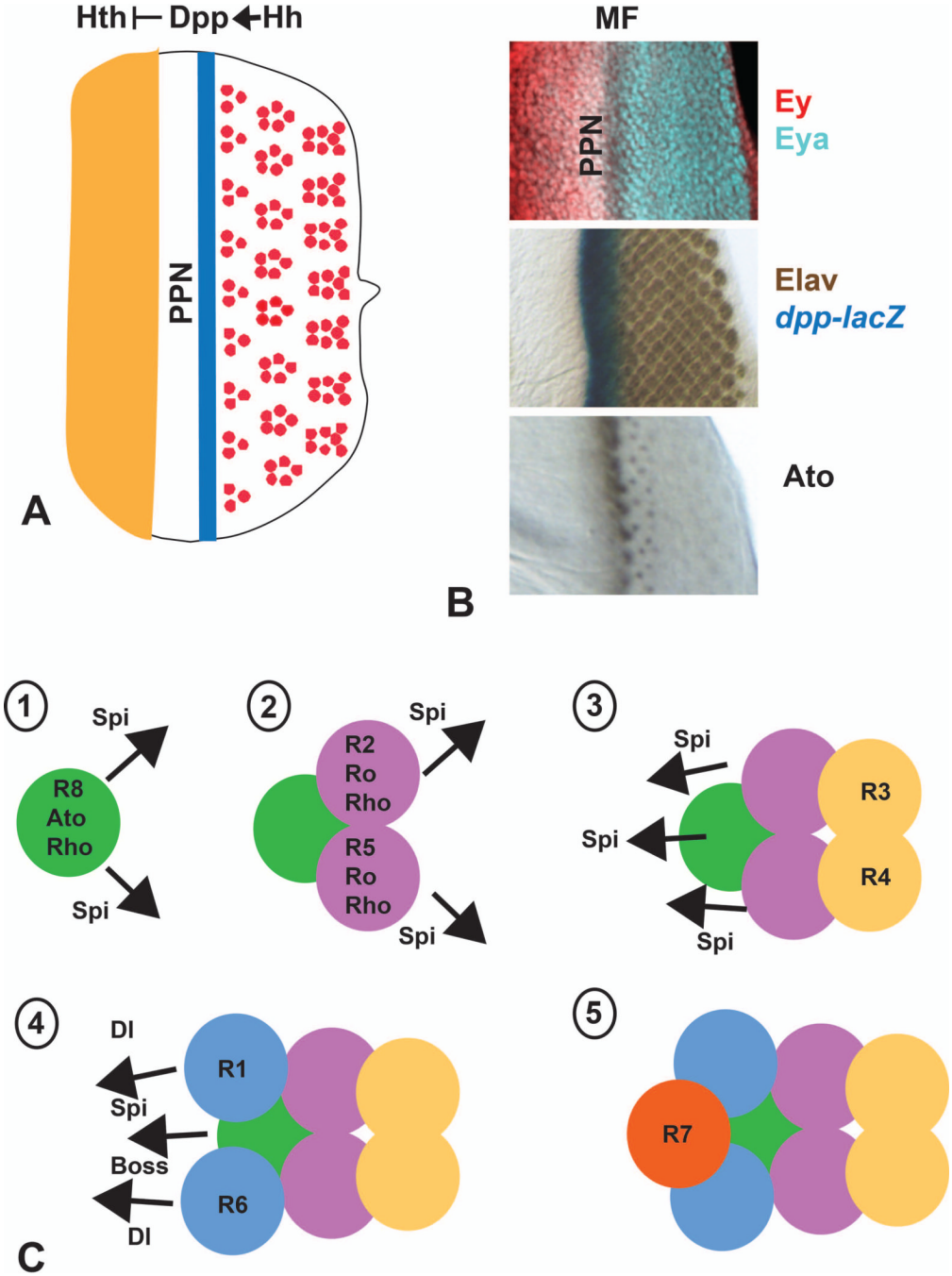

Figure 4. Progression of the morphogenetic furrow

(A) is a diagram showing that Hh expressed in developing photoreceptors activates a stripe of *dpp* expression in the morphogenetic furrow. Dpp then acts at a long range to repress *hth*, limiting it to the anterior of the eye disc and establishing a preproneural zone (PPN) in which cells can respond to Hh. (B) shows regions of third instar eye discs stained for the indicated markers. Elav is a neuronal-specific protein used to mark differentiating photoreceptors. (C) is a diagram of the five signaling steps involved in recruitment of each photoreceptor or photoreceptor pair to the forming ommatidium. Rho proteins expressed in R8, R2 and R5 allow these cells to produce Spi, which is instrumental in recruiting all

photoreceptors other than R8. Dl produced by R1 and R6 and Boss produced by R8 are also necessary to recruit R7.

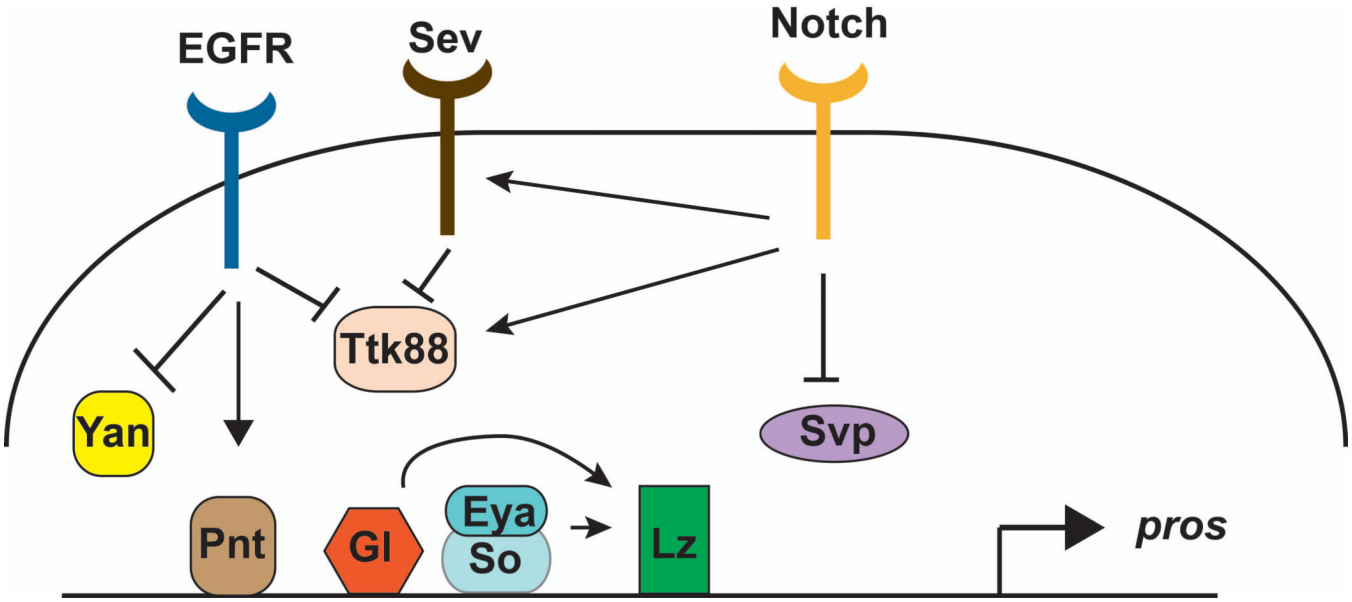

Figure 5. Diagram of the factors involved in regulating *prospero*, a gene expressed in R7

The activator PntP2 and repressors Yan and Ttk88 are regulated by EGFR signaling, and Ttk88 also responds to Sev and Notch signaling. In addition, Notch positively regulates *sev* expression and negatively regulates *svp*, which encodes a repressor of *pros*. The eye-specific transcription factors Gl, Eya/So, and Lz also contribute to pros activation. *lz* is itself a target of Gl, Eya and So. This diagram only indicates the factors that bind to the Pros enhancer, and not the correct number or placement of their binding sites.

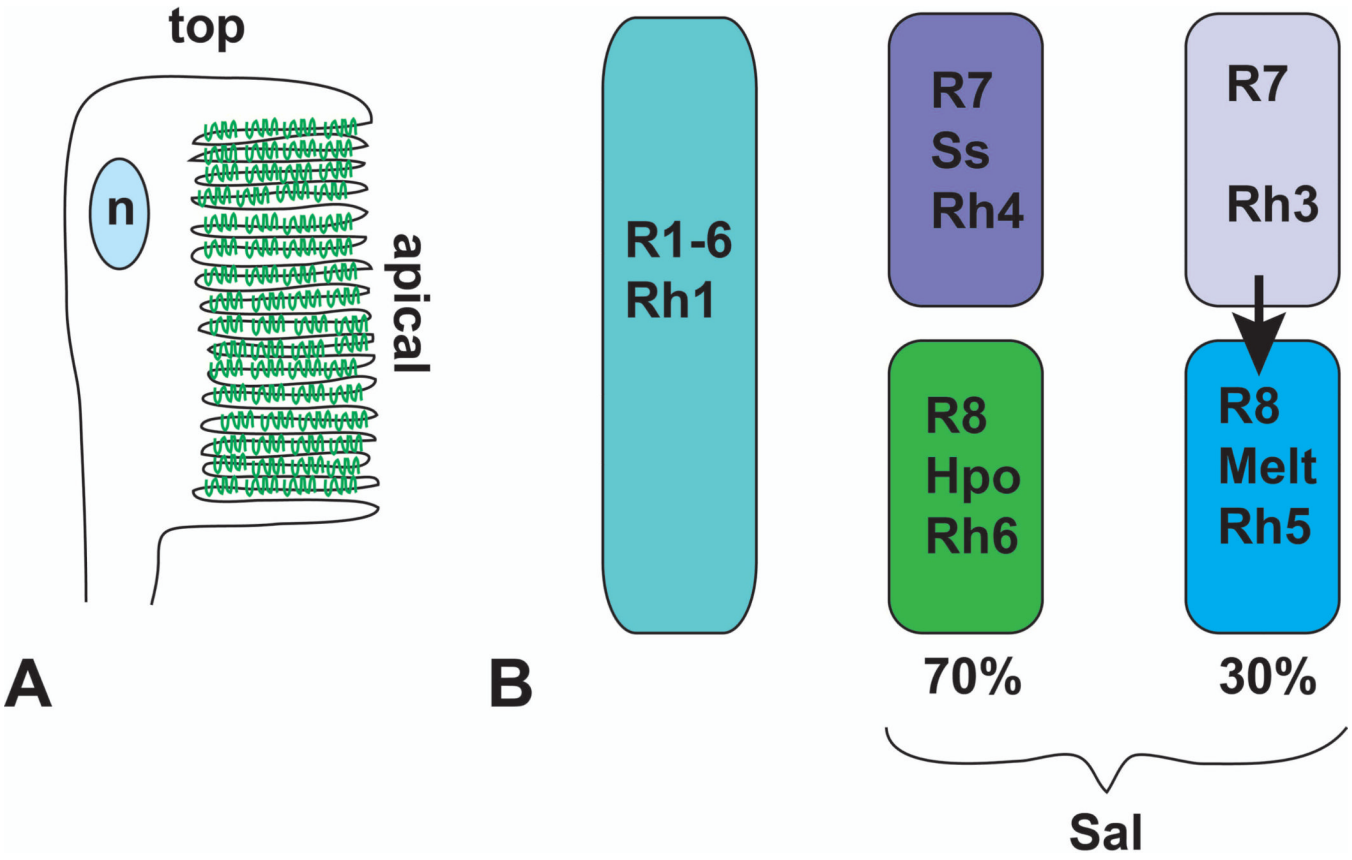

Figure 6. Terminal differentiation involves Rhodopsin expression and localization

(A) is a diagram of the adult rhabdomere, indicating the rotation and folding of the apical surface. Rhodopsin molecules are represented in green. n, nucleus. (B) shows the distribution of the five different rhodopsins between the eight photoreceptors. All R1–6 cells express Rh1. Approximately 70% of R7 cells express Ss and Rh4. The R8 cells in the same ommatidia activate the Hpo pathway and express Rh6. In the absence of Ss, R7 cells express Rh3 and signal to the R8 cells in their ommatidia to express Melt and Rh5