

Genetic and Developmental Mechanisms Underlying the Formation of the *Drosophila* Compound Eye

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The compound eye of *Drosophila melanogaster* consists of individual subunits (“ommatidia”), each containing photoreceptors and support cells. These cells derive from an undifferentiated epithelium in the eye imaginal disc and their differentiation follows a highly stereotypic pattern. Sequential commitment of pluripotent cells to become specialized cells of the visual system serves as a unique model system to study basic mechanisms of tissue development. In the past years, many regulatory genes that govern the development of the compound eye have been identified and their mode of action genetically dissected. Transcription factor networks in combination with cell–cell signalling pathways regulate the development of the eye tissue in a precise temporal and spatial manner. Here, we review the recent advances on how a single-cell-layered epithelium is patterned to give rise to the compound eye. We discuss the molecular pathways controlling differentiation of individual photoreceptors, through which they acquire their functional specificity.

Key words: compound eye; photoreceptors; Rhodopsins; eye disc; morphogenetic furrow

INTRODUCTION

The eye of *Drosophila melanogaster* is widely used to study mechanisms of tissue specification and cell fate determination (Sprecher and Desplan, 2009a). The adult compound eye consists of 750–800 independent subunits, termed “ommatidia,” which form a characteristic neurocrystalline lattice (Fig. 1A) (Ready et al., 1976). Each ommatidium consists of a stereotypic array of photoreceptor neurons (PRs), lens-secreting cone cells, and pigment cells (Charlton-Perkins and Cook, 2010). Cells building ommatidia develop from an epithelial

sac of the larva, the eye-antennal imaginal disc (Fig.1B). In this disc, epithelial cells are sequentially committed to become PRs or support cells. Differentiation starts posterior and terminates anterior of the eye part of the disc. The morphogenetic furrow (MF) crosses the eye disc, patterning proliferating and undifferentiated precursor cells into highly organized developing and differentiating eye tissue (Wolff and Ready, 1991). A number of signalling pathways and downstream genes ensure that cell specification within the MF will occur in a precise spatial and temporal manner.

Each ommatidium is composed of eight PRs, which are further divided into six outer (R1–R6) and two inner PRs (R7 and R8). Spectral sensitivity of the different PR subtypes is defined by the expression of the *rhodopsin* (*rh*) genes, which encode photosensitive G protein–coupled receptors (GPCRs) (Terakita, 2005). Rhodopsins occupy specialized apical membranes, called rhabdomeres, and they form a complex with the chromophore 11-cis retinal. Upon light stimulation, 11-cis retinal is isomerized to the all-trans form, which causes conformational changes in the receptors, activating the coupled G protein. This triggers

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an intracellular cascade that results in the opening of receptor channels and the depolarization of the cell (Yau and Hardie, 2009). Therefore, Rhodopsins are responsible for converting light signals into electrical stimuli.

PRs are sensitive to a certain spectrum of light wavelengths, depending on which *rhodopsin* gene they express. The *Drosophila* genome contains seven *rhodopsin* genes, six of which (Rh1–Rh6) are well described. The seventh *rhodopsin* gene has not been characterized so far (Adams et al., 2000), but its existence has been revealed by the *Drosophila* genome-sequencing project (Brody and Cravchik, 2000; Broeck, 2001). The pattern of expression of this *rhodopsin* gene remains unknown. Outer PRs in the retina express the broad-spectrum Rhodopsin 1 (Rh1) and function in the photoperception under dim light conditions, as well as in the detection of motion. They are often regarded as the equivalent to the vertebrate rods, the PRs responsible for scotopic (“night”) vision. The two inner PRs mediate colour discrimination, functionally resembling the vertebrate cones. Thus, outer and inner PRs fulfil distinct functions. These two types of PRs also project their axons to different optic ganglia: outer PRs terminate in the lamina and inner PRs in the medulla (Clandinin and Zipursky, 2002). R7 PRs express UV-sensitive Rhodopsins, either Rh3 or Rh4. R8 PRs lie underneath the R7 and express either the blue-sensitive Rh5 or the green-sensitive Rh6. The eye is a mosaic of two major types of ommatidia, which differ in the expression of inner PR *rhodopsins*: the “pale” and the “yellow” ommatidia. In pale ommatidia, R7 express Rh3 and R8 express Rh5, while in yellow ommatidia, Rh4 is expressed in R7 and Rh6 in R8. About 70% of the compound eye consists of yellow and 30% of pale ommatidia.

The architecture of the compound eye and precise positioning of cells within the eye is the result of highly orchestrated developmental processes. Seemingly unpatterned pluripotent precursor cells residing in the eye imaginal disc are transformed into highly specialized cells within the MF, in a precise and highly stereotypic manner. First investigations on

the patterning mechanisms of the fly retina started over 30 years ago with the landmark study by Don Ready in the Benzer lab (Ready et al., 1976). Since then, a wealth of studies on the processes acting in forming the compound eye has uncovered many important developmental and genetic mechanisms, signalling pathways, and general concepts in developmental and cell biology (Cagan, 2009).

Here, we provide an overview of the developmental mechanisms governing eye formation, from an unpatterned epithelium to the final step of terminal differentiation and choice of *rhodopsin* expression. We specifically focus on the following aspects of eye development. First, we describe the genes and signalling pathways that regulate early steps controlling retinal determination. Next, we depict the steps defining how the MF patterns the eye disc, which leads to the recruitment and differentiation of the specific PR subtypes. Finally, we describe the developmental program acting in defining *rhodopsin* expression and spectral sensitivity during terminal differentiation of PRs. We discuss recent findings in view of the profound knowledge that has emerged from research during the last decades. At the same time, we integrate past findings with the most important recent advances in the field.

“SELECTOR” GENES IN EYE DEVELOPMENT

Development of the adult eye begins with a cluster of cells at the anterior-dorsal part of the embryo, which upon continuous proliferation give rise during larval stages to an epithelial sac, the eye-antennal imaginal disc (Fig. 1B). This disc consists of an anterior part that will generate the antenna, and a posterior part that will generate the retina, the vertex, and the ocelli. The subdivision of the antennal-eye field is the result of interaction between signalling pathways and selector genes. During embryogenesis, all cells that will give rise to the eye-antennal disc are marked by the expression of the transcription factors Eyeless (Ey) and Twin of eyeless (Toy). During the early second instar larval stage, the two parts of the disc begin to be separated: Ey expression

retracts to the posterior part that will give rise to the eye, whereas the homeodomain transcription factor Cut starts to be expressed exclusively in the antennal region (Kenyon et al., 2003). Two major signalling pathways, the Epidermal Growth Factor Receptor (EGFR) and the Notch (N) pathways, playing diverse roles throughout eye development, are involved in subdividing the eye-antennal disc. The EGFR pathway has been shown to specify an antennal fate by repressing Ey expression at the anterior domain (Kumar and Moses 2001a). Conversely, N signalling promotes eye formation. It has been suggested that N contributes to the establishment of the eye field by promoting the proliferation of retinal precursors and not by directly regulating the expression of selector genes (Dominguez et al., 2004; Kenyon et al., 2003).

Once the region of the eye-antennal disc that will give rise to the eye field has been determined, refined regulatory mechanisms trigger formation of the eye tissue. During this time, undifferentiated cells are sequentially committed to adopt a retinal fate, starting at the posterior edge and ending at the anterior part of the disc. The genes that mediate retinal development form a regulatory network, the Retinal Determination Network (RDN), rather than a single signalling cascade (Fig. 2A). This network is characterized by complex interactions among its members, autoregulatory and feedback loops. Another characteristic of the RDN is that a single gene can participate in several steps and control the expression of more than one gene within the network. This depends on the complexes the gene products form with other RD proteins or co-activators/co-repressors. The RDN genes are expressed in different phases during the patterning of the retina and each of them exhibits a distinct spatial expression pattern (Kumar, 2010).

Members of the RDN are often termed “selector” genes, which means that upon ectopic expression they have the ability of converting the fate of one tissue to that of an eye tissue (Fig. 2C). Depending on the Gal4 driver lines used, it is possible to trigger retinal development in tissues such as the wing, legs, and antennae.

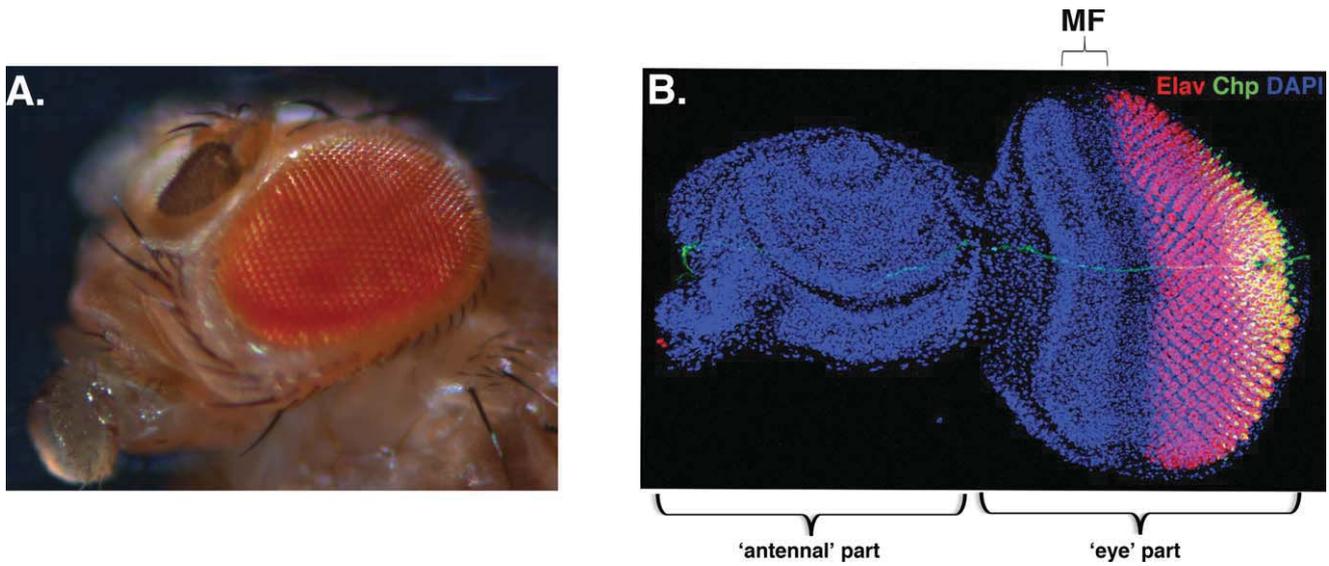


Fig. 1. **A:** Picture of the compound eye of *Drosophila*. The compound eye consists of 750–800 individual subunits, the “ommatidia.” **B:** Confocal image of a larval eye-antennal imaginal disc. The disc is subdivided into the “antennal part” (anterior, to the left of the image) and the “eye” part (posterior, to the right of the image). The cells already determined as neurons express the neuronal marker Elav (red). The cells that are specified as photoreceptors express the photoreceptor-specific marker Chaoptin (Chp, green). All the cell nuclei are stained with DAPI (blue). MF, Morphogenetic furrow.

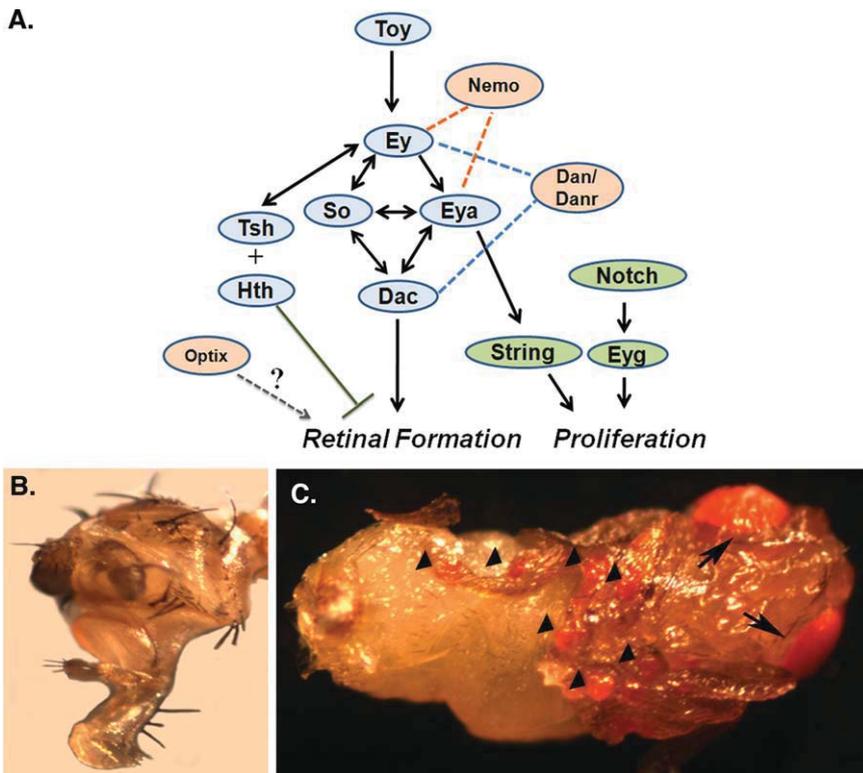


Fig. 2. **A:** The genes comprising the Retinal Determination Network (RDN). The dotted lines represent physical interactions that have not been confirmed *in vivo*. The genes that control cell proliferation are shown in green and those that control retinal formation in blue. The genes for which the relation with the other members has not been determined by genetic interactions are shown in orange (for details see the text). **B:** Adult fly head exhibiting complete absence of eyes, caused by mutation in the RDN gene *eyes absent* (*eya*). **C:** Pupa showing ectopic eye formation after misexpression of *eyeless* (*ey*) using the *dpp-Gal4* driver line. The arrowheads point towards ectopic eyes and the arrows towards the compound eyes.

RDN genes have a different potency in converting tissues into eyes. The location and also the frequency of ectopic eye formation can vary. The tissue specificity was recently genetically dissected for *Ey* and *Toy* and was attributed to distinct domains of the proteins (Weasner et al., 2009). Mutant strains for the RDN genes exhibit severe phenotypes in eye formation. These phenotypes range from malformed, rough eyes to the complete loss of eyes (Fig. 2B). The specific mutant phenotype of an RDN gene depends on their function, their position in the regulatory network, and the timing of expression. Homologues of most RDN genes also exist in mammals (including human) and some of them have been implicated in diseases of the visual system or in the patterning of eye tissue (Silver and Rebay, 2005). This suggests that although the eyes of vertebrates and invertebrates have a different structural organization and developmental origin, many of the genes that specify the eye tissue have been evolutionarily conserved.

In 1995, Halder and colleagues first reported the role of the eye-selector gene *eyeless* (*ey*), capable of transforming non-retinal tissues (wings, legs, antennae) into eyes when expressed ectopically during the development of

these structures (Halder et al., 1995). The *ey* gene was previously characterized and found to belong to the *Pax6* family (Quiring et al., 1994). It encodes for a homeodomain transcription factor of the Paired class, named after the segmentation gene *paired* (Frigerio et al., 1986). Members of this class of transcription factors contain the PAIRED domain critical for DNA binding, subdivided into two distinct domains, the PAI and RED domain (Czerny et al., 1993; Treisman et al., 1991). Loss of the human *Pax6* homologue of *ey* is linked to the genetic eye disorder aniridia, and of the murine homologue to a small eye phenotype (Glaser et al., 1992; Jordan et al., 1992; Quiring et al., 1994). *Ey* is functionally equivalent among many invertebrate species (Lynch and Wagner 2011). However, in species belonging to the stem-lineage of *Drosophila* (for instance, *Drosophila virilis* and *Drosophila willistoni*), *Ey* developed novel functions in response to the new developmental mechanisms controlling eye development and formation. Although in humans there is only one *Pax6* gene, a second member of the family was identified in *Drosophila*, *twin of eyeless* (*toy*). At the same time, with its identification and characterization, *toy* was demonstrated to also have the ability to generate eyes upon ectopic expression, revealing that *ey* is not the only “master regulatory gene” in eye development. Moreover, *toy* acts upstream of *ey* and activates its expression (Czerny et al., 1999).

One of the targets of *ey* is *sine oculis* (*so*) (Niimi et al., 1999). *So* belongs to the Sine oculis homeobox (SIX) transcription factors (Kumar, 2009), which contain a homeobox for DNA binding and the SIX domain for protein–protein interactions. Loss-of-function *so* mutants exhibit severe retinal defects (Cheyette et al., 1994; Serikaku and O’Tousa, 1994). However, initial attempts to recover eyes upon *so* ectopic expression failed (Pignoni et al., 1997). The possible reason behind that was attributed to the fact that *So* requires the presence of the transcriptional co-activator Eyes absent (*Eya*) for strong binding to the DNA. When *so* and *eya* are co-expressed, they can induce ectopic eyes, although *eya* is also able to induce eye formation on its own in

certain tissues (Bonini et al., 1997). Nevertheless, in an extensive screen for the ability of 219 Gal-4 lines to induce ectopic eyes upon solely *so* overexpression, ectopic eye formation was observed in the adult antenna and the ventral portion of the head (Weasner et al., 2007).

Eya is the founding member of a family of transcriptional co-activators and is unable to bind DNA on its own. *Eya* is a particularly interesting member of the RDN, not only because it is one of the few members that are not transcription factors, but also because it possesses an intrinsic tyrosine phosphatase activity. The precise function of this phosphatase activity of *Eya* in retinal development is not completely clear. However, mutations affecting *Eya* phosphatase activity reduce the DNA-binding ability of the *Eya-So* complex (Jemc and Rebay 2007a; Li et al., 2003; Mutsuddi et al., 2005; Rayapureddi et al., 2003; Tootle et al., 2003). *Eya* is expressed both in the cytoplasm and in the nucleus and its expression is regulated in many levels. The EGFR pathway downstream components Yan/anterior open (Yan) and Pointed (Pnt) are regulators of *Eya* phosphorylation (Hsiao et al., 2001). In addition, in a screen for mutants affecting *Eya* expression, Yan and Pnt were identified as regulators of *eya* transcription. Therefore, the EGFR pathway plays a central role in the regulation of both *Eya* expression and function (Salzer et al., 2010).

So binding sites have been identified in the *so* enhancer itself as well as in the *ey* enhancer, implicating *so* in an autoregulatory loop and in a positive feedback loop (Pauli et al., 2005). *So* and *Eya* trigger the expression of Dachshund (*Dac*), a transcription factor containing a winged helix domain that contacts the DNA (Kim et al., 2002; Mardon et al., 1994; Pappu et al., 2005). No downstream target of *Dac* has been identified so far. Importantly, *Dac* and its human homologue Dach are closely related to the Ski/Sno family of co-repressors, suggesting that *Dac* could act as a transcriptional repressor (Hammond et al., 1998). Loss of *dac* in turn results in a loss of *so* and *eya* expression at the posterior–lateral margin of the retinal field, which shows that a feedback loop exists between *Dac* and

So-Eya (Salzer and Kumar, 2009). The *So-Eya* complex not only affects retinal determination, but also cell proliferation. This is achieved by the activation of the cell cycle gene *string* (*stg*), encoding for a phosphatase that regulates the G2/M phase transition of the cell cycle (Jemc and Rebay, 2007b). The vertebrate homologues of *So* and *Dac* have a similar role in the expansion of precursor cells during tissue formation (Li et al., 2002).

In a genome-wide bioinformatic approach, another member of the SIX family, *optix*, was identified as a potential downstream target of *Ey* (Ostrin et al., 2006). Although loss-of-function mutants are not available, *optix* is expressed in the developing eye disc and is able to induce ectopic eye formation (Seimiya and Gehring, 2000). The target genes of *Optix* remain unknown. It is regarded to act as a repressor of transcription, since it shows strong binding affinity with the co-repressor Groucho (*Gro*), (Kenyon et al., 2005a,b).

Although most of the RDN genes act as either activators or suppressors of retinal determination, exceptions exist, as is the case for the zinc-finger protein Teashirt (*Tsh*). Ectopic expression of *tsh* leads to the formation of eyes at the base of the antenna and is able to induce an eye-precursor fate in non-retinal precursors, suggesting that *Tsh* acts as a promoter of eye development (Bessa and Casares, 2005; Datta et al., 2009; Pan and Rubin, 1998). However, in a more recent study, an additional suppressive role of *Tsh* in retinal determination was demonstrated upon over-expression (Singh et al., 2002). Surprisingly, even though *Tsh* is expressed uniformly in the eye disc, the suppressive phenotype is observed exclusively in the ventral but not in the dorsal margin of the eye disc, where it promotes eye development. The suppression of retinal development in the ventral part of the eye is mediated through induction of *hth*, a known suppressor of eye development and requires Wingless (*Wg*) signalling (Pai et al., 1998; Pichaud and Casares, 2000). On the other hand, *tsh* promotes eye growth at the dorsal parts of the eye disc. This function requires genes of the *Iroquois* (*IroC*) complex as well as Delta (*DI*), (Singh et al., 2004). Therefore, *Tsh* has a dual role in

suppression and promotion of eye development, depending on its various interactors in the different fields of the eye disc.

Two genes recently introduced in the RDN are *distal antenna (dan)* and *distal antenna-related (danr)*. These genes were initially identified as regulators of the antennal specification, since loss-of-function mutants led to the transformation of antennae into legs (Emerald et al., 2003). However, they are also capable of coaxing the antennal tissue into an eye fate, triggering at the same time the expression of *ey* (Curtiss et al., 2007). An open question is how Dan and Danr specify both antennal and eye development. It could be assumed that they do so by interacting with different fate determination factors in the two tissues. Dan and Danr are chromatin modifiers and they contain a helix-turn-helix structure, named the *pipsqueack (psq)* motif (Siegmond and Lehmann, 2002). Therefore, their role could be the maintenance of open chromatin states, after initial specification genes have committed the tissue into a certain fate. More studies are needed to explore these possibilities and uncover the mechanism through which these two genes are involved in the development of two different and adjacent imaginal discs.

danr and *dan/danr* double mutants show severe malformations, including small rough eyes and defects in the spacing of ommatidia and PR recruitment, further supporting their role in retinal determination. In addition, Dan and Danr regulate each other's expression and they both physically interact with Ey and Dac (Curtiss et al., 2007). The transcriptional co-regulator C-terminal Binding Protein (CtBP) interacts physically and genetically with Dan and Danr. This interaction could be important for the recruitment of the PRs from the pool of undifferentiated cells. In support of this, *CtBP/dan/danr* triple mutants develop significantly fewer ommatidia than the *dan/danr* double and *CtBP* single mutants. Intriguingly, CtBP also forms complexes with Ey. In *CtBP* mutant clones, there is enhanced cell proliferation anterior to the MF (Hoang et al., 2010). These results raise the possibility that CtBP could

function in conjunction with Ey to promote proliferation of eye precursor cells.

The RDN does not only contain nuclear proteins and transcription factors. Nemo (Nmo), a serine-threonine kinase plays key roles in eye development, equally important to those of nuclear factors that directly affect gene expression. The role of Nmo in eye development was first proposed by the finding that *nmo* mutants exhibit long and narrow eyes with a non-canonical packing of ommatidial clusters (Choi and Benzer, 1994). Nmo is co-expressed with other RDN genes during eye development, such as *eya*, and it is able by itself to specify presumptive head cells to a retinal fate. In addition, it potentiates the ectopic eye formation upon misexpression of *ey* and *eya*, with which it genetically interacts. Since Nmo is expressed in very early developmental stages, it could have additional, unidentified functions in the subdivision of the eye-antennal imaginal disc (Braid and Verheijen, 2008).

For the development of eye, tissue-controlled cell proliferation has to take place during initial developmental stages in order to increase the pool of cells that will follow a retinal fate. In the early eye primordium, N signalling has a leading role in controlling cell proliferation and tissue growth, mainly at the equator that divides the ventral from the dorsal part of the eye (Dominguez and de Celis, 1998). The effect of N in cell proliferation involves the control of the Pax gene *eye gone (eyg)*. Mutants of this gene display a complete lack of eyes and its forced expression induces ectopic eye formation in the ventral head region (Jang et al., 2003). Eyg was shown to be a negative regulator of head cuticle development and its expression has to be actively suppressed in certain regions of the head vertex primordium (Wang et al., 2010). The role of Eyg extends further than that of a cell proliferation regulator. It is also implicated in the initiation of the MF by inhibiting Wg (see below). Although *eyg* belongs to the Pax gene family, it has many structural differences with the Pax-6 gene *ey*. Eyg almost completely lacks the PAI subdomain of the PAIRED domain, present in the Pax proteins,

suggesting that different mechanisms of binding exist between these two transcription factors.

RDN genes can act differently in the different parts of the eye disc and the interactions among them occur in a precise temporal manner. An example is given by the So-Eya and Dac regulation (Salzer and Kumar, 2009). In the region of the eye disc where MF initiation takes place, So and Eya trigger the expression of Dac. In turn, Dac feeds back to maintain So-Eya expression. However, as the MF progresses in the parts of the disc where cells are already differentiated, the So-Eya complex no longer activates *dac*. Instead, So forms a complex with a co-repressor (potentially Gro) to inhibit *dac* expression. Given the complexity of the interactions present in the RDN, the simple discovery of a gene being a part of this network does not always reveal its interrelation with the other RDN genes. Further studies may identify the precise role or the multiple roles of each gene in retinal determination (Baker and Firth, 2011).

INITIATION AND PROGRESSION OF THE MORPHOGENETIC FURROW

The initial pool of cells in the eye disc is increased through proliferative replication, which is critical for the subsequent specification process (Amore and Casares, 2010). The proliferation is mediated through N signalling, which in turn activates the Janus tyrosine kinase/signal transducer and activator of transcription (JAK/STAT) pathway (Chao et al., 2004; Ekas et al., 2006; Reynolds-Kenneally and Mlodzik, 2005; Tsai and Sun, 2004). The ligand of the JAK/STAT pathway acting during eye development is the cytokine-like molecule Unpaired (Upd), (Harrison et al., 1998). Upd bind to the transmembrane receptor Domeless (Dome) (Brown et al., 2001) and triggers the activation/phosphorylation of the associate JAK Hopscotch (Hop), (Perrimon and Mahowald, 1987). Upon activation, JAKs phosphorylate tyrosine residues at the receptor, which constitute docking sites for the STAT transcription factor Stat92E (Hou et al., 1996; Yan et al.,

1996). Subsequently, Stat92E dimerizes and translocates to the nucleus, where it triggers the expression of target genes. Ectopic expression of *upd* causes eye overgrowth and Eye Transformer (ET) mediates this phenotype. ET regulates Stat92E phosphorylation and it is a negative regulator of the JAK/STAT pathway (Kallio et al., 2010). The downstream components of the JAK/STAT pathway regulating cell proliferation in the eye disc are elusive. Nevertheless, a potential downstream effector of Stat92E was found to be Chronologically inappropriate morphogenesis (Chinmo). The loss-of-function phenotypes of *chinmo* include reduction of the eye field and expansion of the head cuticle, similar to *Stat92E* phenotypes (Flaherty et al., 2010). A novel complementation group, the *gang of four* (*gfr*), was recently identified. Mutations in this group cause increase in eye size (Beam and Moberg, 2010). The mutant phenotype of the *gfr* alleles suggests that the factor encoded by this chromosomal region could affect cell proliferation of eye precursors by interacting with the N pathway.

The proliferation of retinal precursors is maintained by Hth, which is essential to regulate the balance between cell proliferation and cell-cycle arrest. Hth acts in conjunction with Ey and Tsh in order to inhibit *stg* (Bessa et al., 2002; Lopes and Casares, 2010). Since *Stg* controls the G2/M transition, a peak of *Stg* expression is apparent right before the cells undergo some last mitotic cycles (termed the first mitotic wave, FMW). After that phase, the cells arrest at the G1 phase of the cell cycle, which marks the onset of differentiation. This stage coincides with a reduction in Hth expression, mediated by two signalling molecules, Decapentaplegic (Dpp) and Hedgehog (Hh) (Escudero and Freeman, 2007; Firth and Baker, 2005; Lopes and Casares, 2010; Vraïlas and Moses, 2006). Dpp is a morphogen, exhibiting graded expression within the eye disc. G1 arrest occurs only at a specific threshold of Dpp levels (Firth et al., 2010). A negative regulator of cell-cycle arrest is *roughex* (*rux*), since in *rux* mutants all the cells circumvent G1 and enter the S phase (Thomas et al., 1994).

The transition from proliferation to differentiation is clearly visible by the passage of the MF, which crosses the eye disc from posterior to anterior. The MF is a narrow channel apparent in the eye disc after the early second/late early larval stage, where differentiation of ommatidia is initiated. The morphological appearance is the result of the contraction of the epithelial cells in the apical/basal dimension. An intriguing feature for developmental studies is that by the passage of the MF, the same disc contains distinct developmental stages of ommatidium formation: cells anterior to the furrow are undifferentiated, cells within the furrow are being patterned, and cells posterior to the furrow are already specified (Roignant and Treisman, 2009).

The initiation of the MF occurs in the posterior margin of the eye-disc. Several signalling cascades participate in this process, including the N, EGFR, Wingless (Wg), Hh, and Dpp signalling pathways (Fig. 3A). The EGFR and N pathways stand on top of the regulatory cascade. Although these pathways act antagonistically during the segregation of the eye-antennal field of the eye disk, with EGFR promoting an antennal fate and N promoting an eye fate, they act in concert to actuate the genesis of the MF (Kumar and Moses, 2001b). EGFR is a cell surface receptor tyrosine kinase (RTK) that is activated upon ligand binding and sets in motion the Ras/Raf/MEK/ERK signalling cascade. Of the various ligands able to activate EGFR, Spitz (Spi) has been shown to be the one involved in eye development. Notch is also a cell surface transmembrane receptor and acts by binding to its ligands, which are also transmembrane receptors expressed in neighbouring cells. Upon interaction with its ligands, Notch is cleaved and releases its intracellular fragment that triggers the expression of target genes (Artavanis-Tsakonas et al., 1999). Two Notch ligands have been identified in *Drosophila*, Dl and Serrate (D'Souza et al., 2008). Whether both or only one of these ligands participate in the initiation of the MF has not been clearly determined.

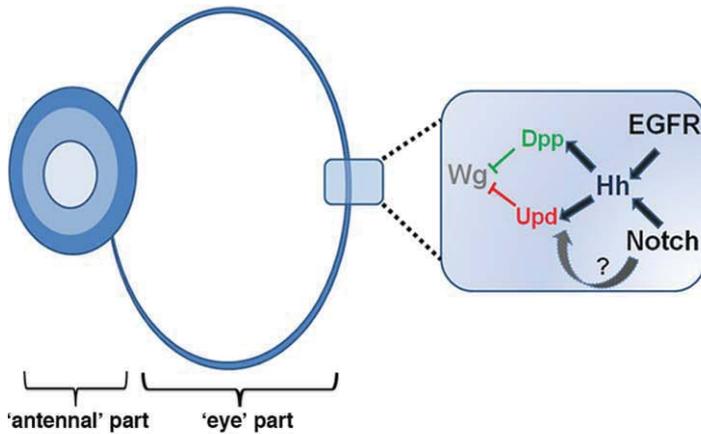
Surprisingly, a microRNA (*miR-7*) has been shown to be involved in the EGFR and N pathways during eye development through interactions with

downstream signalling components. More specifically, *miR-7* was shown to be activated by Pnt and to halt the expression of the repressor Yan (Li et al., 2009). It is not clear, however, if *miR-7* functions in the initiation or in later steps of MF progression. Additionally, the *miR-7* mutant phenotypes are solely observed under conditions of environmental fluctuations, such as temperature variations. Thus, *miR-7* is regarded to act as a stabilization factor rather than an essential component of the EGFR and N pathways.

At the point of furrow initiation both EGFR and N pathways regulate Hh signalling (Dominguez and Hafen, 1997). The Hh signalling pathway has a prominent function in an array of developmental processes in all metazoans (Wilson and Chuang, 2010). Hh acts as a morphogen and binds to receptors at the surface of the target cells. Although Patched (Ptc) has been long regarded to be the receptor of Hh, recent studies implicate two other proteins, Ihog (Interference hedgehog) and Boi (Brother of Ihog), in Hh binding at the eye and the wing disc, acting possibly as co-receptors (Camp et al., 2010; Zheng et al., 2010). Mutations in these genes lead to inhibition of Hh signalling and serious defects in photoreceptor development. In contrast to Ihog and Boi, Ptc has not been shown to be able to bind Hh directly, suggesting that Ihog and Boi might be essential for Hh binding to Ptc (McLellan et al., 2006; Yao et al., 2006).

Ectopic expression of Hh anterior to the MF is able to generate ectopic furrows (Baonza and Freeman, 2002; Ma et al., 1993). Hh acts by regulating at least two downstream signals: the Wg (Baonza and Freeman, 2002) and the Dpp pathway (Heberlein et al., 1993). Wg is the founding member of the Wnt family and it is expressed throughout the entire disc in second instar larvae. In the third instar, its expression is restricted to the anterior-lateral margins of the disc. Its role is to promote head capsule development and to inhibit retinal differentiation (Ma and Moses, 1995; Treisman and Rubin, 1995). At the point of furrow initiation, Dpp is expressed in a narrow ring right in front of the Hh expression field and it is inhibited laterally by Wg. Expression of Dpp at the

A. Initiation



B. Progression and 're-incarnation'

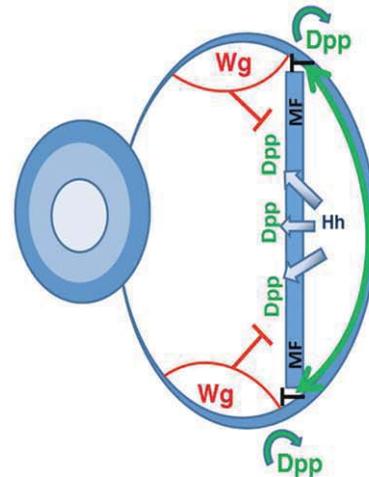


Fig. 3.

posterior margin depends on Hh. Wg is further repressed by Upd (Ekas et al., 2006; Tsai et al., 2007). Although Hh acts upstream to regulate Upd (Reifegerste et al., 1997), Notch signalling has also been implicated as a factor that sets out Upd expression (Chao et al., 2004). Therefore, at the posterior margin of the eye disc, EGFR-N signalling pathways trigger Hh expression, which in turn controls the expression of Dpp at the adjacent region. Dpp and Upd act as promoters of eye differentiation by inhibiting Wg signalling.

Because of the shape of the eye disc, the width of the MF increases as it moves anterior (Fig. 3B). It has been proposed that along the lateral margins of the disc, the differentiation has to be re-initiated, a process termed "re-incarnation" of the furrow (Kumar and Moses, 2001b). The re-incarnation requires Dpp. Dpp regulates its own expression at the lateral margins, where it restricts Wg expression (Borod and Heberlein, 1998).

During progression of the MF, Hh is expressed in already differentiated cells posterior to the MF and signals to a stripe of cells anterior to the MF to express Dpp (Dominguez and Hafen, 1997). Hh utilizes Dpp as a long-range signal that canalizes undifferentiated cells into a pre-proneural state, marked by the expression of the basic helix-loop-helix (bHLH) transcription factor Hairy (H) and Extramacrochaetae (Emc). H and Emc function as

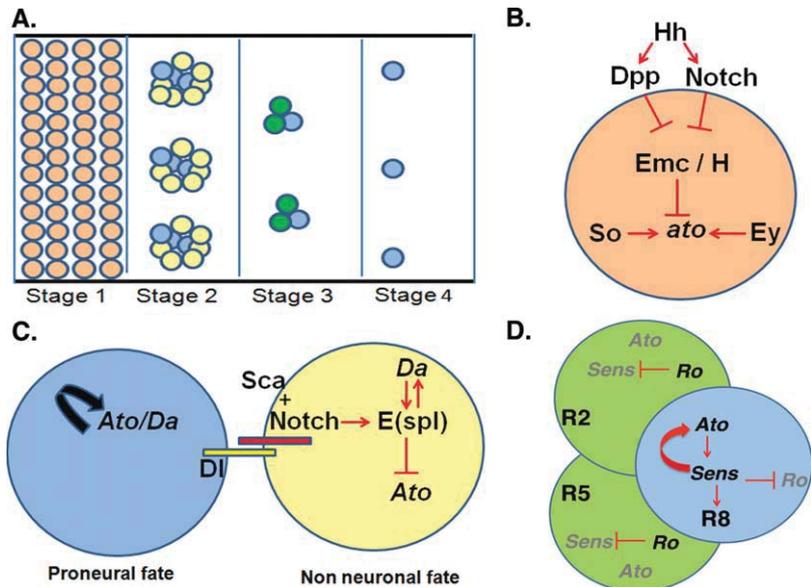
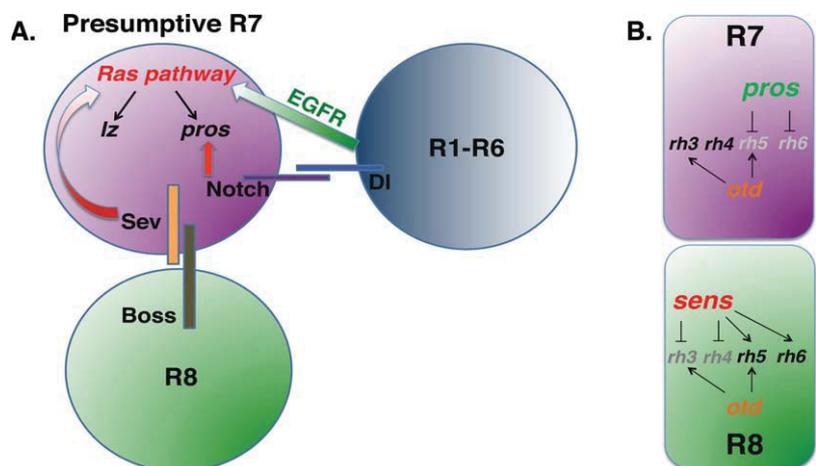


Fig. 4.



repressors of the pre-proneural to the proneural transition. Release from the pre-proneural to the proneural state is believed to take place by a short-range signal induced by Hh. Although the nature of this signal is unknown, it involves the activation of the Ras pathway independently of EGFR (Greenwood and Struhl, 1999).

SPECIFICATION OF THE R8 PR: THE FIRST STEP IN OMMATIDIUM FORMATION

The first cell to be specified within the ommatidium is the R8 founder cell. During ommatidium development, the R8 cell serves as the centre around which all PRs are subsequently incorporated into the ommatidium. Not only the R8 founder recruits the cells that will form the ommatidium in a stereotypical manner, but also its positioning defines the spacing between the ommatidia, scaffolding the whole structural organization of the eye.

Key in the specification of R8 founders is the proneural gene *atonal* (*ato*), a central determinant in peripheral nervous system development (Jarman et al., 1993, 1994). *Ato* is a bHLH transcription factor and acts in conjunction with the transcription factor Daughterless (*Da*), with which it

forms a dimer. It is first expressed in a stripe of 12–15 cells anterior to the MF (stage 1). Later, its expression is limited in a rosette of 10 cells, called Intermediate Group (IG; stage 2), consisting of cells that have the competency of becoming R8 PRs. *Ato* expression is subsequently further limited in a group of 3 cells, the R8 equivalence group (stage 3). Of these 3 cells only one will retain *Ato* expression and will become the R8 founder cell (stage 4) (Fig. 4A). The dynamic pattern of *ato* expression is controlled by two different enhancer elements located 3' and 5' to the *ato* coding sequence (Sun et al., 1998). The 3' enhancer controls the expression of *ato* in a band of cells anterior to the MF and the 5' element controls its expression in the IG, the R8 equivalence group, and the R8 founder. The 5' element depends on *ato* expression, suggesting that later steps of specification are at least reinforced by an *ato* autoregulatory loop. Hence, the expression of *ato* is under differential control before and after the formation of the IG.

For the initial expression of *ato* in a stripe of cells anterior to the MF, the Hh signalling pathway plays an inducing role by repressing the expression of the pre-proneural genes *emc* and *h*. Hh also promotes the N signalling pathway, which inhibits *emc* and *h* (Baonza

and Freeman, 2001). Recently, Ey and So were shown to be able to bind in vitro to sites within the 3' enhancer of *ato* that are crucial for its expression (Zhang et al., 2006).

Late *ato* expression is controlled by lateral inhibition (Fig. 4C). Lateral inhibition is a widely used developmental mechanism mediated by N signalling. It aims at selecting cells that will acquire a certain fate among a group of neighbouring cells that have the same developmental potential. N and its ligands are initially symmetrically distributed in this equivalence group. Through a regulatory feedback loop, cells that receive the highest N signal down-regulate the ligand Dl. This in turn reduces N signalling in neighbouring cells, thereby leading to asymmetric levels of N signalling activity within an equivalence group. N interaction with its ligands leads to its proteolytic cleavage and the activation of *Enhancer of split -E(spl)*, which acts as a suppressor of a certain fate. Therefore, the cells that don't express N are selected to acquire a specific cell fate.

Even though N initially promotes *ato* expression, in the R8 IG it will finally be expressed at the surface of cells that will not acquire a neuronal fate. Its action is potentiated by Scabrous (*Sca*), a secreted fibronogen-like protein, which interacts with N (Powell et al., 2001). The N ligand Dl is expressed in the cells that will maintain *ato* expression and will comprise the R8 equivalence group. Upon interaction with Dl, N is cleaved and triggers the expression of *E(spl)* at the cells that will not maintain *ato* expression. *E(spl)* acts as an inhibitor of *ato*.

Interestingly, the binding partner of *Ato* *Da* has an anti-proneural function when expressed in high levels. Neighbouring cells of the *Ato*-positive cells express high *Da* levels, induced by the N-*E(spl)* pathway. *Da* in turn further triggers the expression of *E(spl)* in these cells. In contrast, in the *Ato*-positive cells *Da* is present in low levels and forms heterodimers with *Ato* (Lim et al., 2008). Therefore, the double nature of *Da* as a proneural and an anti-proneural factor is decisive for the initial patterning of eye precursors.

Hh at this stage of development represses *ato* expression (Dominguez

Fig. 3. A: Schematic representation of the initiation of the MF. The interactions taking place at the posterior part of the disc are depicted in the inset. **B:** Progression and “re-incarnation” of the MF. The progression of the MF depends on Hh signalling sent from the cells that are already specified to a stripe of cells anterior to the MF. This Hh signal activates Dpp expression. Dpp is repressed more anterior by Wg, which is expressed at the lateral margins of the disc and inhibits eye development. The MF is re-initiated at the lateral margins, a process called re-incarnation of the MF. Dpp controls its own expression at the lateral margins, where it inhibits Wg. Dpp, decapentaplegic; Hh, Hedgehog; Wg, Wingless; Upd, Unpaired; MF, Morphogenetic Furrow.

Fig. 4. A: The different stages of *atonal* (*ato*) expression. *ato* is first expressed in a stripe of cells anterior to the MF (stage 1). Later, its expression is restricted to a rosette of 10 cells, the intermediate group (IG) (stage 2), and subsequently to three cells that comprise the R8 equivalence group (stage 3). Finally, *ato* expression is maintained only in one cell that will give rise to the R8 founder (stage 4). **B:** Genetic control of *ato* expression at stage 1. **C:** Mechanism of lateral inhibition. Through the action of N signalling, 3 of the cells at the IG will maintain *ato* expression. **D:** Selection of the R8 founder among the three cells of the R8 equivalence group. This choice depends on a bi-stable negative loop between Senseless (*Sens*) and Rough (*Ro*).

Fig. 5. A: The specification of R7. The interaction between Sevenless (*Sev*) and Boss (*Bride of Sevenless*) at the surface of the presumptive R7 and the R8 triggers the expression of the Ras pathway. EGFR signalling from the R1–R6 outer photoreceptors activates the same pathway. The Ras pathway turns on the expression of *logpense* (*lz*) and *prospero* (*pros*). A Notch signal from the R1–R6 is also essential for *pros* activation. **B:** Discrimination of the R7 and R8 inner photoreceptors. R7 express *prospero* (*pros*), which inhibits the expression of *rh5* and *rh6*. R8 express *senseless* (*sens*), which promotes the expression of *rh5* and *rh6* and inhibits the expression of the *rh3* and *rh4*. *orthodenticle* (*otd*) is expressed in both the R7 and R8, but has only a permissive and not a sufficient role for *rh3* and *rh5* expression.

1999). In order to explain how Hh initially activates and later represses *ato*, it is assumed that Hh acts as morphogen: low levels anterior to the MF induce *ato*, whereas higher levels posterior inhibit its expression. High levels of Ato in the cells that comprise the IG are maintained through *ato* autoregulation.

Only three of the cells in the R8 IG will escape lateral inhibition and will continue expressing high levels of *ato*. The selection of the R8 founder among these cells is the result of competition between two transcription factors, Senseless (Sens) and Rough (Ro). Sens is a zinc-finger transcription factor that plays a key role in the development of the sensory organ precursors, being involved in a feedback loop that activates proneural gene expression (Nolo et al., 2000). In the retinal precursors, Sens is activated by Ato and represses Ro, which acts as a negative regulator of differentiation (Frankfort et al., 2001). A single cell in the R8 equivalence group starts expressing high levels of *sens*. This initial expression needs to be maintained through *sens* repression by Ro. In the rest of the cells, Ro is expressed in high levels and maintains *sens* in a repressed state (Fig. 4D) (Pepple et al., 2008). This exemplary regulation strategy integrates a repressive signal mediated by lateral inhibition with the potential for developmental plasticity retained by a single cell within an equipotent cluster. Bistable networks, such as that formed by Sens and Ro, constitute a common developmental strategy for cell-fate choices (Graham et al., 2010).

The recruitment of the PRs into the developing ommatidium by the R8 founder follows a stereotypic pattern (Tomlinson and Ready, 1987). The first PRs to be recruited are R2 and R5, followed by R3 and R4, and subsequently by R1 and R6. The EGFR ligand Spi is expressed by the R8 PR and triggers the specification of all the outer PRs through the Ras pathway (Freeman, 1994). Ro, which is expressed in the R2 and R5 PRs, acts to suppress *seven-up* (*svp*). *Svp* is a member of the COUP subfamily of orphan nuclear receptors and is expressed exclusively in the R3/4 and R1/R6. In *ro* mutants, R2/R5 acquire R1/R3/R4/R6 fates (Kramer et al.,

1995). Furthermore, the R3/R4 and R1/R6 PRs depend on *svp* for their specification, in the absence of which they acquire an R7 fate (Mlodzik et al., 1990). The R3/R4 PR specification also depends on the *spalt* gene complex, encoding for the zinc-finger transcription factors Spalt major (Salm) and Spalt related (Salr). Intriguingly, the *spalt* genes are expressed early in the R3/R4 PRs in order to promote the expression of *svp*. Later, *svp* represses *spalt* expression, in order to prevent R3/R4 PRs from adopting an R7 fate (Domingos et al., 2004b). Finally, the development of the R1/R6 pair requires Logenze (Lz), a member of the RUNX family of transcription factors (Daga et al., 1996). Lz promotes the expression of BarH1, a homeodomain transcription factor essential for the differentiation of R1/R6 PRs (Higashijima et al., 1992).

The last cell to be specified is the R7 PR and its specification includes several factors and signals from the R8 and the R1/R6 PRs (Fig. 5A). First, the presumptive R7 PR expresses the cell surface RTK Sevenless (Sev), which binds to its membrane-tethered ligand Boss (Bride of Sevenless) in the surface of the R8 PR (Kramer et al., 1991; Simon et al., 1991). This binding triggers the activation of the Ras signal transduction cascade in the presumptive R7 PR. Also activating the same cascade is EGFR.

Since *sev* is expressed in other PRs except for the presumptive R7, a regulatory mechanism exists to restrict the R7 fate to a single cell. The Suppressor of cytokine signalling 36E (SOCS36E) is a negative regulator of *sev*, not expressed in the presumptive R7 PR (Almudi et al., 2009). In contrast, the homologue of the mammalian Grb2 adaptor protein Downstream of receptor kinase (Drk) is expressed only in the presumptive R7 PR and positively regulates the Ras pathway, by linking Sev to its downstream effectors (Olivier et al., 1993; Simon et al., 1993). SOCS36E and Drk bind directly to Sev as shown by FRET assays in *Drosophila* S2 cells (Almudi et al., 2010). It has been suggested that SOCS36E and Drk compete for binding to Sev and define the activation or attenuation of the Ras pathway, thereby leading to a robust

choice between different developmental programs.

A downstream target of the Ras pathway is *lz* (Daga et al., 1996). In non-specified cells, the zinc-finger DNA binding protein Tramtrack69 (Ttk69), a member of the BTB/POZF family, represses *lz*. However, upon activation of the Ras pathway, the repression of *lz* is relieved, allowing the differentiation of the R7 (Siddall et al., 2009). In addition, the development of R7 requires the absence of *svp* (Begemann et al., 1995; Kramer et al., 1995). The R7 PR receives an N signal from the R1/R6 PRs, in the absence of which it adopts an R1/R6 fate (Cooper and Bray 2000). A homeodomain transcription factor that genetically differentiates R7 from R8 PRs is Prospero (Pros). In the absence of Pros the presumptive R7 PR is canalized towards an R8 fate (Cook et al., 2003). The N and EGFR pathways act in a combinatorial manner to regulate *pros* expression. This is achieved through the control by EGFR and N of several factors that bind cooperatively to the *pros* enhancer. These factors include Lz and So/Eya (Hayashi et al., 2008). Another factor necessary for *pros* transcription is the zinc-finger transcription factor Glass (Gl), (Moses and Rubin 1991). Glass is specifically expressed in the visual system and it is essential for the differentiation of all the PRs (Moses et al., 1989). The *pros* enhancer contains one Glass binding site that is essential for the transcription of *pros*. Mutant clones for *glass* show a dramatic decrease of Pros expression. The current evidence suggests that Lz, So/Eya, and Glass act in a combinatorial manner to regulate *pros* expression (Hayashi et al., 2008).

SUBTYPE SPECIFICATION OF PHOTORECEPTORS

Once the PRs of the compound eye have been determined, they undergo terminal differentiation, which includes the choice to express a specific *rhodopsin* gene. This step is of particular importance, since the PRs must select to express only one among the seven *rhodopsin* genes present in the fly genome, in order to acquire their functional identity. At the same time, the expression of the rest of the genes

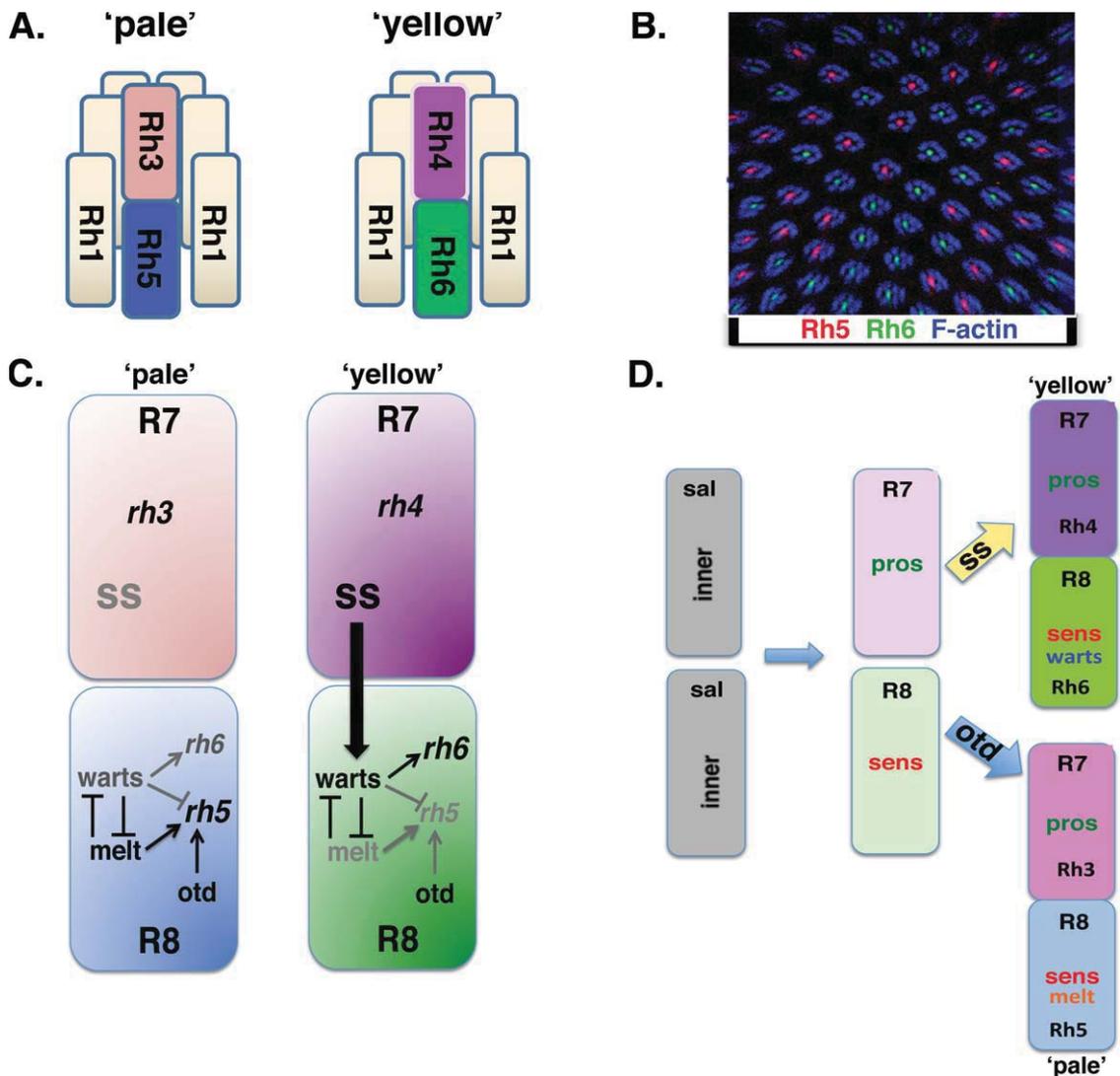


Fig. 6. **A:** Schematic representation of “pale” and “yellow” ommatidia. Pale ommatidia express Rh3 in R7 and Rh5 in R8 and yellow ommatidia express Rh4 in R7 and Rh6 in R8. **B:** Confocal image of a compound eye, showing the mosaic of pale and yellow ommatidia. The pale R8 express Rh5 (red) and the yellow Rh6 (green). The cytoplasm of all the ommatidia is stained with phalloidin that binds to F-actin (blue). **C:** The choice between pale and yellow ommatidia. A part of the R7 starts to express *spineless* (*ss*) stochastically, which defines these R7 as yellow. Subsequently, *ss* sends a signal to the overlying R8 to express *warts*, which promotes *rh6* expression. The R8 that do not turn on *warts* expression express *melt*, which activates *rh5* expression. *warts* and *melt* suppress each other’s expression. **D:** Overview of the subtype specification of R7 and R8 PRs.

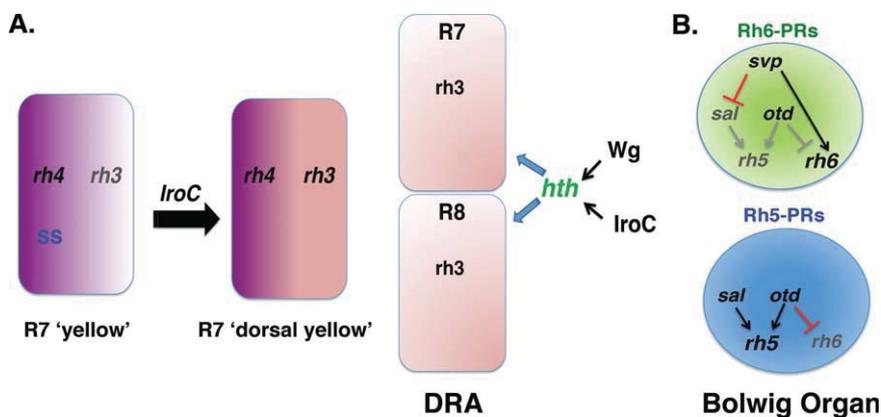


Fig. 7.

Fig. 7. **A:** The specification of the “dorsal yellow” ommatidia. These ommatidia first express *ss* and activate *rh4* expression, which specifies them as yellow. Subsequently, *rh3* is turned on by the action of genes of the *IroC* complex (left). The Dorsal Rim Area (DRA) ommatidia specification. These ommatidia express *rh3* in both R7 and R8 inner photoreceptors under the control of *hth*, which is specifically expressed in the DRA. The expression of *hth* is controlled by *Wg* signaling from the head cuticle and by the *IroC* genes expressed in the dorsal tissue (right). **B:** Subtype specification of the Bolwig Organ (BO) photoreceptors. The Rh5 subtype is specified by the action of *spalt* (*sal*) and *orthodenticle* (*otd*), which triggers *rh5* expression. At the same time, *otd* inhibits *rh6* expression. In the Rh6 subtype, *seven-up* (*svp*) inhibits *sal* and activates *rh6*.

is suppressed. Similar to the olfactory receptors, the photoreceptors have been proposed to follow the “one receptor rule” (Mazzoni et al., 2004). This rule dictates that only one receptor gene is expressed in one sensory neuron subtype, so that the different subtypes are functionally distinct. In the case of PRs, the different subtypes express one *rhodopsin* gene that is sensitive to different wavelengths of light. An intriguing exception to this rule are the “dorsal yellow” R7, which are specified to co-express Rh3 and Rh4 (see below).

First, the inner PRs are discriminated from the outer PRs by the *spalt* complex. Loss-of-function of *spalt* converts the R7 and R8 into outer-like PRs. Surprisingly, even though these PRs express Rh1, they project to the medulla, exhibiting an inner PR axonal pattern. This suggests that axonal targeting of PRs occurs earlier than terminal differentiation (Mollereau et al., 2001). The *spalt* complex is not required for the early specification of R8 PRs, but only for their terminal differentiation. In contrast, *spalt* is expressed earlier in the R7 PRs and it has been suggested to be required for their specification soon after their commitment as R7 (Domingos et al., 2004a). In a next step, R7 and R8 PRs are differentially specified (Fig. 5B). R7 PRs specifically express Pros, whose function is to repress both *rh5* and *rh6* (Cook et al., 2003). The R8 PRs express Sens, which has an opposite function to that of Pros, by promoting *rh5* and *rh6* expression. Therefore, Sens not only has a role early in R8 selection, but plays also a central role in the terminal differentiation of R8 PRs. Although the initial expression of *sens* is Ato-dependent, its expression at this stage depends on the presence of Sal (Xie et al., 2007).

Subsequently, ommatidia are differentially specified in order to become pale and yellow subtypes (Fig. 6A and B). This stochastic choice of a given ommatidium to become yellow or pale is initiated in R7 PRs (Fig. 6C). During mid-pupation, a subset of R7 PRs begin to express the transcription factor Spineless (Ss), a member of the bHLH-PAS family of transcription factors (Wernet et al., 2006). Ss is necessary and sufficient to induce the yellow ommatidial fate; the ommati-

dia devoid of *ss* expression become pale. What triggers the expression of *ss* only in a subset of ommatidia and leads to the accurate generation of a retinal mosaic of *rhodopsin* expression remains enigmatic. Once an R7 cell has been defined as yellow, it sends a signal to the underlying R8 cell to adopt the same fate and express *rh6*. The nature of the signal sent by the R7 PRs and received by the R8 is unknown, although two components of the signalling cascade it triggers have been described. These are the tumour suppressor gene *warts* specifically expressed in the yellow R8 and the growth regulator gene *melted* expressed in the pale R8 PRs (Mikelladze-Dvali et al., 2005). *warts* and *melt* negatively regulate each other's expression. They create a bistable loop, which ensures that a robust decision is made by the R8 PRs regarding their yellow-pale fate. Since *warts* and *melt* do not encode for transcription factors, downstream genes involved in this regulation await discovery.

Orthodenticle (Otd) is a direct regulator of ommatidial fate, although its relation with the above signalling components has not been defined. Otd is a Paired class homeodomain transcription factor and belongs to the well-conserved Otd/Crx family, having four paralogs in mice (Otx1, Otx2, Otx3, and Crx), (Simeone et al., 1993; Zhang et al., 2002). Crx is the most closely related to Otd. Strikingly, Crx affects the development of cones and rods, as well as gene expression of PR-specific genes (Chau et al., 2000; Freund et al., 1997; Furukawa et al., 1997). Otd acts as a permissive factor for the specification of the pale ommatidia, by directly binding to well-characterized sites in the promoters of *rh3* and *rh5* (Tahayato et al., 2003). Loss of *otd* leads to absence of Rh3 and Rh5 in pale ommatidia. However, yellow ommatidia do not acquire a pale fate in the absence of *otd*, suggesting that it has a permissive role and that additional factors are needed to turn on *rh3* and *rh5* expression. Otd also acts further to repress *rh6* in the outer ommatidia, since in the absence of *otd* they activate *rh6* expression. Distinct structural domains of Otd are responsible for exerting diverse effects on Rhodopsin expression (McDonald et al., 2010). The C-termi-

nus mediates Rh5 activation, whereas the N-terminus mediates the activation of Rh3 in pale ommatidia. Deletion of the N-terminus also leads to de-repression of Rh6 in the pale R8 PRs. In addition, removal of a region placed centrally in the primary structure of the protein (termed AB) causes extended co-expression of Rh5 and Rh6 in R8. These data suggest that Otd could also play a role in the mutual exclusion of blue versus green Rhodopsins. The function of Otd in *rhodopsin* expression remains one of the best characterized, since the transcription factors binding directly to the *rhodopsin* enhancers to regulate gene expression have been largely unknown. An overview of the subtype specification of R7 and R8 is shown in Figure 6D.

Recently, null mutants of the homeodomain transcription factor PvuII-PstI homology 13 (Pph13) were shown to exhibit a loss of Rh6 in the R8 (Mishra et al., 2010). Pph13 is a PR-specific transcription factor and it has been shown to act in concert with Otd to control the formation of rhabdomeres (Zelhof et al., 2003). Whether its effect on Rh6 expression is direct or is the consequence of general PR malformations due to the disorganized rhabdomeres in *pph13* mutants deserves further investigation.

Apart from pale and yellow, another type of ommatidia, termed dorsal yellow, resides in the dorsal part of the eye, starting from the dorsal rim and extending up to one-third of the eye. Surprisingly, the R7 PRs of these ommatidia co-express the two UV-sensitive Rhodopsins Rh3 and Rh4, violating the one-receptor rule. However, the R8 PRs in these ommatidia express either Rh5 or Rh6, following the pattern of the rest R8 inner PRs. After the pale and yellow specification throughout the retina, the dorsal yellow ommatidia are firstly defined as yellow and express Rh4. Otd is expressed in all the PRs, but it only enables Rh3 expression in the pale R7. Therefore, Rh3 has to be silenced in yellow R7. The genes of the *IroC* complex (Cavodeassi et al., 1999) are necessary and sufficient to promote the co-expression of Rh3 and Rh4, raising the exciting possibility that they could act as the signal that de-represses *rh3* in the dorsal yellow

ommatidia (Fig. 7A) (Mazzoni et al., 2008). The identification of genes acting downstream of *IroC* would be a critical step in the understanding of the elaborate mechanism by which the co-expression of Rhodopsins in the different PR subtypes is excluded.

A highly specialized type of ommatidia is found at the Dorsal Rim Area (DRA) of the eye (Fig. 7A). At the DRA, one or two rows of ommatidia display special morphological features and are enabling the ommatidium to detect polarized light (Labhart and Meyer, 1999). In the DRA, both R7 and R8 PRs express the same Rhodopsin, Rh3. Their specification involves the activation of *hth* by the Wg signaling from the head cuticle, as well as the *IroC* genes expressed dorsally (Tomlinson 2003; Wernet et al., 2003). *Hth* is both necessary and sufficient for a DRA fate, thereby allowing the DRA ommatidia to perceive polarized light.

Distinct mechanisms of terminal differentiation and specification of *rhodopsin* fates are acting in the development of the larval eye (also named Bolwig organ, BO). Since the larval eye and the adult compound eye of the insects have likely evolutionarily emerged by partitioning of an ancestral adult eye, studies of larval eye development provided an intriguing entry point into common mechanisms governing eye formation (Friedrich, 2006a,b; Liu and Friedrich, 2004). In contrast to the comparably complex organization of the adult compound eye, the larval counterpart consists of only 12 PRs, which are further divided into two subtypes: eight PRs express Rh6 and four PRs express Rh5. Therefore, larval PRs express the same Rhodopsins as the adult R8-PRs. However, this does not imply that all BO PRs are homologs of the adult R8 cells. Several lines of evidence suggest that the larval Rh5-PRs of the BO share similarities with a subset of R8 PRs while the larval Rh6-PRs are similar to adult outer PRs (Friedrich, 2008).

The Rh5-PRs are specified by the combinatorial action of *Sal* and *Otd*, which trigger *rh5* expression (Fig. 7B). At the same time, *Otd* represses Rh6 expression in the Rh5-PRs. This function of *Otd* is reminiscent of its function in the outer PRs of the retina,

where it also represses *rh6* in outer PRs. Although *sal* expression defines the inner PRs in the retina, in the BO it is exclusively expressed in the Rh5-PRs. In Rh6-PRs, *sal* expression is repressed by *svp*, leading to inhibition of *rh5* expression. Although *Otd* is equally expressed in all the BO PRs, it is not sufficient to turn on *rh5* in the absence of *sal*, suggesting that as in the retina its role in activating *rhodopsin* expression is permissive, but not sufficient. At the same time, *Svp* is required for *rh6* expression in the Rh6-PRs, since in *svp* mutant embryos all PRs express only Rh5 (Sprecher et al., 2007). *svp* has a role only in the initial specification of the R3/R4 and R1/R6 PRs of the retina and not in their terminal specification (Mlodzik et al., 1990). Thus, even though larval and adult R8 PRs express the same set of *rhodopsin* genes, the genetic mechanisms controlling the expression of Rh5 and Rh6 are surprisingly distinct.

During metamorphosis, the larval eye gives rise to the adult eyelet, a small visual organ important for circadian rhythm control (Helfrich-Forster 2002; Helfrich-Forster et al., 2002; Veleri et al., 2007; Yasuyama and Meinertzhagen 1999). The eyelet contains only 4 PRs, all of which express Rh6, whereas the BO consists of 12 PRs expressing either Rh5 or Rh6. At early pupation, the Rh6-PRs of the BO undergo apoptotic cell death, while Rh5-PRs are maintained giving rise to cells of the eyelet. At the same time as Rh6-PRs degenerate, the Rh5-subtype cease to express *rh5* and start to turn on *rh6* expression in late pupal stages. Therefore, during the transformation of the BO to the eyelet, terminally differentiated and functional neurons retain the potential to change their identity and are re-specified by expressing a different receptor molecule (Sprecher and Desplan, 2009b). The Ecdysone hormone, which is the trigger for many developmental transformations during pupation, was found to be responsible for the switch of Rhodopsins as well as for the degeneration of the Rh6-PRs of the BO. Surprisingly, *Sens* is specifically expressed in the Rh5-PRs, preventing them from undergoing apoptosis (Sprecher and Desplan, 2008). Therefore, an additional function dur-

ing PR development is attributed to *Sens*, this time as a survival factor.

PERSPECTIVES

A central question in developmental biology is how various highly specialized cell types of an organism are formed, starting from a uniform population of unspecified cells. It is now apparent that this process involves many intermediate steps in order to ensure precise specification of cells. The eye of *Drosophila* provides a prototype experimental system to study these processes, since it consists of various different types of cells that are organized in a highly stereotypic manner. For instance, different subtypes of PRs, with different spectral sensitivity, are organized in an accurate way within the ommatidia. The research in the development of the fly eye has flourished in the last 30 years and has yielded many interesting results.

Despite the progress that has been achieved, many aspects of the development of the eye remain enigmatic. For example, although many selector genes that are sufficient to induce a general eye fate in other imaginal discs have been discovered, their precise role in the differentiation process is cryptic. Thus, the way that these genes contribute in the specification of an initially uniform developmental field in an imaginal disc deserves further examination. In this context, it is important to identify downstream targets of these master regulatory genes, which would be either transcription factors or effector proteins. Currently, only certain steps of the molecular machinery controlling eye development in *Drosophila* have been identified. Common mechanisms of cell fate determination are likely to exist in many organisms, including vertebrates. Moreover, most of the genes that control eye specification in *Drosophila* have vertebrate homologues, which could have common functions in tissue determination.

Although the initial steps of the formation of the MF have been well studied, little is known about the genes and cellular pathways controlling the transition from the neuronal identity to the photoreceptor identity. The processes by which a neuron

develops to a specific neuronal type (for example a sensory neuron, an interneuron, or a motor neuron) have been the focus of intense research. Since several photoreceptor-specific genes have been identified, the mechanisms that lead to their activation would give insights into the choice of a neuron to adopt a certain fate (in this case a retinal fate).

The presence of different PR subtypes in the compound eye of *Drosophila* is the result of terminal differentiation events, converting a generic PR into a functional neuron. During terminal differentiation, a certain PR subtype activates the expression of one *rhodopsin* gene and suppresses the expression of the others. Since the Rhodopsins are regulated in a transcriptional level, activators or suppressors of transcription bind to the *rhodopsin* enhancers and dictate whether a certain gene will be expressed or not. The identification of transcription factors that directly regulate *rhodopsin* expression will shed light on the regulatory mechanisms discriminating neurons into highly specialized subtypes with unique functions.

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REFERENCES

- Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, George RA, Lewis SE, Richards S, Ashburner M, Henderson SN, Sutton GG, Wortman JR, Yandell MD, Zhang Q, Chen LX, Brandon RC, Rogers YH, Blazej RG, Champe M, Pfeiffer BD, Wan KH, Doyle C, Baxter EG, Helt G, Nelson CR, Gabor GL, Abril JF, Agbayani A, An HJ, Andrews-Pfannkoch C, Baldwin D, Ballew RM, Basu A, Baxendale J, Bayraktaroglu L, Beasley EM, Beeson KY, Benos PV, Berman BP, Bhandari D, Bolshakov S, Borkova D, Botchan MR, Bouck J, Brokstein P, Brottier P, Burtis KC, Busam DA, Butler H, Cadieu E, Center A, Chandra I, Cherry JM, Cawley S, Dahlke C, Davenport LB, Davies P, de Pablos B, Delcher A, Deng Z, Mays AD, Dew I, Dietz SM, Dodson K, Doup LE, Downes M, Dugan-Rocha S, Dunkov BC, Dunn P, Durbin KJ, Evangelista CC, Ferraz C, Ferreira S, Fleischmann W, Fosler C, Gabrielian AE, Garg NS, Gelbart WM, Glasser K, Glodek A, Gong F, Gorrell JH, Gu Z, Guan P, Harris M, Harris NL, Harvey D, Heiman TJ, Hernandez JR, Houck J, Hostin D, Houston KA, Howland TJ, Wei MH, Ibegwam C, Jalali M, Kalush F, Karpen GH, Ke Z, Kennison JA, Ketchum KA, Kimmel BE, Kodira CD, Kraft C, Kravitz S, Kulp D, Lai Z, Lasko P, Lei Y, Levitsky AA, Li J, Li Z, Liang Y, Lin X, Liu X, Mattei B, McIntosh TC, McLeod MP, McPherson D, Merkulov G, Milshina NV, Mobarry C, Morris J, Moshrefi A, Mount SM, Moy M, Murphy B, Murphy L, Muzny DM, Nelson DL, Nelson DR, Nelson KA, Nixon K, Nusskern DR, Pacleb JM, Palazzolo M, Pittman GS, Pan S, Pollard J, Puri V, Reese MG, Reinert K, Remington K, Saunders RD, Scheeler F, Shen H, Shue BC, Sidón-Kiamos I, Simpson M, Skupski MP, Smith T, Spier E, Spradling AC, Stapleton M, Strong R, Sun E, Svirskas R, Tector C, Turner R, Venter E, Wang AH, Wang X, Wang ZY, Wassarman DA, Weinstock GM, Weissenbach J, Williams SM, Woodage T, Worley KC, Wu D, Yang S, Yao QA, Ye J, Yeh RF, Zaveri JS, Zhan M, Zhang G, Zhao Q, Zheng L, Zheng XH, Zhong FN, Zhong W, Zhou X, Zhu S, Zhu X, Smith HO, Gibbs RA, Myers EW, Rubin GM, Venter JC. 2000. The genome sequence of *Drosophila melanogaster*. *Science* 287:2185–2195.
- Almudi I, Stocker H, Hafen E, Corominas M, Serras F. 2009. SOCS36E specifically interferes with Sevenless signaling during *Drosophila* eye development. *Dev Biol* 326:212–223.
- Almudi I, Corominas M, Serras F. 2010. Competition between SOCS36E and Drk modulates Sevenless receptor tyrosine kinase activity. *J Cell Sci* 123:3857–3862.
- Amore G, Casares F. 2010. Size matters: the contribution of cell proliferation to the progression of the specification *Drosophila* eye gene regulatory network. *Dev Biol* 344:569–577.
- Artavanis-Tsakonas S, Rand MD, Lake RJ. 1999. Notch signaling: cell fate control and signal integration in development. *Science* 284:770–776.
- Baker NE, Firth LC. 2011. Retinal determination genes function along with cell-cell signals to regulate *Drosophila* eye development: examples of multi-layered regulation by master regulators. *Bioessays* 33: 538–546.
- Baonza A, Freeman M. 2001. Notch signalling and the initiation of neural development in the *Drosophila* eye. *Development* 128:3889–3898.
- Baonza A, Freeman M. 2002. Control of *Drosophila* eye specification by Wingless signalling. *Development* 129:5313–5322.
- Beam CK, Moberg K. 2010. The gang of four gene regulates growth and patterning of the developing *Drosophila* eye. *Fly (Austin)* 4:104–116.
- Begemann G, Michon AM, vd Voorn L, Wepf R, Mlodzik M. 1995. The *Drosophila* orphan nuclear receptor seven-up requires the Ras pathway for its function in photoreceptor determination. *Development* 121:225–235.
- Bessa J, Casares F. 2005. Restricted teashirt expression confers eye-specific responsiveness to Dpp and Wg signals during eye specification in *Drosophila*. *Development* 132:5011–5020.
- Bessa J, Gebelein B, Pichaud F, Casares F, Mann RS. 2002. Combinatorial control of *Drosophila* eye development by eyeless, homothorax, and teashirt. *Genes Dev* 16:2415–2427.
- Bonini NM, Bui QT, Gray-Board GL, Warlick JM. 1997. The *Drosophila* eyes *absent* gene directs ectopic eye formation in a pathway conserved between flies and vertebrates. *Development* 124:4819–4826.
- Borod ER, Heberlein U. 1998. Mutual regulation of decapentaplegic and hedgehog during the initiation of differentiation in the *Drosophila* retina. *Dev Biol* 197: 187–197.
- Braid LR, Verheyen EM. 2008. *Drosophila* nemo promotes eye specification directed by the retinal determination gene network. *Genetics* 180:283–299.
- Brody T, Cravchik A. 2000. *Drosophila* melanogaster G protein-coupled receptors. *J Cell Biol* 150:F83–88.
- Broeck JV. 2001. Insect G protein-coupled receptors and signal transduction. *Arch Insect Biochem Physiol* 48:1–12.
- Brown S, Hu N, Hombria JC. 2001. Identification of the first invertebrate interleukin JAK/STAT receptor, the *Drosophila* gene domeless. *Curr Biol* 11:1700–1705.
- Cagan R. 2009. Principles of *Drosophila* eye differentiation. *Curr Top Dev Biol* 89:115–135.
- Camp D, Currie K, Labbe A, van Meyel DJ, Charron F. 2010. Ihog and Boi are essential for Hedgehog signaling in *Drosophila*. *Neural Dev* 5:28.
- Cavodeassi F, Diez Del Corral R, Campuzano S, Dominguez M. 1999. Compartments and organising boundaries in the *Drosophila* eye: the role of the homeodomain Iroquois proteins. *Development* 126:4933–4942.
- Chao JL, Tsai YC, Chiu SJ, Sun YH. 2004. Localized Notch signal acts through eyg and upd to promote global growth in *Drosophila* eye. *Development* 131:3839–3847.
- Charlton-Perkins M, Cook TA. 2010. Building a fly eye: terminal differentiation events of the retina, corneal lens, and pigmented epithelia. *Curr Top Dev Biol* 93:129–173.
- Chau KY, Munshi N, Keane-Myers A, Cheung-Chau KW, Tai AK, Manfioletti G, Dorey CK, Thanos D, Zack DJ, Ono SJ. 2000. The architectural transcription factor high mobility group I(Y) participates in photoreceptor-specific gene expression. *J Neurosci* 20:7317–7324.

- Cheyette BN, Green PJ, Martin K, Garren H, Hartenstein V, Zipursky SL. 1994. The *Drosophila sine oculis* locus encodes a homeodomain-containing protein required for the development of the entire visual system. *Neuron* 12:977–996.
- Choi KW, Benzer S. 1994. Rotation of photoreceptor clusters in the developing *Drosophila* eye requires the *nemo* gene. *Cell* 78:125–136.
- Clandinin TR, Zipursky SL. 2002. Making connections in the fly visual system. *Neuron* 35:827–841.
- Cook T, Pichaud F, Sonnevile R, Papatzenko D, Desplan C. 2003. Distinction between color photoreceptor cell fates is controlled by Prospero in *Drosophila*. *Dev Cell* 4:853–864.
- Cooper MT, Bray SJ. 2000. R7 photoreceptor specification requires Notch activity. *Curr Biol* 10:1507–1510.
- Curtiss J, Burnett M, Mlodzik M. 2007. *distal antenna* and *distal antenna-related* function in the retinal determination network during eye development in *Drosophila*. *Dev Biol* 306:685–702.
- Czerny T, Schaffner G, Busslinger M. 1993. DNA sequence recognition by Pax proteins: bipartite structure of the paired domain and its binding site. *Genes Dev* 7:2048–2061.
- Czerny T, Halder G, Kloter U, Souabni A, Gehring WJ, Busslinger M. 1999. *twin of eyeless*, a second Pax-6 gene of *Drosophila*, acts upstream of *eyeless* in the control of eye development. *Mol Cell* 3:297–307.
- Daga A, Karlovich CA, Dumstrei K, Banerjee U. 1996. Patterning of cells in the *Drosophila* eye by Lozenge, which shares homologous domains with AML1. *Genes Dev* 10:1194–1205.
- Datta RR, Lurye JM, Kumar JP. 2009. Restriction of ectopic eye formation by *Drosophila* *teashirt* and *tiptop* to the developing antenna. *Dev Dyn* 238:2202–2210.
- Domingos PM, Brown S, Barrio R, Ratnakumar K, Frankfort BJ, Mardon G, Steller H, Mollereau B. 2004a. Regulation of R7 and R8 differentiation by the spalt genes. *Dev Biol* 273:121–133.
- Domingos PM, Mlodzik M, Mendes CS, Brown S, Steller H, Mollereau B. 2004b. Spalt transcription factors are required for R3/R4 specification and establishment of planar cell polarity in the *Drosophila* eye. *Development* 131:5695–5702.
- Dominguez M. 1999. Dual role for Hedgehog in the regulation of the proneural gene *atonal* during ommatidia development. *Development* 126:2345–2353.
- Dominguez M, de Celis JF. 1998. A dorsal/ventral boundary established by Notch controls growth and polarity in the *Drosophila* eye. *Nature* 396:276–278.
- Dominguez M, Hafen E. 1997. Hedgehog directly controls initiation and propagation of retinal differentiation in the *Drosophila* eye. *Genes Dev* 11:3254–3264.
- Dominguez M, Ferrer-Marco D, Gutierrez-Avino FJ, Speicher SA, Beneyto M. 2004. Growth and specification of the eye are controlled independently by *Eyeless* and *Eyeless* in *Drosophila melanogaster*. *Nat Genet* 36:31–39.
- D'Souza B, Miyamoto A, Weinmaster G. 2008. The many facets of Notch ligands. *Oncogene* 27:5148–5167.
- Ekas LA, Baeg GH, Flaherty MS, Ayala-Camargo A, Bach EA. 2006. JAK/STAT signaling promotes regional specification by negatively regulating wingless expression in *Drosophila*. *Development* 133:4721–4729.
- Emerald BS, Curtiss J, Mlodzik M, Cohen SM. 2003. *Distal antenna* and *distal antenna-related* encode nuclear proteins containing pipsqueak motifs involved in antenna development in *Drosophila*. *Development* 130:1171–1180.
- Escudero LM, Freeman M. 2007. Mechanism of G1 arrest in the *Drosophila* eye imaginal disc. *BMC Dev Biol* 7:13.
- Firth LC, Baker NE. 2005. Extracellular signals responsible for spatially regulated proliferation in the differentiating *Drosophila* eye. *Dev Cell* 8:541–551.
- Firth LC, Bhattacharya A, Baker NE. 2010. Cell cycle arrest by a gradient of Dpp signaling during *Drosophila* eye development. *BMC Dev Biol* 10:28.
- Flaherty MS, Salis P, Evans CJ, Ekas LA, Marouf A, Zavadil J, Banerjee U, Bach EA. 2010. *chinmo* is a functional effector of the JAK/STAT pathway that regulates eye development, tumor formation, and stem cell self-renewal in *Drosophila*. *Dev Cell* 18:556–568.
- Frankfort BJ, Nolo R, Zhang Z, Bellen H, Mardon G. 2001. *senseless* repression of *rough* is required for R8 photoreceptor differentiation in the developing *Drosophila* eye. *Neuron* 32:403–414.
- Freeman M. 1994. The *spitz* gene is required for photoreceptor determination in the *Drosophila* eye where it interacts with the EGF receptor. *Mech Dev* 48:25–33.
- Freund CL, Gregory-Evans CY, Furukawa T, Papaioannou M, Looser J, Ploder L, Bellingham J, Ng D, Herbrick JA, Duncan A, Scherer SW, Tsui LC, Loutradis-Anagnostou A, Jacobson SG, Cepko CL, Bhattacharya SS, McInnes RR. 1997. Cone-rod dystrophy due to mutations in a novel photoreceptor-specific homeobox gene (CRX) essential for maintenance of the photoreceptor. *Cell* 91:543–553.
- Friedrich M. 2006a. Ancient mechanisms of visual sense organ development based on comparison of the gene networks controlling larval eye, ocellus, and compound eye specification in *Drosophila*. *Arthropod Struct Dev* 35:357–378.
- Friedrich M. 2006b. Continuity versus split and reconstitution: exploring the molecular developmental corollaries of insect eye primordium evolution. *Dev Biol* 299:310–329.
- Friedrich M. 2008. Opsins and cell fate in the *Drosophila* Bolwig organ: tricky lessons in homology inference. *Bioessays* 30:980–993.
- Frigerio G, Burri M, Bopp D, Baumgartner S, Noll M. 1986. Structure of the segmentation gene *paired* and the *Drosophila* PRD gene set as part of a gene network. *Cell* 47:735–746.
- Furukawa T, Morrow EM, Cepko CL. 1997. *Crx*, a novel *otx-like* homeobox gene, shows photoreceptor-specific expression and regulates photoreceptor differentiation. *Cell* 91:531–541.
- Glaser T, Walton DS, Maas RL. 1992. Genomic structure, evolutionary conservation and aniridia mutations in the human *PAX6* gene. *Nat Genet* 2:232–239.
- Graham TG, Tabei SM, Dinner AR, Rebay I. 2010. Modeling bistable cell-fate choices in the *Drosophila* eye: qualitative and quantitative perspectives. *Development* 137:2265–2278.
- Greenwood S, Struhl G. 1999. Progression of the morphogenetic furrow in the *Drosophila* eye: the roles of Hedgehog, Decapentaplegic and the Raf pathway. *Development* 126:5795–5808.
- Halder G, Callaerts P, Gehring WJ. 1995. Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*. *Science* 267:1788–1792.
- Hammond KL, Hanson IM, Brown AG, Lettice LA, Hill RE. 1998. Mammalian and *Drosophila dachshund* genes are related to the *Ski proto-oncogene* and are expressed in eye and limb. *Mech Dev* 74:121–131.
- Harrison DA, McCoon PE, Binari R, Gilman M, Perrimon N. 1998. *Drosophila* unpaired encodes a secreted protein that activates the JAK signaling pathway. *Genes Dev* 12:3252–3263.
- Hayashi T, Xu C, Carthew RW. 2008. Cell-type-specific transcription of *prospero* is controlled by combinatorial signaling in the *Drosophila* eye. *Development* 135:2787–2796.
- Heberlein U, Wolff T, Rubin GM. 1993. The TGF beta homolog *dpp* and the segment polarity gene *hedgehog* are required for propagation of a morphogenetic wave in the *Drosophila* retina. *Cell* 75:913–926.
- Helfrich-Forster C. 2002. The circadian system of *Drosophila melanogaster* and its light input pathways. *Zoology (Jena)* 105:297–312.
- Helfrich-Forster C, Edwards T, Yasuyama K, Wisotzki B, Schneuwly S, Stanewsky R, Meinertzhagen IA, Hofbauer A. 2002. The extraretinal eyelet of *Drosophila*: development, ultrastructure, and putative circadian function. *J Neurosci* 22:9255–9266.
- Higashijima S, Kojima T, Michiue T, Ishimaru S, Emori Y, Saigo K. 1992. 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* 6:50–60.
- Hoang CQ, Burnett ME, Curtiss J. 2010. *Drosophila* CtBP regulates proliferation and differentiation of eye precursors and complexes with *Eyeless*, *Dachshund*, *Dan*, and *Danr* during eye and

- antennal development. *Dev Dyn* 239: 2367–2385.
- Hou XS, Melnick MB, Perrimon N. 1996. Marelle acts downstream of the Drosophila HOP/JAK kinase and encodes a protein similar to the mammalian STATs. *Cell* 84:411–419.
- Hsiao FC, Williams A, Davies EL, Rebay I. 2001. Eyes absent mediates cross-talk between retinal determination genes and the receptor tyrosine kinase signaling pathway. *Dev Cell* 1:51–61.
- Jang CC, Chao JL, Jones N, Yao LC, Besarab DA, Kuo YM, Jun S, Desplan C, Beckendorf SK, Sun YH. 2003. Two Pax genes, *eye gone* and *eyeless*, act cooperatively in promoting Drosophila eye development. *Development* 130:2939–2951.
- Jarman AP, Grau Y, Jan LY, Jan YN. 1993. *atonal* is a proneural gene that directs chordotonal organ formation in the Drosophila peripheral nervous system. *Cell* 73:1307–1321.
- Jarman AP, Grell EH, Ackerman L, Jan LY, Jan YN. 1994. *Atonal* is the proneural gene for Drosophila photoreceptors. *Nature* 369:398–400.
- Jemc J, Rebay I. 2007a. The eyes absent family of phosphotyrosine phosphatases: properties and roles in developmental regulation of transcription. *Annu Rev Biochem* 76:513–538.
- Jemc J, Rebay I. 2007b. Identification of transcriptional targets of the dual-function transcription factor/phosphatase eyes absent. *Dev Biol* 310:416–429.
- Jordan T, Hanson I, Zaletayev D, Hodgson S, Prosser J, Seawright A, Hastie N, van Heyningen V. 1992. The human PAX6 gene is mutated in two patients with aniridia. *Nat Genet* 1:328–332.
- Kallio J, Myllymaki H, Gronholm J, Armstrong M, Vanha-aho LM, Makinen L, Silvennoinen O, Valanne S, Ramet M. 2010. Eye transformer is a negative regulator of Drosophila JAK/STAT signaling. *Faseb J* 24:4467–4479.
- Kenyon KL, Ranade SS, Curtiss J, Mlodzik M, Pignoni F. 2003. Coordinating proliferation and tissue specification to promote regional identity in the Drosophila head. *Dev Cell* 5:403–414.
- Kenyon KL, Li DJ, Clouser C, Tran S, Pignoni F. 2005a. Fly SIX-type homeodomain proteins Sine oculis and Optix partner with different cofactors during eye development. *Dev Dyn* 234:497–504.
- Kenyon KL, Yang-Zhou D, Cai CQ, Tran S, Clouser C, Decene G, Ranade S, Pignoni F. 2005b. Partner specificity is essential for proper function of the SIX-type homeodomain proteins Sine oculis and Optix during fly eye development. *Dev Biol* 286:158–168.
- Kim SS, Zhang RG, Braunstein SE, Joachimiak A, Cvekl A, Hegde RS. 2002. Structure of the retinal determination protein Dachshund reveals a DNA binding motif. *Structure* 10:787–795.
- Kramer H, Cagan RL, Zipursky SL. 1991. Interaction of bride of sevenless membrane-bound ligand and the sevenless tyrosine-kinase receptor. *Nature* 352: 207–212.
- Kramer S, West SR, Hiromi Y. 1995. Cell fate control in the Drosophila retina by the orphan receptor seven-up: its role in the decisions mediated by the ras signaling pathway. *Development* 121: 1361–1372.
- Kumar JP. 2009. The sine oculis homeobox (SIX) family of transcription factors as regulators of development and disease. *Cell Mol Life Sci* 66:565–583.
- Kumar JP. 2010. Retinal determination the beginning of eye development. *Curr Top Dev Biol* 93:1–28.
- Kumar JP, Moses K. 2001a. EGF receptor and Notch signaling act upstream of Eyeless/Pax6 to control eye specification. *Cell* 104:687–697.
- Kumar JP, Moses K. 2001b. The EGF receptor and notch signaling pathways control the initiation of the morphogenetic furrow during Drosophila eye development. *Development* 128:2689–2697.
- Labhart T, Meyer EP. 1999. Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microsc Res Tech* 47:368–379.
- Li X, Perissi V, Liu F, Rose DW, Rosenfeld MG. 2002. Tissue-specific regulation of retinal and pituitary precursor cell proliferation. *Science* 297:1180–1183.
- Li X, Cassidy JJ, Reinke CA, Fischboeck S, Carthew RW. 2009. A microRNA imparts robustness against environmental fluctuation during development. *Cell* 137:273–282.
- Lim J, Jafar-Nejad H, Hsu YC, Choi KW. 2008. Novel function of the class I bHLH protein Daughterless in the negative regulation of proneural gene expression in the Drosophila eye. *EMBO Rep* 9:1128–1133.
- Liu Z, Friedrich M. 2004. The Tribolium homologue of glass and the evolution of insect larval eyes. *Dev Biol* 269:36–54.
- Lopes CS, Casares F. 2010. hth maintains the pool of eye progenitors and its downregulation by Dpp and Hh couples retinal fate acquisition with cell cycle exit. *Dev Biol* 339:78–88.
- Lynch VJ, Wagner GP. 2011. Revisiting a classic example of transcription factor functional equivalence: are Eyeless and Pax6 functionally equivalent or divergent? *J Exp Zool B Mol Dev Evol* 316B:93–98.
- Ma C, Moses K. 1995. Wingless and patched are negative regulators of the morphogenetic furrow and can affect tissue polarity in the developing Drosophila compound eye. *Development* 121:2279–2289.
- Ma C, Zhou Y, Beachy PA, Moses K. 1993. The segment polarity gene hedgehog is required for progression of the morphogenetic furrow in the developing Drosophila eye. *Cell* 75:927–938.
- Mardon G, Solomon NM, Rubin GM. 1994. dachshund encodes a nuclear protein required for normal eye and leg development in Drosophila. *Development* 120:3473–3486.
- Mazzoni EO, Desplan C, Celik A. 2004. “One receptor” rules in sensory neurons. *Dev Neurosci* 26:388–395.
- Mazzoni EO, Celik A, Wernet MF, Vasilauskas D, Johnston RJ, Cook TA, Pichaud F, Desplan C. 2008. Iroquois complex genes induce co-expression of rhodopsins in Drosophila. *PLoS Biol* 6: e97.
- McDonald EC, Xie B, Workman M, Charlton-Perkins M, Terrell DA, Reischl J, Wimmer EA, Gebelein BA, Cook TA. 2010. Separable transcriptional regulatory domains within Otd control photoreceptor terminal differentiation events. *Dev Biol* 347:122–132.
- McLellan JS, Yao S, Zheng X, Geisbrecht BV, Ghirlando R, Beachy PA, Leahy DJ. 2006. Structure of a heparin-dependent complex of Hedgehog and Ihog. *Proc Natl Acad Sci USA* 103:17208–17213.
- Mikeladze-Dvali T, Wernet MF, Pistillo D, Mazzoni EO, Teleman AA, Chen YW, Cohen S, Desplan C. 2005. The growth regulators *warts/lats* and *meltd* interact in a bistable loop to specify opposite fates in Drosophila R8 photoreceptors. *Cell* 122:775–787.
- Mishra M, Oke A, Lebel C, McDonald EC, Plummer Z, Cook TA, Zelhof AC. 2010. Pph13 and orthodenticle define a dual regulatory pathway for photoreceptor cell morphogenesis and function. *Development* 137:2895–2904.
- Mlodzik M, Hiromi Y, Weber U, Goodman CS, Rubin GM. 1990. The Drosophila *seven-up* gene, a member of the steroid receptor gene superfamily, controls photoreceptor cell fates. *Cell* 60:211–224.
- Mollereau B, Dominguez M, Weibel R, Colley NJ, Keung B, de Celis JF, Desplan C. 2001. Two-step process for photoreceptor formation in Drosophila. *Nature* 412:911–913.
- Moses K, Rubin GM. 1991. Glass encodes a site-specific DNA-binding protein that is regulated in response to positional signals in the developing Drosophila eye. *Genes Dev* 5:583–593.
- Moses K, Ellis MC, Rubin GM. 1989. The *glass* gene encodes a zinc-finger protein required by Drosophila photoreceptor cells. *Nature* 340:531–536.
- Mutsuddi M, Chaffee B, Cassidy J, Silver SJ, Tootle TL, Rebay I. 2005. Using Drosophila to decipher how mutations associated with human branchio-oto-renal syndrome and optical defects compromise the protein tyrosine phosphatase and transcriptional functions of eyes absent. *Genetics* 170: 687–695.
- Niimi T, Seimiya M, Kloter U, Flister S, Gehring WJ. 1999. Direct regulatory interaction of the eyeless protein with an eye-specific enhancer in the sine oculis gene during eye induction in Drosophila. *Development* 126:2253–2260.
- Nolo R, Abbott LA, Bellen HJ. 2000. Senseless, a Zn finger transcription factor, is necessary and sufficient for sensory organ development in Drosophila. *Cell* 102:349–362.
- Olivier JP, Raabe T, Henkemeyer M, Dickson B, Mbamalu G, Margolis B, Schlessinger J, Hafen E, Pawson T. 1993. A Drosophila SH2-SH3 adaptor

- protein implicated in coupling the sevenless tyrosine kinase to an activator of Ras guanine nucleotide exchange, Sos. *Cell* 73:179–191.
- Ostrin EJ, Li Y, Hoffman K, Liu J, Wang K, Zhang L, Mardon G, Chen R. 2006. Genome-wide identification of direct targets of the Drosophila retinal determination protein Eyeless. *Genome Res* 16:466–476.
- Pai CY, Kuo TS, Jaw TJ, Kurant E, Chen CT, Bessarab DA, Salzberg A, Sun YH. 1998. The Homothorax homeoprotein activates the nuclear localization of another homeoprotein, extradenticle, and suppresses eye development in Drosophila. *Genes Dev* 12:435–446.
- Pan D, Rubin GM. 1998. Targeted expression of *teashirt* induces ectopic eyes in Drosophila. *Proc Natl Acad Sci USA* 95:15508–15512.
- Pappu KS, Ostrin EJ, Middlebrooks BW, Sili BT, Chen R, Atkins MR, Gibbs R, Mardon G. 2005. Dual regulation and redundant function of two eye-specific enhancers of the Drosophila retinal determination gene *dachshund*. *Development* 132:2895–2905.
- Pauli T, Seimiya M, Blanco J, Gehring WJ. 2005. 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* 132:2771–2782.
- Pepple KL, Atkins M, Venken K, Wellnitz K, Harding M, Frankfort B, Mardon G. 2008. Two-step selection of a single R8 photoreceptor: a bistable loop between senseless and rough locks in R8 fate. *Development* 135:4071–4079.
- Perrimon N, Mahowald AP. 1987. Multiple functions of segment polarity genes in Drosophila. *Dev Biol* 119:587–600.
- Pichaud F, Casares F. 2000. *homothorax* and *iroquois-C* genes are required for the establishment of territories within the developing eye disc. *Mech Dev* 96:15–25.
- Pignoni F, Hu B, Zavitz KH, Xiao J, Garrity PA, Zipursky SL. 1997. The eye-specification proteins So and Eya form a complex and regulate multiple steps in Drosophila eye development. *Cell* 91:881–891.
- Powell PA, Wesley C, Spencer S, Cagan RL. 2001. Scabrous complexes with Notch to mediate boundary formation. *Nature* 409:626–630.
- Quiring R, Walldorf U, Kloter U, Gehring WJ. 1994. Homology of the eyeless gene of Drosophila to the Small eye gene in mice and Aniridia in humans. *Science* 265:785–789.
- Rayapureddi JP, Kattamuri C, Steinmetz BD, Frankfort BJ, Ostrin EJ, Mardon G, Hegde RS. 2003. Eyes absent represents a class of protein tyrosine phosphatases. *Nature* 426:295–298.
- Ready DF, Hanson TE, Benzer S. 1976. Development of the Drosophila retina, a neurocrystalline lattice. *Dev Biol* 53:217–240.
- Reifegerste R, Ma C, Moses K. 1997. A polarity field is established early in the development of the Drosophila compound eye. *Mech Dev* 68:69–79.
- Reynolds-Kenneally J, Mlodzik M. 2005. Notch signaling controls proliferation through cell-autonomous and non-autonomous mechanisms in the Drosophila eye. *Dev Biol* 285:38–48.
- Roignant JY, Treisman JE. 2009. Pattern formation in the Drosophila eye disc. *Int J Dev Biol* 53:795–804.
- Salzer CL, Kumar JP. 2009. Position dependent responses to discontinuities in the retinal determination network. *Dev Biol* 326:121–130.
- Salzer CL, Elias Y, Kumar JP. 2010. The retinal determination gene eyes absent is regulated by the EGF receptor pathway throughout development in Drosophila. *Genetics* 184:185–197.
- Seimiya M, Gehring WJ. 2000. The Drosophila homeobox gene *optix* is capable of inducing ectopic eyes by an *eyeless*-independent mechanism. *Development* 127:1879–1886.
- Serikaku MA, O'Tousa JE. 1994. *sine oculis* is a homeobox gene required for Drosophila visual system development. *Genetics* 138:1137–1150.
- Siddall NA, Hime GR, Pollock JA, Batterham P. 2009. Ttk69-dependent repression of lozenge prevents the ectopic development of R7 cells in the Drosophila larval eye disc. *BMC Dev Biol* 9:64.
- Siegmund T, Lehmann M. 2002. The Drosophila Pipsqueak protein defines a new family of helix-turn-helix DNA-binding proteins. *Dev Genes Evol* 212:152–157.
- Silver SJ, Rebay I. 2005. Signaling circuitries in development: insights from the retinal determination gene network. *Development* 132:3–13.
- Simeone A, Acampora D, Mallamaci A, Stornaiuolo A, D'Apice MR, Nigro V, Boncinelli E. 1993. A vertebrate gene related to orthodenticle contains a homeodomain of the bicoid class and demarcates anterior neuroectoderm in the gastrulating mouse embryo. *Embo J* 12:2735–2747.
- Simon MA, Bowtell DD, Dodson GS, Laverty TR, Rubin GM. 1991. Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. *Cell* 67:701–716.
- Simon MA, Dodson GS, Rubin GM. 1993. An SH3-SH2-SH3 protein is required for p21Ras1 activation and binds to sevenless and Sos proteins in vitro. *Cell* 73:169–177.
- Singh A, Kango-Singh M, Sun YH. 2002. Eye suppression, a novel function of *teashirt*, requires Wingless signaling. *Development* 129:4271–4280.
- Singh A, Kango-Singh M, Choi KW, Sun YH. 2004. Dorso-ventral asymmetric functions of *teashirt* in Drosophila eye development depend on spatial cues provided by early DV patterning genes. *Mech Dev* 121:365–370.
- Sprecher SG, Desplan C. 2008. Switch of rhodopsin expression in terminally differentiated Drosophila sensory neurons. *Nature* 454:533–537.
- Sprecher SG, Pichaud F, Desplan C. 2007. Adult and larval photoreceptors use different mechanisms to specify the same Rhodopsin fates. *Genes Dev* 21:2182–2195.
- Sprecher SG, Desplan C. 2009a. Development of the Drosophila eye, from precursor specification to terminal differentiation. In: Tsonis PA, Wittbrodt J, editors. *Animal models for eye research*. New York: Elsevier.
- Sprecher SG, Desplan C. 2009b. Plasticity of terminally differentiated neurons by switching rhodopsin expression. *J Neurogenetics* 23:S22.
- Sun Y, Jan LY, Jan YN. 1998. Transcriptional regulation of atonal during development of the Drosophila peripheral nervous system. *Development* 125:3731–3740.
- Tahayato A, Sonnevill R, Pichaud F, Wernet MF, Papatsenko D, Beaufils P, Cook T, Desplan C. 2003. Otd/Crx, a dual regulator for the specification of ommatidia subtypes in the Drosophila retina. *Dev Cell* 5:391–402.
- Terakita A. 2005. The opsins. *Genome Biol* 6:213.
- Thomas BJ, Gunning DA, Cho J, Zipursky L. 1994. Cell cycle progression in the developing Drosophila eye: roughex encodes a novel protein required for the establishment of G1. *Cell* 77:1003–1014.
- Tomlinson A. 2003. Patterning the peripheral retina of the fly: decoding a gradient. *Dev Cell* 5:799–809.
- Tomlinson A, Ready DF. 1987. Neuronal differentiation in Drosophila ommatidium. *Dev Biol* 120:366–376.
- Tootle TL, Silver SJ, Davies EL, Newman V, Latek RR, Mills IA, Selengut JD, Parlikar BE, Rebay I. 2003. The transcription factor Eyes absent is a protein tyrosine phosphatase. *Nature* 426:299–302.
- Treisman JE, Rubin GM. 1995. wingless inhibits morphogenetic furrow movement in the Drosophila eye disc. *Development* 121:3519–3527.
- Treisman J, Harris E, Desplan C. 1991. The paired box encodes a second DNA-binding domain in the paired homeo domain protein. *Genes Dev* 5:594–604.
- Tsai YC, Sun YH. 2004. Long-range effect of upd, a ligand for Jak/STAT pathway, on cell cycle in Drosophila eye development. *Genesis* 39:141–153.
- Tsai YC, Yao JG, Chen PH, Posakony JW, Barolo S, Kim J, Sun YH. 2007. Upd/Jak/STAT signaling represses wg transcription to allow initiation of morphogenetic furrow in Drosophila eye development. *Dev Biol* 306:760–771.
- Veleri S, Rieger D, Helfrich-Forster C, Stanewsky R. 2007. Hofbauer-Buchner eyelet affects circadian photosensitivity and coordinates TIM and PER expression in Drosophila clock neurons. *J Biol Rhythms* 22:29–42.
- Vraïlas AD, Moses K. 2006. Smoothed, thickveins and the genetic control of cell cycle and cell fate in the developing Drosophila eye. *Mech Dev* 123:151–165.
- Wang LH, Huang YT, Tsai YC, Sun YH. 2010. The role of *eyg* Pax gene in the

- development of the head vertex in *Drosophila*. *Dev Biol* 337:246–258.
- Weasner B, Salzer C, Kumar JP. 2007. Sine oculis, a member of the SIX family of transcription factors, directs eye formation. *Dev Biol* 303:756–771.
- Weasner BM, Weasner B, Deyoung SM, Michaels SD, Kumar JP. 2009. Transcriptional activities of the Pax6 gene *eyeless* regulate tissue specificity of ectopic eye formation in *Drosophila*. *Dev Biol* 334:492–502.
- Wernet MF, Labhart T, Baumann F, Mazzoni EO, Pichaud F, Desplan C. 2003. Homothorax switches function of *Drosophila* photoreceptors from color to polarized light sensors. *Cell* 115:267–279.
- Wernet MF, Mazzoni EO, Celik A, Duncan DM, Duncan I, Desplan C. 2006. Stochastic spineless expression creates the retinal mosaic for colour vision. *Nature* 440:174–180.
- Wilson CW, Chuang PT. 2010. Mechanism and evolution of cytosolic Hedgehog signal transduction. *Development* 137:2079–2094.
- Wolff T, Ready DF. 1991. The beginning of pattern formation in the *Drosophila* compound eye: the morphogenetic furrow and the second mitotic wave. *Development* 113:841–850.
- Xie B, Charlton-Perkins M, McDonald E, Gebelein B, Cook T. 2007. Senseless functions as a molecular switch for color photoreceptor differentiation in *Drosophila*. *Development* 134:4243–4253.
- Yan R, Small S, Desplan C, Dearolf CR, Darnell JE Jr. 1996. Identification of a Stat gene that functions in *Drosophila* development. *Cell* 84:421–430.
- Yao S, Lum L, Beachy P. 2006. The ihog cell-surface proteins bind Hedgehog and mediate pathway activation. *Cell* 125:343–357.
- Yasuyama K, Meinertzhagen IA. 1999. Extraretinal photoreceptors at the compound eye's posterior margin in *Drosophila melanogaster*. *J Comp Neurol* 412:193–202.
- Yau KW, Hardie RC. 2009. Phototransduction motifs and variations. *Cell* 139:246–264.
- Zelhof AC, Koundakjian E, Scully AL, Hardy RW, Pounds L. 2003. Mutation of the photoreceptor specific homeodomain gene Pph13 results in defects in phototransduction and rhabdomere morphogenesis. *Development* 130:4383–4392.
- Zhang T, Ranade S, Cai CQ, Clouser C, Pignoni F. 2006. Direct control of neurogenesis by selector factors in the fly eye: regulation of atonal by Ey and So. *Development* 133:4881–4889.
- Zhang Y, Miki T, Iwanaga T, Koseki Y, Okuno M, Sunaga Y, Ozaki N, Yano H, Koseki H, Seino S. 2002. Identification, tissue expression, and functional characterization of Otx3, a novel member of the Otx family. *J Biol Chem* 277:28065–28069.
- Zheng X, Mann RK, Sever N, Beachy PA. 2010. Genetic and biochemical definition of the Hedgehog receptor. *Genes Dev* 24:57–71.