Human eye conditions: insights from the fly eye

Pedro Gaspar^{1,*}, Isabel Almudi^{2,*}, Maria D. S. Nunes¹ and Alistair P. McGregor¹

¹ Department of Biological and Medical Sciences, Oxford Brookes University, Gipsy Lane, Oxford UK OX3 0BP.

² Centro Andaluz de Biología del Desarrollo, CSIC/ Universidad Pablo de Olavide, Carretera de Utrera Km1, 41013 Sevilla, Spain.

*Contributed equally

1 **Abstract**

2 The fruit fly *Drosophila melanogaster* has served as an excellent model to study and
3 understand the genetics of many human diseases from cancer to neurodegeneration.
4 Studying the regulation of growth, specification and differentiation of the compound eyes
5 of this fly, in particular, have provided key insights into a wide range of diseases. Here we
6 review the regulation of the development of fly eyes in light of shared aspects with human
7 eye development. We also show how understanding conserved regulatory pathways in
8 eye development together with the application of tools for genetic screening and functional
9 analysis make *Drosophila* a powerful model to diagnose and characterise the genetics
10 underlying many human eye conditions, such as aniridia and retinitis pigmentosa. This
11 further emphasises the importance and vast potential of basic research to underpin
12 applied research including identifying and treating the genetic basis of human diseases.

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14

1 **Introduction**

2 The sequencing of the genome of the fruit fly *Drosophila melanogaster* in 2000 (Adams et
3 al. 2000; Myers et al. 2000; Rubin and Lewis 2000) and subsequent comparative genomic
4 studies showed that approximately 70% of human disease-associated genes have a single
5 *Drosophila* homolog, and that there is even greater enrichment for genes that cause
6 lethality in *Drosophila* that are represented by multiple homologues in humans (Reiter et
7 al. 2001; Yamamoto et al. 2014). This highlights the relevance of this model organism to
8 study the function of conserved genes and their effect in human disease (Wangler et al.
9 2015). Indeed, studies of the genetic regulation of *Drosophila* development over the last
10 30 years have provided many crucial insights into the genetic basis and progression of a
11 wide range of human conditions from cancer to neurodegeneration and aging (Burke et al.
12 2017; Kreipke et al. 2017; Michno et al. 2005; Sen and Cox 2017; Sonoshita and Cagan
13 2017).

14 The compound eye of *Drosophila* in particular has proven to be an excellent model
15 for many diseases despite the noticeable anatomical differences between insect and
16 vertebrate eyes (Fig. 1; Table 1). The *Drosophila* eye is constituted of a regular array of
17 several hundred individual light-sensing hexagonal structures called ommatidia (Cagan
18 2009; Hilbrant et al. 2014; Kumar 2012; Posnien et al. 2012), each of which transmits a
19 single image to the brain that must then project it onto one spherical retinal image (Land
20 2005) (Fig. 1). In contrast, the human “camera” eye is a globular structure with a single
21 lens leading to the formation of a single image (Fig. 1). As a consequence, humans have a
22 much narrower field-of-view than fruit flies (Borst 2009). However, the human eye has
23 thousands of sensory cells (rods and cones) packed tightly into its retina (Jonas et al.
24 1992) while each *Drosophila* ommatidia only has eight photoreceptors and 12 accessory
25 cells (Treisman 2013), a difference that contributes to the larger spatial resolution and
26 acuity of the human eye (Borst 2009) (Fig. 1). At the basic structural level, however, both

insect and vertebrate eyes are made up of a lens to focus light, a neural retina with photoreceptors to sense the light and a pigmented epithelium to protect photoreceptor signalling from scattered or diffracted light (Charlton-Perkins et al. 2011; Sanes and Zipursky 2010) (Fig. 1). More importantly, however, decades of research has revealed that the development of *Drosophila* eyes is regulated by conserved genetic pathways that are conserved between flies and humans, and consequently studies of *Drosophila* have taught us much about human eyes (Gehring 2005; Halder et al. 1995; Kumar and Moses 2001; Vopalensky and Kozmik 2009; Wawersik and Maas 2000). In this review we focus on how research on the genetic regulation of *Drosophila* eye development has informed our understanding of many eye conditions in humans (Table 1), highlighting the power and potential of research on fly eyes to better understand and potentially treat human conditions.

Eye development

Master regulators of invertebrate and vertebrate eye development

The *Drosophila* eye develops from an evagination of the embryonic neuroectoderm to form two epithelial sacs, the so-called eye-antenna imaginal disc (Casares and Almudi 2016; Green et al. 1993; Younossi-Hartenstein et al. 1993) (Figs 1 and 2). This region is characterised by the early expression of the conserved transcription factors, *orthodenticle* (*otd*)/*Otx* and *twin of eyeless* (*toy*), and later by expression of the *Pax6* homologue *eyeless* (*ey*) in the posterior region of the epithelial sac, which will give rise to the eye, ocelli, and head capsule and expression of *Cut* in the anterior region, which will mostly give rise to the antenna and maxillary palps (Czerny et al. 1999; Kenyon et al. 2003) (Fig. 2).

Much like in *Drosophila*, vertebrate eye development is centred on the activity of master control genes, namely the *Pax6* gene. Vertebrate *Pax6* genes encode two alternatively spliced variants that differ in the presence of exon 5a. In contrast, *Drosophila*

1 *Pax6* and *Pax6(5a)* homologues arose as separate loci from a relatively recent gene
2 duplication event (Aldaz et al. 2003; Jun et al. 1998). Like in vertebrates, the two
3 *Drosophila Pax6* genes, *ey* and *toy* (Czerny et al. 1999; Quiring et al. 1994), and two
4 *Pax6(5a)* genes, *eyegone (eyg)* and *twin-of-eyegone (toe)* (Jang et al. 2003; Jun et al.
5 1998) have distinct roles in eye development. *ey* and *toy* promote primarily retinal
6 specification, whereas *eyg* mainly promotes cell proliferation (Chao 2004; Dominguez et
7 al. 2004). Each *Pax6/Pax6(a)* orthologue also acts through distinct transcriptional
8 mechanisms, with *Ey* acting as a transcriptional activator, while *Eyg* seems to act as a
9 transcriptional repressor (Chao 2004; Dominguez et al. 2004; Punzo 2004; Punzo et al.
10 2001; Yao and Sun 2005).

11 Based on its sufficiency for eye development in *Drosophila* and vertebrates, it was
12 originally proposed that *Pax6* functions at the highest level of a hierarchy of genes whose
13 sequential expression lead to eye development (Gehring 1996). Indeed, three core
14 members of this postulated hierarchy: *eya* (EYA1, EYA2, EYA3), *sine oculis (so)*, SIX1,
15 SIX2 and SIX3), and *dac* (DACH1) have been identified in *Drosophila* and vertebrates, all
16 sharing similar temporal expression patterns during eye development (Bonini et al. 1997;
17 Chen et al. 1997; Pignoni et al. 1997; Shen and Mardon 1997; Treisman 1999; Zuber
18 2003). Rather than acting in a simple linear pathway, positive transcriptional feedback
19 organizes these master control genes into an interconnected network (Desplan 1997). This
20 regulatory structure appears critical to induce retinal tissue as evidenced by mis-
21 expression experiments where core genes fail to induce ectopic eyes if any of the other
22 critical factors is absent (Bonini et al. 1997). Positive transcriptional feedback seems
23 relevant in this case for activating a second, independently regulated phase of gene
24 expression, as many of these genes indeed function at several stages of eye
25 development. For instance, *ey*, *eya* and *so* are critical for growth of the eye primordium
26 and initiation of differentiation (Bonini et al. 1993; Cheyette et al. 1994; Halder et al. 1998;

Jang et al. 2003; Mardon et al. 1994; Pignoni et al. 1997), while *eya* and *so* are then also required for progression of the MF and photoreceptor differentiation (Pignoni et al. 1997), and *ey* is needed for rhodopsin gene expression (Papatsenko et al. 2001; Sheng et al. 1997).

Cellular differentiation

The differentiation of the cells that form the ommatidia in *Drosophila* is a sequential process. A morphogenetic wave, designated as the morphogenetic furrow (MF, Figs 1 and 2), sweeps across the epithelium from the most posterior region towards the anterior promoting the recruitment and differentiation of the different types of cells that constitute the ommatidium (reviewed in Kumar 2011; Lee and Treisman 2002). During the first larval stages of *Drosophila*, the epithelial cells are undifferentiated progenitor cells that proliferate continuously due to the combined activity of the genes *ey*, *homothorax* (*hth*), *toy*, *teashirt* (*tsh*), *tiptop* and *yorkie* (*yki*) (Figs 1 and 2) (Bessa et al. 2009; Bessa and Casares 2005; Datta et al. 2009; Laugier et al. 2005). The growth of the *Drosophila* eye is interconnected with the organization of both antero-posterior and dorso-ventral axes during development, such as established through the action of Wingless (Wg) and Notch (N) (Dominguez and de Celis 1998; Heberlein et al. 1998; Papayannopoulos et al. 1998). In particular, N induces proliferation by activating the expression of *eyg* (Dominguez et al. 2004). At the same time, the first wave of retinal determinants, mainly *ey* and *toy*, induce and reinforce the expression of other retinal determinants, *eya*, *so* and *dachsund* (*dac*) (Fig. 2) (reviewed in Casares and Almudi 2016; Davis and Rebay 2017).

During differentiation, the activity of Hedgehog ahead of the MF triggers N dependent activation of the proneural gene *atonal* (*ato*) and restriction of its expression in a single cell in each proneural cluster, the R8 cell, which differentiates into the first photoreceptor of the ommatidial cluster (Jarman et al. 1994; Jarman et al. 1995). Similarly,

1 in zebrafish, retinal neurogenesis has been shown to occur in a wave starting in the optic
2 cup adjacent to the optic stalk and spreading outwards, being preceded by expression of
3 Sonic Hedgehog and the Ato homologue Ath5 (Masai et al. 2000; Neumann and
4 Nüsslein-Volhard 2000). In primates, differentiation also follows sequential waves of gene
5 expression, emanating from the optic disc outward (Cornish et al. 2005; Hendrickson et al.
6 2008) (Fig. 1). Much like Ato in *Drosophila*, Ath5 in mice seems to be essential to specify
7 the first retinal neurons, the retinal ganglion cells (Brzezinski et al. 2012; Sun et al. 2003).
8 Furthermore, in zebrafish the early differentiating red cones recruit undifferentiated cells to
9 drive further cone cell differentiation, in a process analogous to R8 recruitment of other
10 photoreceptors in *Drosophila* (Raymond and Barthel 2004). Therefore, the developmental
11 patterning of photoreceptor recruitment from undifferentiated progenitor cells appears to
12 have many similarities between flies and vertebrates (Fig. 1).

13 In flies, Ato activates the expression of *senseless* (*sens*), whereas in the two
14 adjacent remaining cells, Rough inhibits *sens* to specify the R2 and R5 photoreceptors
15 (Pepple et al. 2008). The rest of the photoreceptors are specified in a sequential manner,
16 involving the activity of the Epidermal Growth Factor Receptor (EGFR) signalling pathway,
17 much of which was first delineated in *Drosophila* by the analysis of mutants affecting
18 photoreceptor differentiation (Freeman 1996; Freeman 1997; Kumar et al. 1998). During
19 this specification process, photoreceptors are specialized by expression of specific colour-
20 detecting Rhodopsins, a process that shares some similarities again between flies and
21 vertebrates (Fig. 1). In flies, the Otx-family transcription factor Otd regulates the
22 transcriptional repressor Defective Proventriculus (Dve) to regulate rhodopsin gene
23 expression in specific photoreceptor subtypes (Johnston et al. 2011; Yan et al. 2017). In
24 mice, the genes *Nr2e3* and *Nrl* also act with Otx-family members, like Crx, the homologue
25 of fly Otd, to activate rod opsin expression and repress cone opsin expression (Cheng et
26 al. 2004; Hennig et al. 2008; Kaewkhaw et al. 2015; Peng et al. 2005). Interestingly, like

1 Otd, human OTX1 and OTX2 can induce *dve* expression in *Drosophila* photoreceptors,
2 suggesting conserved mechanisms of OTX-mediated regulation of photoreceptor
3 specification between flies and humans (Terrell et al. 2012a). Differentiation of yellow and
4 pale ommatidial subtypes in *Drosophila* is based on the stochastic expression of the PAS-
5 bHLH transcription factor Spineless, which determines Rh3 expression, being
6 counteracted by Spalt-major (Sal) and Otd to select expression of Rh4 (Johnston et al.
7 2011; Tahayato et al. 2003; Wernet et al. 2006). Interestingly, in mice, Sall3 (homolog of
8 *sal*) has been shown to activate opsins and in humans, expression of red versus green
9 opsins also relies on a stochastic mechanism (de Melo et al. 2011; Nathans et al. 1989;
10 Wang et al. 1992). Although different from the one reported in *Drosophila*, this stochastic
11 mechanism may share aspects of counter-regulation by Sal and OTX factors.

12 Overall, current research suggests that late photoreceptor specification by
13 regulation of Rhodopsin gene expression shares many conserved mechanisms between
14 flies and vertebrates, which highlights this as an area of important focus for understanding
15 human retinal diseases using the *Drosophila* model.

16

17 **Insights into human eye conditions from *Drosophila***

18 ***Drosophila* research on *Pax6* genes and its impact on understanding eye** 19 **developmental diseases**

20 As mentioned above, in both flies and humans, oculogenesis is the product of a conserved
21 gene regulatory network centered on the activity of the master regulator gene *Pax6*
22 (Gehring 2014; Quiring et al. 1994). The *Drosophila* gene *ey* was first cloned and
23 functionally analyzed, being shown to share a high level of sequence conservation with the
24 *Small eye* gene in mice and the *Aniridia* gene in humans, all now collectively established
25 as *Pax6* functional homologues. Nearly 300 dominant mutations in the human *Pax6* locus
26 have been described, which cause haploinsufficiency. Most of these mutations lead to iris

1 hypoplasia or total loss of the iris associated with cataracts and corneal changes, a
2 condition designated as aniridia (Glaser et al. 1992; Hanson et al. 1994; Jordan et al.
3 1992; Kaplan and Rozet 2008). Other mutations in *Pax6* or other functionally conserved
4 genes in eye development are also the cause of failed embryonic optic fissure closure in
5 MAC spectrum diseases, like anophthalmia, characterized by absence of one or both
6 eyes, microphthalmia, characterised by abnormally small eyes with various malformations,
7 to colobomas, characterized by an open iris, retina, choroid or optic disc (Kaplan and
8 Rozet 2008). Of the eye master regulators, apart from *Pax6*, mutations in *Six5*, for
9 example, have been shown promote cataract formation in mice, (Klesert et al.
10 2000; Sarkar et al. 2000) and mutations in *EYA1* have been associated with congenital
11 cataracts (Azuma et al. 2000). Despite the differences in development of the optic
12 primordium between *Drosophila* and vertebrates, *Pax6/Ey* are necessary to induce eye
13 formation, and ectopic expression of *ey* or mouse *Pax6* is sufficient to induce ectopic eyes
14 (Halder et al. 1995). Additionally, a *Drosophila* eye-enhancer of *ey* is capable of driving
15 many features of endogenous *Pax6* expression in mice (Xu et al. 1999). These
16 experiments have established the *Drosophila* model as suitable to study conserved *Pax6*
17 functions in eye development and disease. It is however, worth noting that based on the
18 human and mice mutant phenotypes, *Pax6* in vertebrates seems more critical for lens
19 development.

20 The fly eye, which develops as an evagination of the embryonic ectoderm, much
21 like the vertebrate lens vesicle, may thus bear greater similarities in genetic regulation to
22 this structure than to the whole vertebrate eye (Charlton-Perkins et al. 2011). Of special
23 mention in this respect is the highly conserved regulation of lens Crystallin proteins by
24 *Drosophila Pax2* through the same binding sites as vertebrate *Pax6* (Blanco et al. 2005;
25 Kozmik et al. 2003). Lens crystallins are a family of ancient proteins, found even in
26 jellyfish, where their expression in lentoid bodies relies on the activity of the ancestral

PaxB transcription factor (Kozmik et al. 2003). In *Drosophila*, the *Pax2* gene appears to have been co-opted into lens development, whereas vertebrate *Pax6* has retained or required that function. As such, *Drosophila* research into Pax2 regulation of *Crystallin* gene expression may prove essential to uncover mechanisms of vertebrate lens abnormalities in *Pax6* mutations.

Regulatory switching of *Pax6/ey* in eye development is an area of intense research to which *Drosophila* has contributed significantly. Switching off *ey* expression is required for photoreceptor fate specification, since mitotic clones that inappropriately maintain high *Ey* levels fail to activate markers of neuronal differentiation (Atkins et al. 2013). Similarly, in vertebrates, *Pax6* is lowly expressed in fully differentiated photoreceptors and high levels of *Pax6* have been shown to inhibit differentiation of retinal progenitors (Belecky-Adams et al. 1997; Canto-Soler et al. 2008; Toy et al. 2002).

To achieve this transition, the positive feedback loop that maintains anterior *ey* expression must be interrupted. In *Drosophila*, this inhibition results from rewiring the network such that *Eya-So* directly represses *ey* transcription in differentiating cells (Atkins et al. 2013). Interestingly, increasing the levels of *Eya* and *So* anterior to the MF reduces *ey* expression (Atkins et al. 2013). *Dac* appears to be crucial for this switching, as *Dac* is required for *ey* repression posterior to the MF and can cooperate with *Eya* and *So* to inhibit *ey* transcription in anterior overexpressing clones (Atkins et al. 2013). One idea is that *Dac* joins *Eya-So* to switch the complex into a repressive activity (Davis and Rebay 2017). Although a direct repressive function has not been confirmed in *Drosophila*, this may be a conserved function of *Dac*, as mammalian DACH1 can recruit co-repressors and directly repress target gene transcription (Chen et al. 2013; Chu et al. 2014; Li et al. 2003; Sundaram et al. 2008; Wu et al. 2011; Wu et al. 2008; Wu et al. 2009; Wu et al. 2006; Wu et al. 2003; Zhao et al. 2015). On the other hand, mammalian *Eya* is thought to convert

repressive SIX-DACH complexes to activating EYA-SIX-DACH complexes (Li et al. 2003; Li et al. 2002).

A second example of regulatory switching is the coordination of proliferation with specification in retinal progenitors. Competency to switch from proliferation to specification is initiated when Ey activates transcription of *eya* and *so*, which in turn reinforces *ey* expression and promotes *dac* transcription (Fig. 1) (Anderson et al. 2006; Atkins et al. 2013; Bonini et al. 1997; Chen et al. 1997; Halder et al. 1998; Niimi et al. 1999; Ostrin 2006; Pappu 2005; Pignoni et al. 1997; Salzer and Kumar 2009). *Dac* then terminates the pro-proliferative role of Hth-Yki complexes by inhibiting Hth expression and interfering with the ability of Hth-Yki to activate transcription of the pro-growth *bantam* microRNA (Fig. 1) (Bras-Pereira et al. 2015). Subsequently, Ey cooperates with Eya-So to activate *ato* transcription, which specifies the first photoreceptor to initiate ommatidial assembly (Fig. 1) (Jemc and Rebay 2007; Zhang et al. 2006; Zhou et al. 2014). This results in a mutual inhibition between the Ey-Hth-Tsh and Ey-Eya-So-Dac signaling networks, which drives precursors from asynchronous proliferation to coordinated differentiation. As in the first switching example, *Dac* appears to be a key player in the transcriptional repression events that drive developmental transition. How these transitions are orchestrated at the level of chromatin regulation and transcriptional regulation is poorly understood. One idea stemming from *Drosophila* studies is that core eye determination factors recruit Polycomb group proteins (PcG) to promote switching from proliferative precursors to differentiating retinal cells. Indeed, in a phenotype reminiscent of *eya*, *so*, or *dac* loss, deletion of repressive Polycomb Group genes leads to ectopic Hth and Tsh expression posterior to the MF (Janody et al. 2004). Another intriguing observation is that mutation of *skuld* or *kohtalo*, two Trithorax Group genes, leads to inappropriate maintenance of Ey posterior to the MF (Janody et al. 2004). Consistent with this, Eya1 and Six1 recruit the SWI/SNF complex to activate downstream target transcription that drives cochlear neurogenesis

(Ahmed et al. 2012), while Dach1 primarily associates with co-repressors, as discussed above.

As illustrated in the mechanisms delineated in the *Drosophila* eye model, and by the conservation of molecular mechanisms involving the main conserved retinal determinants in vertebrates, human eye developmental diseases may arise from failure to properly regulate rewiring of the retinal determination network during critical developmental switches, such as the transition from proliferative precursors to differentiating retinal cells.

Retinal Degeneration Models

As highlighted earlier in this review, the *Drosophila* compound eye has little resemblance to the human eye morphologically and yet the gene regulatory networks governing their development are remarkably similar. At a cellular level, the *Drosophila* and human retinas share many similarities, namely in the structure of photoreceptors and in the genetic and molecular basis of phototransduction, both of which are frequently affected in retinal disease. Since the mid-70s genetic screens have been designed in *Drosophila* to identify mutations where the morphogenesis of the eye is normal, but photoreceptor cells degenerate from the onset of adult visual function (Harris and Stark 1977; Harris et al. 1976; Hotta and Benzer 1970; Steele and O'Tousa 1990; Yoon et al. 2000). These studies revealed mutants that lead to either a light independent (Bentrop 1998; Lee et al. 1996; Raghu et al. 2000) (Dolph et al. 1993) or light-dependent photoreceptor degeneration (Meyertholen et al. 1987; Stark and Sapp 1987; Steele and O'Tousa 1990). This was of great relevance to understanding the mechanisms of many inherited degenerative diseases that cause blindness in humans, such as Retinitis Pigmentosa, a disease that affects 1 in 3000 people, being associated with progressive degeneration from night blindness to full blindness, or Leber Congenital Amaurosis, the most early and severe form of inherited retinal dystrophy, accounting for at least 5% of inherited retinal diseases

1 (Kaplan and Rozet 2008). Animal models have shown that retinal degeneration in these
2 cases results from cell death via conserved mechanisms (Chang et al. 1993; Chen et al.
3 1999; Liu et al. 1999). Mutations in humans that cause Retinitis Pigmentosa have been
4 mapped to 35 known genes, and the genetics of their inheritance is complex, from
5 autosomal dominant or recessive to X-linked (Kaplan and Rozet 2008). Mutations in the
6 human rhodopsin genes account for nearly 25% of the autosomal-dominant retinitis
7 pigmentosa (ADPR) cases (Alemaghteh et al. 1993; Bhattacharya et al. ; Shokravi and
8 Dryja 1993; Sung et al. 1993). Importantly, many of the mutations uncovered in *Drosophila*
9 genetic screens affect the synthesis, maturation, intracellular transport, chemical recycling
10 or degradation of the light-sensitive G-protein coupled receptor Rhodopsin.

11 As in vertebrates, the phototransduction cascade in *Drosophila* is initiated by the
12 photoactivation of a G-protein coupled receptor Rhodopsin covalently linked to a
13 chromophore, the carotenoid 3-hydroxylm, 11-cis retinal (Fig. 2). Light-stimulation induces
14 isomerization of the chromophore thereby inducing a conformational change of Rhodopsin
15 to Metarhodopsin that allows its interaction with a Gq α protein, thereby inducing multiple
16 downstream processes, namely metabolism of diacylglycerol and the ensuing regulation of
17 calcium influx (Fig. 2) (reviewed in Wang and Montell 2007; Xiong and Bellen 2013). The
18 phototransduction is terminated upon Metarhodopsin phosphorylation by GPRK1 and
19 upon Arrestin2 binding, which lead to endocytic internalization (Fig. 2). Early studies in
20 *Drosophila* revealed that mutations in genes involved in the termination of Metarhodopsin
21 activity, including *arr2*, *rdgB*, *rdgC*, *norpA* and *Camta*, cause light-dependent retinal
22 degeneration (Fig. 2) (Wang and Montell 2007). In these cases, cell death is light
23 dependent because it depends on the light-induced association of Metarhodopsin with
24 Arrestin, the internalised accumulation of which triggers apoptosis in photoreceptors
25 (Alloway et al. 2000; Kiselev et al. 2000; Kristaponyte et al. 2012). Similar to what happens
26 in *Drosophila*, the vertebrate Rhodopsin mutant RhK296E, common in autosomal

1 dominant Retinitis Pigmentosa (ADPR), forms a stable complex with Arrestin and
2 accumulates in the inner segment of photoreceptors (Chen et al. 2006), suggesting this is
3 a conserved mechanism in retinal degeneration between flies and vertebrates. Other
4 *Drosophila* and vertebrate mutants highlight toxicity due to internalised accumulation of
5 Rhodopsin, such as in genes affecting endolysosomal degradation or the autophagy
6 pathway (Chinchore et al. 2009; Hara et al. 2006; Komatsu et al. 2006; Xu et al. 2004).
7 Furthermore, dominant mutations in *Drosophila* Rh1 were isolated that cause
8 photoreceptor degeneration only in the heterozygous state (Colley et al. 1995; Kurada and
9 O'Tousa 1995), many being identical to those found in ADPR patients, implying that
10 degeneration is dependent on the existence of both wild type and mutant Rhodopsin and
11 revealing that dominant mutant Rhodopsin interferes with the maturation of wild type
12 Rhodopsin (Colley et al. 1995). Toxicity due to incorrect folding or maturation of Rhodopsin
13 has also been implicated by mutations in the *Drosophila* chaperones Calnexin and Xport,
14 which lead to Rh1 accumulation in the ER and reduced Rh1 levels in rhabdomeres and
15 thereby cause light-enhanced retinal degeneration (Rosenbaum et al. 2011; Rosenbaum
16 et al. 2006). On the other hand, uncontrolled activity of Rhodopsin can also result in
17 retinal degeneration. Indeed this is the case in the *Drosophila* Rh1 PP100 mutant, where
18 the mutant opsin persistently binds Arrestin and there is a constitutive activity of the
19 phototransduction cascade (Iakhine 2004). In this case, loss of either Arrestin or the Gqα
20 rescues the degenerative phenotype. This mechanism may be relevant to autosomal
21 dominant Congenital Night Blindness, where a mild retinal degeneration is seen as a result
22 of constitutively active forms of rod opsin (Dryja 2000).

23 The transcriptional mechanisms regulating the expression of Rhodopsin genes is
24 yet another important factor commonly affected in retinal diseases. In *Drosophila*, the
25 transcriptional activation of the Rh3 and Rh5 genes and transcriptional repression of Rh6
26 is orchestrated by the homeodomain protein Otd, and these functions can be partially

1 complemented by the Human Otd-related genes *OTX2* and *Crx*, both of which are
2 expressed in human cones and rods and regulate many photoreceptor-specific genes
3 (Terrell et al. 2012b). Importantly, several homeodomain mutations in *CRX* lead to LCA
4 and this mutations have been used to replace *Drosophila otd* in genetic complementation
5 experiments, revealing differential effects of these mutations on Rhodopsin gene
6 expression and rhabdomeric structure (Terrell et al. 2012b).

7 Concerning rhabdomeric structure, some *Drosophila* mutants have been particularly
8 useful in elucidating how this impacts retinal degeneration. In particular mutations affecting
9 the Crumbs complex, formed by the proteins Stardust, Discs-lost/PATJ and Crumbs, were
10 shown to disrupt the correct separation of the rhabdomere from the stalk membrane in
11 photoreceptors (Fig. 3) (Berger et al. 2007; Hong et al. 2003; Pellikka et al. 2002; Richard
12 et al. 2006). Furthermore, mutations affecting the GPI-anchored protein Choptin and the
13 transmembrane protein Prominin along with its associated extracellular glycoprotein Eyes
14 Shut, prevent the separation of adjacent rhabdomeric membranes and disrupt the inter-
15 rhabdomeral space (Fig. 3) (Cook and Zelhof 2008; Gurudev et al. 2014; Husain et al.
16 2006; Nie et al. 2012; Zelhof et al. 2006). These mutations result in light-dependent
17 photoreceptor degeneration possibly as a direct consequent of loss of localisation of
18 Rhodopsin and other proteins to the rhabdomeric membrane. Indeed, these effects are
19 similar to architectural defects seen in the rhabdomeres of null or strong mutants of
20 *Drosophila* Rh1 (Kurada and O'Tousa 1995; Leonard et al. 1992). Importantly, Crumbs
21 has been shown to interact and stabilise Myosin V and thereby promote trafficking of Rh1
22 to the rhabdomeres (Pocha et al. 2011). This mechanism may bear relevance to cases of
23 autosomal recessive Retinitis Pigmentosa and Leber Congenital Amaurosis and
24 autosomal dominant pigmented paravenous chorioretinal atrophy associated with
25 mutations in the human *CRB1* gene (Cremers et al. 2002; den Hollander et al. 2010; den
26 Hollander et al. 2001; Den Hollander et al. 1999; Jacobson et al. 2003; Lotery et al. 2001;

McKay et al. 2005), or in autosomal recessive retinal degeneration caused by mutation in human *PROMINI-1* (Maw et al. 2000), or *RP25*, orthologue of *Drosophila Eyes Shut* (Abd El-Aziz et al. 2008; Alfano et al. 2016; Yu et al. 2016).

Critically, *Drosophila* research has contributed to highlight targets for therapeutical approaches to many of the degenerations reported above. The chemical transformations of dietary carotenoids into 3-hydroxylm, 11-cis retinal, were shown to be important modulators of light-induced retinal degeneration (Voolstra et al. 2010; Wang et al. 2010; Wang et al. 2012). Limiting de-novo chromophore synthesis, via B-carotene/Vitamin-A dietary depletion, can greatly reduce Rhodopsin levels, thus significantly rescuing light-induced retinal degeneration in mutants affecting the stability of the metharodopsin-arrestin2 complex (Alloway et al. 2000; Berger et al. 2007; Kiselev et al. 2000; Richard et al. 2006), and similarly in *crumbs*, *stardust* and *PATJ* mutant eyes (Johnson et al. 2002).

On the other hand, in cases of accumulation of immature Rhodopsin, a *Drosophila* study has shown that boosting ER-associated degradation (ERAD), by overexpression of the ERAD factors Hrd1 and EDEM2, reduces mutant Rh1 levels in dominant RhG69D mutants, thereby delaying retinal degeneration (Kang and Ryoo 2009). Intriguingly, genetic inactivation of the ERAD effector chaperone VCP/ter94 in *Drosophila* or its chemical inhibition with Eeyarestatin I, led to strong suppression of retinal degeneration caused by accumulation of the immature RhP23H mutant (Griciuc et al. 2010). These apparently conflicting observations hint at different mechanisms of dominance of Rh1 mutations and argue that manipulation of ERAD for therapeutic purposes should consider these differences carefully. Similar to the effects observed for manipulation of ERAD, potentiating autophagy or lysosomal degradation pathways, can effectively reduce Rhodopsin accumulation and ameliorate retinal degeneration (Lee et al. 2013; Wang et al. 2009).

1 A possible mechanism underlying the degeneration upon subcellular Rhodopsin
2 accumulation is an overload of cytoplasmic Ca²⁺ influx (Orrenius et al. 2003). Cytoplasmic
3 Ca²⁺ influx induces the dephosphorylation of metharhodopsin by CAMKII, and therefore,
4 low levels of Ca²⁺ can lead to accumulation of internalized Metharhodopsin-Arrestin
5 complexes and cause photoreceptor degeneration (Chinchore et al. 2009; Kiselev et al.
6 2000; Orem and Dolph 2002). This is also seen in loss of function mutations in TRP
7 calcium channels, but simultaneous counterbalancing mutations in the Ca²⁺/Na²⁺
8 exchanger CalX or the Diacylglycerol Kinase, RDGA, can greatly suppress light-induced
9 retinal degeneration, indicating that balanced Ca²⁺ levels are critical for photoreceptor
10 survival (Fig. 3) (Raghu et al. 2000; Wang et al. 2005). Cytoplasmic Ca²⁺ influx during the
11 photo-response is modulated by the levels of PIP2 and their effect on calcium channels.
12 Interestingly, an altered PIP2 regeneration cycle in *Drosophila* photoreceptors, such as
13 through mutations affecting the Diacylglycerol Kinase, RDGA, or the Phosphatidate
14 phosphatase, Lazaro, or overexpression of phospholipase D, can all modify light-induced
15 neurodegeneration phenotypes (Garcia-Murillas et al. 2006; Inoue et al. 1989; Kwon and
16 Montell 2006; LaLonde et al. 2006; Masai et al. 1993).

17 It has been suggested that dominant negative mutations in Rhodopsin associated
18 with Retinitis Pigmentosa could be especially amenable to gene therapy, as providing
19 extra dosage of Rhodopsin was shown to significantly ameliorate the cell death observed
20 in the Rh P23H mouse model (Lewin et al. 2014; Mao et al. 2011). However, too much
21 Rhodopsin overexpression was also shown to cause retinal degeneration in this case,
22 indicating that Rhodopsin augmentation needs to be tightly controlled for therapeutic
23 purposes. In *Drosophila*, it was also noticed that expression of phosphorylation deficient
24 Rhodopsin could offer protective effects in *norpA* mutant photoreceptors, indicating that
25 modulation of Rhodopsin phosphorylation is critical to prevent its high levels of toxic
26 internalization in light-dependent retinal degeneration (Kristaponyte et al. 2012).

With the advent of CRISPR/Cas9 technology, it is now possible to realize many of these gene therapy changes, albeit cautiously considering possible off-target effects. Recently, AAV-mediated CRISPR/Cas9 targeting of Nrl, a rod-specific transcription factor, was shown to improve rod survival in a mouse Rh P374S mutant (Yu et al. 2017). Furthermore, CRISPR/Cas9 targeting of the Rh P23H allele has been achieved efficiently in a null background both in the mouse and pig retina, offering a great promise for gene therapy (Burnight et al. 2017; Latella et al. 2016). Alternatively, using an artificial transcription factor, it was recently possible to repress a faulty Rhodopsin gene in pigs, while maintaining normal expression of the wild type Rhodopsin (Botta et al. 2016).

Future perspectives

The genetic basis of most eye conditions, for example anophthalmia and microphthalmia, has only been characterised in less than 30% of cases (Chassaing et al. 2014; Williamson and FitzPatrick 2014). This low rate of diagnosis is caused in part by the polygenic nature of eye development and by the fact that many cases are caused by rare variants. This is exacerbated by a poor understanding of the function of most human genes. Further functional analysis of known disease causing genes and screens for new candidates in *Drosophila* has great potential to improve diagnosis and better understand the underlying mechanisms (Wangler et al. 2017). This was exemplified recently by an elegant screen in *Drosophila* for lethal mutations in genes involved in sensory functions and cross-referencing with human exome data (Yamamoto et al. 2014). This study allowed the diagnosis of conditions in several individuals including in a new role for *Crx* in bull's eye maculopathy where the phenotypic effect on photoreceptors was similar in flies and humans (Yamamoto et al. 2014). This was facilitated by the availability of sophisticated approaches for screening and powerful tools for genetic and phenotypic analysis in this model. Therefore it is clear that the *Drosophila* eye will continue to be an excellent and

economic model to study eye conditions as well as other diseases (Chow and Reiter 2017; Kumar 2018; Senturk and Bellen 2017; Yamamoto et al. 2014). This can be enhanced by further collaborations between clinicians and researchers working on *Drosophila* (Wangler et al. 2015) as part of national and international initiatives such as the Undiagnosed Diseases Network (Gahl et al. 2016) and tools such as GeneMatcher (Sobreira et al. 2015a; Sobreira et al. 2015b).

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Conflict of interest statement

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Figure legends

Figure 1. Comparative overview of human and *Drosophila* eye development. Eye development from week 4 to week 20 of human embryogenesis a. At week 4 of development, the optic vesicles evaginate from the forebrain neuroectoderm inducing the adjacent ectoderm to form the lens placode. Between weeks 4 and 6, the optic vesicle invaginates on itself, forming the optic cup, which partially encapsulates the invaginating lens vesicle (interrupted posteriorly by the optic fissure). After week 6 and until week 20, the optic cup differentiates into two main layers, the neuroretina and the pigmented epithelium, while the lens vesicle thickens and forms the crystalline lens. The neural cells in the neuroretina differentiate from the center to the periphery of the optic disc, such that the adult retina pattern is set with circumferential bands or zones enriched in specific photoreceptors. Eye development and adult retina patterning of *Drosophila* b. The eye imaginal disc develops from an invagination of the embryonic ectoderm, being subdivided early to form precursor regions to the eye and antenna (1st larval instar imaginal disc). During the 3rd larval instar, differentiation of ommatidia into clusters of 8 photoreceptor cells progresses from the posterior to the anterior of the disc, as a band of apical cell constrictions termed the morphogenetic furrow sweeps across the tissue. The longitudinal structure of the adult ommatidium shows an apical facet lens lined basally by primary pigment cells and focusing into a central rhabdomere projected by the lateral membrane of the photoreceptor cells. The cluster of photoreceptor cells is surrounded by secondary and tertiary pigment cells. The adult retina is patterned dorso-ventrally into two main zones, a dorsal third consisting of the dorsal rim area and dorsal yellow ommatidia, and a ventral area with a stochastic pattern of yellow and pale ommatidia. Ommatidia in these areas have a specific repertoire of rhodopsin (Rh) gene expression in photoreceptors R7 and R8 (A—anterior, P—posterior, D—dorsal, V—ventral, R—photoreceptor, Rh—rhodopsin)

1

2 **Figure 2.** *Drosophila melanogaster* eye development. (A-B) *Drosophila* adult head (A) and

3 eye-antennal imaginal disc stained with anti-Eya (magenta), DAPI (blue) and phalloidin-

4 AlexaFluor488 (Green) (B). Colour code represents the larval structures that will give rise

5 to the adult structures of the head: compound eye (CE, magenta), ocelli (oc, dark

6 magenta), antenna (a, dark green), maxillary palp (mp, light green), and head capsule (hc,

7 blue). (C) Section of an eye imaginal disc showing the compartments where proliferation

8 (most anterior), determination (around MF) and specification (posterior to MF) are

9 occurring. From anterior to posterior (left to right): progenitors are characterised by high

10 levels of proliferation (mitotic cells in red), followed by synchronic cells at G1, the First

11 Mitotic Wave (FMW), Morphogenetic Furrow (MF), cells differentiating (second mitotic

12 wave, SMW) into ommatidia most posteriorly. Mitotic cells are stained using an anti-PH3

13 antibody (red), MF is marked with Phalloidin-AlexaFluor488 (green) and Photoreceptors

14 are shown using anti-Elav, in blue. A simplified gene network showing the main molecules

15 involved in the three processes is displayed. Colour code highlights the process in which

16 each molecule has its main role. Scale bar 50 μ m. All the panels show anterior to the left

17 and posterior to the right.

18

19 **Figure 3.** The *Drosophila* phototransduction apparatus. The *Drosophila* compound eye (A)

20 is composed of hundreds of ommatidia units (B) made up of a cluster of 8 photoreceptor

21 cells surrounded by pigment cells and mechanosensory bristles. Each photoreceptor cell

22 projects a microvilli dense membrane towards the centre of the ommatidium, making up

23 the light-sensitive rhabdomere (C). The rhabdomere membrane is supported at its base by

24 the Crumbs/PatJ/Stardust complex and at its core by the structural proteins Chaoptin,

25 Prominin and Eyes Shut. Within the rhabdomere membrane (D), the phototransduction

26 cascade is initiated by the light-induced conformational change of Rhodopsin into

1 Metarhodopsin, which in turn is able to become phosphorylated by GPRK1, interact with a
2 Gαq protein and bind Arrestin2. This initiates a series of other reactions, from the
3 conversion of PIP2 into IP3 and DAG to the internalisation of Metarhodopsin-Arrestin
4 complexes. DAG is able to stimulate Ca^{2+} influx via TRP channels, which in turn can be
5 counterbalanced by $\text{Ca}^{2+}/\text{Na}^{+}$ antiport by the CalX channel. Light-induced conformational
6 change of Metarhodopsin can promote its dephosphorylation by RdgC and
7 phosphorylation of Arrestin2 by CAMKII, which promotes its dissociation, thus ensuing the
8 recycling of Rhodopsin's original state.

9

10

1 Table 1.

Human gene	Phenotype	References	<i>Drosophila</i> gene	Phenotype	References
PAX6	Aniridia, anophthalmia, microphthalmia	Glaser et al. (1992); Jordan et al. (1992); Ton et al. (1991)	eyeless	Reduced eyes	Patterson and Muller (1930)
EYA1	Congenital cataracts; anterior segment anomalies	Azuma et al. (2000)	eyes absent	Eye facets completely absent in strong alleles	Bonini et al. (1993)
SIX1	Cataracts; branchio-oto-renal syndrome; deafness	Salam et al. 2000	sine oculis	Eyes reduced to small groups of ommatidia, occasionally missing	Cheyette et al. (1994)
SIX5	branchio-oto-renal syndrome; deafness	Hoskins et al. (2007)			
SIX3	holoprosencephally and microphthalmia	Wallis et al. (1999)	optix	Eye defects	Weasner et al. (2007)
SIX6	Optic disc anomalies with retinal and/or macular dystrophy	Aldahmesh et al. (2013)			
DACH1	Microphthalmia, anophthalmia, and coloboma	Warburg (1993)	dachshund	Severely reduced or no eyes	Shen and Mardon (1997)
MEIS2	Cleft palate; cardiac defects; mental retardation	Crowley et al. (2010)	homothorax	Overgrowths of eye tissue; loss of ventral and dorsal head structures	Pichaud and Casares (2000)
ATOH7	Congenital blindness; nystagmus; shallow anterior chamber; massive retrolental mass; retinal	Ghiasvand et al. (2011)	atonal	Neuroanatomy defective; adult photoreceptors missing	Jarman et al. (1995)

	nonattachment				
OTX2	Microphthalmia, syndromic 5; Retinal dystrophy; Early-onset, with or without pituitary dysfunction; Cone-rod retinal dystrophy-2	Freund et al. (1997); Ragge et al. (2005)	oc	Eyes somewhat reduced and body size dwarfed. Phototaxis normal	Benzer (1967)
CRX	cone-rod dystrophy; retinitis pigmentosa; LCA	Freund et al. (1997); Freund et al. (1998); Sohocki et al. (1998)			
CRB1	Pigmented paravenous chorioretinal atrophy; Retinitis pigmentosa-12, autosomal recessive	Den Hollander et al. (1999)	Crumbs	Mild overgrowth of adult eyes	Chen Ling, ... Pan 2010
EYS	Retinitis pigmentosa 25	Abd El-Aziz et al. (2008)	Eyes Shut	Rhabdomere defects	Zelhof et al. (2006)
PAX2	optic nerve coloboma	Favor et al. (1996); Sanyanusin et al. (1995)	D-Pax2	Eyes small, rough, and glazed	Fu et al. (1998)
SHH	holoprosencephally	Roessler et al. (1996)	hedgehog	Eye small and narrow with about 150 facets	Lee et al. (1992); Renfranz and Benzer, 1989, Dev. Biol. 136: 411-29). Heberlein et al, Nature 1995
RHO	Autosomal dominant retinitis pigmentosa	Bhattacharya et al. (); Dryja et al. (1991); Dryja et al. (1990)	ninaE	rhabdomeres 1-6 is smaller than normal; light-independent degeneration of R1-6 rhabdomeres	Kurada and O'Tousa (1995) Colley et al, PNAS, 1995;
PROMININ-1	autosomal recessive retinal degeneration	Maw et al. (2000)	Prominin	defective photoreceptor morphogenesis; disrupted interrhabdomeral space	Zelhof et al. (2006)

A Human eye development

B *Drosophila* eye development

PROLIFERATION DETERMINATION SPECIFICATION

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3