Zebrafish Mutants as Models for Congenital Ocular Disorders in Humans

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INTRODUCTION

In Western societies, the vast majority of people (>68%) will experience some form of vision loss due to cataracts, glaucoma, or retinopathies such as age-related macular degeneration and retinitis pigmentosa. Although many causes of blindness throughout the world are preventable (e.g., poor nutrition, parasites), genetic disorders underlie the majority of visual impairments in developed countries. As these diseases exhibit simple to complex genetic characteristics, the ability to develop therapies that prevent or correct vision loss will depend on a more thorough understanding of the genes and mechanisms involved in ocular dysfunction. To rapidly identify genes involved in blindness, researchers rely on model organisms that mimic the physiology and pathology of the human eye and can be used for high-throughput genetic screens. Recent advances demonstrate that the zebrafish serves as an ideal model for human ocular disorders.

In this review, we focus exclusively on those identified zebrafish mutants that serve as models for inherited forms of human blindness (Table 1). Zebrafish models exist for human diseases affecting ocular morphogenesis (coloboma), the anterior chamber (glaucoma and cataracts), as well as several types of photoreceptor disorders (Usher Syndrome, Bardet–Biedl Syndrome (BBS), achromotopsia, choroideremia, and retinitis pigmentosa). In many cases, the mutated zebrafish gene is an ortholog to a known human disease gene. Furthermore, of the dozens of zebrafish mutants presenting with ocular phenotypes, almost all are represented by only a single allele, which indicates we remain a long way from reaching saturation in genetic screens. While some zebrafish mutations may not yet correspond to known disease-causing loci in humans, analysis of these mutants will none the less provide a better understanding of mechanisms leading to ocular dysfunction and hopefully direct clinical geneticists toward novel gene products or molecular pathways involved in human ocular pathologies. As the purpose of this review is to focus solely on models of human disease, we refer readers to several recent reviews that describe numerous other zebrafish mutations affecting different aspects of eye development and function as well as the methodology to identify those mutants (Goldsmith and Harris, 2003; Tsujikawa and Malicki, 2004b; Morris and Fadool, 2005).

Development and Anatomy of the Zebrafish Eye

Eye development in zebrafish begins at the six-somite stage (SS) when the optic lobes evaginate from the diencephalon (Schmitt and Dowling, 1994). Development is rapid thereafter; lens induction occurs at the 14–15 SS, the retina and retinal pigment epithelium (RPE) become morphologically distinct at 18–19 SS, choroid fissure formation begins at 18 SS and the fissure closes at around 24 hr post-fertilization (hpf). The first post-mitotic neurons of the retina form at 28hpf (Hu and Easter, 1999; Schmitt and Dowling, 1999) and zebrafish exhibit visual function by 72hpf (Easter and Nicola, 1996).

Vertebrate eyes can be subdivided into an anterior segment composed of the cornea, lens, iris, ciliary body, and other specialized tissues at the iridocorneal angle, and a posterior segment composed of the retina, RPE, and choroid (Fig. 1). Anterior segment formation in embryonic and adult zebrafish has been thoroughly described (Soules and Link, 2005; Zhao et al., 2006; Dahm et al., 2007), and zebrafish possess many stereotypical anterior segment structures. The anatomy of the zebrafish anterior segment differs to some extent from that in humans; for instance, the zebrafish lens develops as a solid mass of cells that delaminate from the surface ectoderm, rather than as a hollow vesicle. Zebrafish also do not possess iris muscles, instead, the zebrafish iris contains several types of pigment cells that may function in preventing light from entering the eye outside of the pupil (Soules and Link, 2005). Nonetheless, there is

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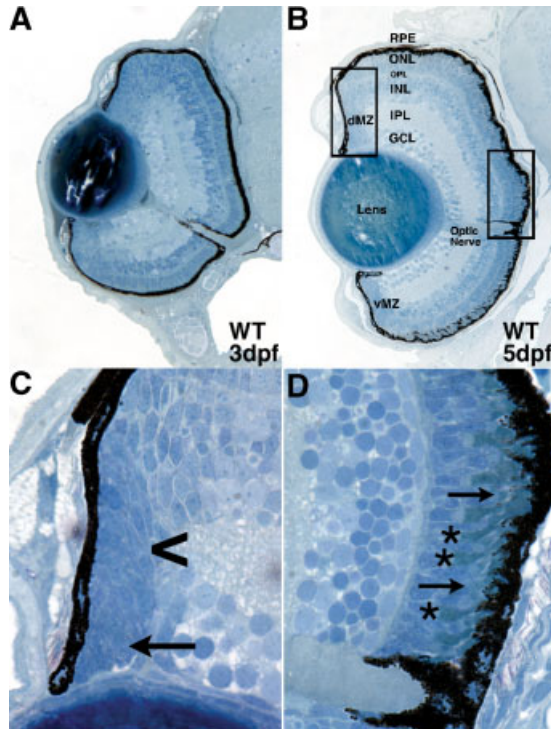
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TABLE 1. Zebrafish Ocular Mutants

Mutant	Gene	Human disease OMIM	Ocular phenotype	References
adenomatous polyposis coli (apc)	apc	175100	Coloboma	Nadauld et al. (2006)
bleached (blc)	Unknown	—	RPE/retinal degeneration	Neuhauss et al. (2003)
brass	Unknown	—	Elevated IOP, iris hypoplasia	Link et al. (2004)
blowout (blw)	Unknown	—	Coloboma	Karlstrom et al. (1996)
brudas (bru)	Unknown	—	Photoreceptor defects	Malicki et al. (1996)
cloche (clo)	Unknown	—	Cataracts	Goishi et al. (2006)
elipsa (eli)	Unknown	—	Photoreceptor defects	Malicki et al. (1996)
fade out (fad)	Unknown	—	RPE/retinal degeneration	Bahadori et al. (2006)
fading vision (fdv)	silva	193500	RPE pigmentation, visual function (embryonic)	Schonthaler et al. (2005)
fleer (flr)	Unknown	—	Photoreceptor defects	Malicki et al. (1996)
gantenbein (grnn)	Unknown	—	RPE/retinal degeneration	Biehlmaier et al. (2003)
ift57	ift57	—	Photoreceptor defects	Gross et al. (2005)
ift172	ift172	—	Photoreceptor defects	Gross et al. (2005)
laminin α1 (lama1)	lama1	—	Anterior segment dysgenesis, visual function	Semina et al. (2006); Biehlmaier et al. (2007)
laminin β1 (lamb1)	lamb1	—	Coloboma, lens dysgenesis, visual function	Lee and Gross (2007); Biehlmaier et al. (2007)
laminin γ1 (lamc1)	lamc1	—	Coloboma, lens dysgenesis, visual function	Lee and Gross (2007); Biehlmaier et al. (2007)
mariner (mar)	myo7a	276900	Photoreceptor defects	Ernest et al. (2000)
mikre oko (mok)	Unknown	—	Photoreceptor defects	Malicki et al. (1996)
n-cadherin (ncad)	ncad	—	Coloboma, retinal disorganization	Masai et al. (2003); Erdmann et al. (2003); Malicki et al. (2003)
niezerka (nie)	Unknown	—	Photoreceptor defects	Malicki et al. (1996)
Night blindness (nba, nbb, nbc, nbd, nbe, nbf, nbg)	Unknown	—	Night blindness (heterozygotes)	Li and Dowling (1997, 2000); Maaswinkel et al. (Maaswinkel et al., 2003a,b, 2005)
no optokinetic response a (noa)	dlat	245348	Visual function	Taylor et al. (2004)
no optokinetic response f (nof)	gnat2	139340	Visual function	Brockerhoff et al. (2003)
oval (ovl)	ift88	—	Photoreceptor defects	Tsujikawa and Malicki (2004a)
Rab escort protein 1 (rep1)	rep1	303100	Retinal degeneration (choroideremia)	Krock et al. (2007)

Zebrafish mutants discussed in this review that serve as useful models for human ocular disorders and their associated pathologies. On-line Mendelian Inheritance in Man (OMIM) entries are listed for those mutants whose disrupted gene is orthologous to a human disease locus. A number of additional zebrafish ocular mutants exist and interested readers are directed to several recent reviews describing some of these mutants (Goldsmith and Harris, 2003; Tsujikawa and Malicki, 2004; Morris and Fadool, 2005).

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Fig. 1. Wild-type development of the zebrafish retina. **A**: Three dpf retina with well-formed laminae in the central retina and large regions of undifferentiated cells at the retinal periphery. **B**: Five dpf retina exhibiting nuclear laminae and cell types characteristic of the mature retina. **C**: High magnification view of the dorsal peripheral retina. Note the change in cell shape as epithelial progenitor cells divide at the peripheral-most dMZ (arrow) and are subsequently displaced into the central retina to differentiate into more rounded appearing retinal neurons (arrowhead). **D**: High magnification view of the central retina and optic nerve. Note the prominent short single cones in the innermost region of the ONL (asterisks) and the elongated rod outer segments in the outer ONL, protruding into the RPE (arrows). Dorsal is up in all panels. Scale bar is 100 μm. RPE, retinal pigment epithelium; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; dMZ, dorsal marginal zone; vMZ, ventral marginal zone. (Reprinted with permission from Gross et al., 2005).

an overall conservation in the temporal aspects of morphogenetic events in the anterior segment, similar identities of the cell lineages that generate these cell types and, with some caveats, a general conservation of the overall anatomical structures in the anterior segment. Combined, these attributes make zebrafish an excellent model for studying anterior segment disorders such as glaucoma, cataracts, and corneal dystrophies.

Within the posterior segment, the organization and function of the retina is also highly conserved among vertebrate species. Like humans, zebrafish possess seven major retinal cell types: rod and cone photoreceptors, horizontal cells, bipolar cells, amacrine cells, ganglion cells, and Müller glia. In addition, the RPE provides physiological and trophic support to the neural retina. The timing of key morphogenetic events in retina and RPE development is conserved between zebrafish and other vertebrates, and the ontogeny of these structures is

similar. Thus, these attributes strongly support the utility of zebrafish as a model for studying congenital disorders of the human posterior segment as well.

Zebrafish Genetics

George Streisinger first demonstrated the use of zebrafish as a vertebrate model for genetic studies more than 25 years ago (Streisinger et al., 1981). Streisinger realized zebrafish possessed many qualities that make it an ideal genetic model system. Zebrafish are small (3–4 cm long as adults), freshwater fish that can be easily maintained in large numbers and at reasonable expense by individual laboratories. The transparency of the developing embryos permits developmental processes to be viewed ex utero. The combination of a short generation time (3–4 months) and the ability of a single mating pair to produce 100 or more offspring about once per week make zebrafish an ideal model for genetic studies.

The explosion of interest in zebrafish as a model organisms occurred in the mid-1990s when several laboratories, most notably the Driever and Nüsslein-Volhard groups, began publishing the results of large-scale forward genetic screens that identified thousands of mutations affecting zebrafish development (Driever et al., 1996; Haffter et al., 1996). These early screens used chemical mutagens and morphological criteria to visually identify mutants, and many of the mutants exhibited phenotypes that resembled human congenital disorders. Moreover, the development of more sophisticated mutagenesis and screening techniques (e.g., retroviral insertional mutagenesis, transgenesis, automated behavioral screens), and the positional cloning of many of the affected loci, has revealed a number of additional zebrafish mutants that model congenital disorders in humans, and more specifically, that resemble congenital ocular disorders. Finally, while not discussed in this review, reverse genetic techniques (e.g., morpholino antisense oligonucleotide "knock-down") have also contributed substantially to the use of zebrafish in studying human ocular disorders (Malicki et al., 2002).

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Morphogenetic Disorders—Coloboma

Congenital coloboma are defined as clefts of absent tissue in the eye that result from a failure of the choroid fissure to close during the early phases ocular morphogenesis. The incidence of coloboma is 2.6 in 10,000 births in the US and coloboma have been estimated to be present in 5–10% of all blind children worldwide (Porges et al., 1992). Coloboma are a phenotypic manifestation in over 50 distinct human genetic disorders (OMIM: http://www.ncbi.nlm.nih.gov/) and are often associated with other congenital abnormalities in the eye, including microphthalmia and anophthalmia (Bermejo and Martinez-Frias, 1998), as well as those of other organ systems. Many genetic loci have been associated with

coloboma in both humans and animal models, however, the molecular mechanisms that underlie these defects have not been well described (reviewed in Chang et al., 2006). Several recessive mutations have been identified in zebrafish that serve as models for human coloboma: *n-cadherin* (*ncad*), *laminin β1* (*lamb1*), *laminin γ1* (*lamc1*), *adenomatous polyposis coli* (*apc*), and *blowout* (*blw*) (Karlstrom et al., 1996; Erdmann et al., 2003; Malicki et al., 2003; Masai et al., 2003; Gross et al., 2005; Nadauld et al., 2006).

lamb1 and *lamc1* mutants present with complex ocular phenotypes that include coloboma, retina, and anterior segment defects (Biehlmaier et al., 2007; Lee and Gross, 2007). The morphogenetic mechanisms underlying the coloboma in these mutants have not yet been identified, however. Laminin proteins play critical roles in the assembly and maintenance of basement membranes and cell-extracellular matrix (ECM) interactions are required for a number of morphogenetic events in developing organisms. This suggests that the molecular basis of the coloboma in these mutants is directly related to pathological changes in ECM deposition or cell–ECM interactions. *blw* mutants possess coloboma but this mutant is unique in that it presents no other obvious ocular or systemic defects. The molecular nature of the coloboma in *blw* have yet to be determined; however, preliminary studies from our laboratory indicate that they may result from defects in morphogenesis of the optic stalk, an epithelial tissue that connects the developing eye to the forebrain during early eye development (*J.M.G.*—unpublished observations). Positional cloning of *blw* and a detailed characterization of choroid fissure closure in this mutant should further our understanding of the etiology of human coloboma.

Patients with familial adenomatous polyposis present with adenomatous colonic polyps that can lead to adenocarcinomas, and in many cases these patients also present with severe ocular defects that include RPE hypertrophy and coloboma. Heterozygous mutations in the adenomatous polyposis coli (*APC*) gene underlie the disease but it remains unclear how these mutations lead to ocular defects. Recently, Nadauld et al. (2006) characterized a recessive zebrafish *apc* mutant and demonstrated through an elegant series of experiments that defects in retinoic acid (RA) production may be the cause of coloboma in these mutants. RA signaling has been shown to be critical for formation of the ventral retina in zebrafish (Marsh-Armstrong et al., 1994) and rodents (Wilson et al., 1953). Moreover, RA is derived from vitamin A and several ocular pathologies in humans, including coloboma, may stem from deficiencies in vitamin A (Dowling and Wald, 1958; Seeliger et al., 1999; Hornby et al., 2003). In their studies, Nadauld et al. demonstrated that Apc regulates the expression of Rdh5, a retinol dehydrogenase, and loss of Rdh5 function leads to coloboma. Interestingly, coloboma in *apc* mutants could be rescued by exogenous RA suggesting that coloboma in familial adenomatous polyposis patients may stem from RA deficiencies.

Anterior Segment Disorders—Cataracts

Cataracts are generally defined as any opacification of the lens that results in light scattering and visual impairment. Cataracts can be congenital or age-related and are the leading cause of blindness in humans (Vijaya et al., 1997). Congenital cataracts, while less prevalent than age-related cataracts are thought to be responsible for roughly one-tenth of blindness worldwide (Gilbert et al., 1993). The vertebrate lens forms as a result of interactions between the optic cup and the surface epithelium during the early stages of embryonic development. The lens placode, derived from the surface epithelium, invaginates to form the lens pit and subsequently undergoes dramatic morphogenetic changes to give rise to the mature lens. Mature lenses can be divided into three general regions; (1) an anterior lens epithelium that remains proliferative throughout life, (2) an equatorial region of epithelial cells that are beginning to differentiate, and (3) a dense core of differentiated lens fibers. Differentiation is peculiar in that lens epithelial cells massively elongate and lose all of their light-scattering organelles to achieve maximal transparency. Defects both in the lens epithelium and in lens fibers contribute to cataractogenesis, which highlights the importance of understanding the biology of these distinct cell types.

Several lens mutants have been identified in zebrafish mutagenesis screens (Fadool et al., 1997; Vihtelic et al., 2001; Vihtelic and Hyde, 2002; Gross et al., 2005; Glass and Dahm, 2004a) and many of these mutants serve as models of congenital lens malformations and human cataracts. Goishi et al. (2006) characterized cataracts in the *cloche* mutant, a recessive mutation in which hematopoiesis and blood vessel development are defective, in addition to the cataracts. Lens fiber cells in *cloche* mutants did not undergo terminal differentiation and retained their nuclei leading to cataracts. Utilizing a proteomic approach, γ-crystallin was identified as a protein whose soluble levels were substantially lower in *cloche* mutants. Crystallins play important roles in maintaining the human lens, as highlighted by the significant number of crystallin mutations that lead to congenital cataracts (Graw, 2003). Interestingly, in *cloche* mutants, αA-crystallin transcript and protein levels were also diminished and experimental overexpression of αA-crystallin in *cloche* mutants rescued their lens defects. αA-crystallin is a member of the small heat shock family of proteins and may serve as a chaperone to keep γ-crystallins soluble and thereby keep the lens transparent.

In addition to the *lamb1* and *lamc1* mutations described above, *laminin α1* (*lama1*) mutations have also been identified in zebrafish (Semina et al., 2006). Like *lamb1* and *lamc1* mutants, *lama1* mutants also present with a number of anterior segment defects that include focal corneal dysplasias, lens degeneration, and cataracts (Semina et al., 2006). *lama1* mutants have reduced levels of focal adhesion kinase activation as well as defects in paxillin localization suggesting that proper

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anterior segment formation requires adhesion to an intact laminin rich extracellular matrix and the activation of adhesion-dependent signaling pathways. Additionally, *lama1* mutants also showed hyaloid vasculature defects where endothelial cell differentiation was delayed and the hyaloid often lacked differentiated capillary structures.

Anterior Segment Disorders—Glaucoma

Glaucoma is a group of blinding conditions that result from progressive retinal ganglion cell death and optic nerve head damage. In the United States alone, over 3 million people are afflicted with glaucoma and another 5–10 million are at risk for developing glaucoma. Glaucoma is known to have genetic origins; however, glaucoma is a complex disease that often is multigenic in its origin making it more difficult to identify causative genetic factors. Intraocular pressure (IOP) elevation is a hallmark of glaucoma and a rise in IOP is triggered by defects in the iridocorneal angle, an anterior segment structure located between the iris and cornea. Normally, aqueous humor is secreted by the ciliary process and drained by the trabecular meshwork at the iridocorneal angle. In patients with glaucoma, drainage is impaired and the buildup of aqueous triggers a rise in IOP leading to retinal ganglion cell death, optic nerve head injury, and ultimately to blindness. Understanding how structures of the anterior segment form during early ocular development, and how congenital defects in their function prevents a rise in IOP, is integral for understanding glaucoma and identifying possible therapeutic interventions.

Zebrafish are an ideal model system to study genetic mutations and multigenic interactions that lead to glaucoma, as researchers can screen large numbers of families for glaucoma-like phenotypes and subsequently identify the affected loci (McMahon et al., 2004). Link et al. (2004) have established methods to measure IOP in zebrafish and their studies have demonstrated that genetically distinct zebrafish strains have differing levels of IOP, which provide a baseline for measurement in mutants derived from these strains. Indeed, one mutation has been identified thus far with elevated IOP, *brass* (Link et al., 2004). *Brass* mutants, in addition to high IOP, present with iris hypoplasia but interestingly, they do not display ganglion cell death. Thus, *brass* mutants, while not a glaucoma model per se, are still quite useful as a sensitized strain in which one can screen for modifier loci that genetically interact with *brass* to contribute to glaucoma progression.

Posterior Segment Disorders—Photoreceptors

Vertebrate photoreceptors are highly polarized neurons capable of detecting a dynamic range of stimuli over several orders of magnitude. Under dark-adapted conditions, rod photoreceptors can respond to a single photon of light. Meanwhile, cone photoreceptors can respond to changes in light intensity even in conditions of bright sunlight that are 11 orders of magnitude brighter than the dark-adapted conditions for rod responses. Photoreceptors have a unique morphology consisting of an elongated outer segment, connecting cilium, inner segment, cell body, and synaptic terminal. The outer segments function as the sensory end of the photoreceptor. Each outer segment contains hundreds of tightly stacked disk membranes that contain the biochemical machinery for phototransduction. The outer segment connects to the rest of the cell via the connecting cilium, which is an elaboration of the primary cilium.

Zebrafish possess one-rod photoreceptor and four-cone photoreceptor subtypes, which absorb light maximally in the red (570 nm), green (480 nm), blue (415 nm), and ultraviolet (362 nm) regions of the spectrum (Robinson et al., 1993). Each of these cone subtypes can be distinguished morphologically; the short single cones absorbing in the ultraviolet, the long single cones responding to blue light, and the red and green sensitive cones forming a double-cone pair. Unlike the rod-dominated rodent retina, zebrafish have a cone-rich retina more similar to humans. These characteristics make zebrafish an excellent model for studying human retinal degenerations such as retinitis pigmentosa, Usher syndrome, cone-rod dystrophies, congenital stationary night blindness, and RPE/photoreceptor degenerations.

Mutations affecting photoreceptor development and function often reduce the overall volume of the eye and many small-eyed mutants identified by morphological analysis in the early genetic screens exhibited defects in photoreceptor development (Malicki et al., 1996; Fadool et al., 1997). In the *oval* (*ovl*), *mikre oko* (*mok*), *fleer* (*flr*), *elipsa* (*eli*), *niezerka* (*nie*), *brudas* (*bru*), and *photoreceptors absent* (*pca*) mutants, the outer retina fails to develop normally and photoreceptors degenerate by 5 days post-fertilization (dpf).

The *flr*, *eli*, and *ovl* mutants block outer segment formation and also develop kidney cysts, phenotypes often associated with BBS and other diseases affecting cilia (Mykytyn and Sheffield, 2004). Positional cloning of the *oval* locus identified a point mutation in the zebrafish ortholog of the *Chlamydomonas* IFT88 gene (Tsujikawa and Malicki, 2004a). IFT88 is a component of the intraflagellar transport (IFT) machinery (reviewed in Scholey, 2003). Defects in IFT disrupt cilia formation in a wide variety of tissues, including photoreceptors, auditory hair cells, olfactory sensory neurons, kidney epithelia, nodal cilia, and the central canal of the spinal cord. Using histology as the primary screening method to identify retinal abnormalities in a collection of retroviral insertion mutants, we found mutations in the zebrafish orthologs of IFT57 and IFT172 also disrupted photoreceptor outer segment formation (Gross et al., 2005). Further analysis of these mutants will provide insight into the formation and maintenance of ciliated structures like the photoreceptor outer segment.

Zebrafish are highly visual animals by 5 dpf and >95% of the animals will display an optokinetic response (OKR) by this timepoint. The OKR consists of a smooth pursuit movement of a visual stimulus, followed by

a quick saccade to reset the eye position in preparation for another stimulus (Neuhauss et al., 2003). Several groups have used the OKR as a behavioral screen with great success to identify dozens of mutants affecting processes throughout the visual system (Brockerhoff et al., 1995; Brockerhoff et al., 1998; Neuhauss et al., 1999; Muto et al., 2005). As the visual response originates in the photoreceptors, many of these mutants exclusively affect photoreceptor biochemistry or physiology.

The cone-rich retina of zebrafish permits analysis of cone-specific responses in animals lacking specific proteins. The *no optokinetic response f* (*nof*) mutant was found to have a nonsense mutation in the α-subunit of cone transducin, which is the G-protein required for the phototransduction pathway (Brockerhoff et al., 2003). In humans, mutations in the cone transducin α-subunit lead to loss of all color vision, a condition known as achromatopsia (OMIM: 139340). As rods do not contribute to the zebrafish visual response until the second week of age (Bilotta et al., 2001), the *nof* mutants were blind and the cones were more than 1,000-fold less sensitive than the wild-type cones. Surprisingly, analysis of the *nof* mutant revealed that photoresponses and Ca^{2+} influx could be detected under very bright light that was within physiological ranges. These studies revealed a light-dependent but transducin-independent phototransduction pathway that is present in cone, but not in rod photoreceptors.

The *no optokinetic response a* (*noa*) mutant was shown to be a potential model for pyruvate dehydrogenase (PDH) deficiency (OMIM: 245348), a disorder causing neurological defects, growth retardation, and early death (Taylor et al., 2004). PDH activity links glycolysis to the Kreb's cycle and stems from a multi-enzyme complex located in the mitochondria. PDH helps regulate energy production and defects in PDH reduce acetyl-CoA levels and limit ATP synthesis. As photoreceptors are one of the biggest energy-consuming cells in the body and contain large numbers of mitochondria, defects in PDH activity are predicted to cause blindness. The *noa* mutant has a mutation in the dihydrolipoamide S-acetyltransferase (*dlat*) gene, which encodes the E2 subunit of the PDH complex. As expected, the *noa* mutants lack swimming and feeding behavior, are lethargic, and display no visual response. Interestingly, *noa* mutants raised on a normal diet of paramecia supplemented with an emulsion of medium- and long-chain fatty acids (e.g., ketogenic diet) exhibited swimming and feeding behaviors similar to wild-type. Importantly, visual responses were restored in *noa* mutants within 24 hr of exposure to the new ketogenic diet, thereby demonstrating that neurological defects can be reversed. The *noa* mutants continued to exhibit retarded growth, indicating that not all PDH phenotypes are rescued by the diet.

Recently, Herwig Baier's group at UCSF used the OKR behavioral assay in combination with a second behavioral assay, the optomotor response (OMR), to identify 53 recessive mutations in 41 genes (Muto et al.,

2005). Larval stage zebrafish will swim to follow a moving visual stimulus in a behavior known as the optomotor response (Neuhauss et al., 1999). In this screening paradigm, a computer monitor displays moving horizontal stripes below a transparent chamber containing larvae zebrafish. Wild-type fish will swim in the direction of the moving stripes and gather at the edge of the chamber within about a minute, whereas mutants will swim randomly. The OKR is a more reliable and accurate behavioral screen but is labor-intensive and can only test a few individuals at once. In contrast, the OMR can be used to screen entire clutches at the same time. Baier's group initially selected mutants detected by either of these two assays but ultimately kept only those mutants that were morphologically indistinguishable from wild-type fish. By only selecting those mutants with normal swimming behavior and no overt developmental defects, it is not surprising that many of the mutants survived to adulthood. Seven mutations affected photoreceptor differentiation and/or maintenance and four of these were adult viable. Ten mutations caused defects in OMR and/or OKR behaviors without affecting morphological development of the animal and at least five of these mutants survived to adulthood. One of these mutations was a new allele of *nof*. The characterization of the many adult-viable recessive mutants will provide a rich source of information regarding the requirements for visual function throughout the life of the animal and serve as useful models of hereditary blindness. Importantly, as these mutations are not required for embryonic survival, the genes are likely to be specific to the visual system.

To identify dominant mutations affecting visual function, Li and co-workers successfully utilized an escape response behavioral assay to measure visual thresholds in adult animals (Li, 2001). This test is performed on the F1 generation fish following ENU mutagenesis to detect night blindness beginning as early at 2–3 months of age. Night blindness can result from nondegenerative diseases (i.e., congenital stationary night blindness) or degenerative diseases such as retinitis pigmentosa. Screens utilizing the escape response assay have identified seven *night blindness* loci (*nba, nbb, nbc, nbd, nbe, nbf, nbg*) (Li and Dowling, 1997, 2000; Maaswinkel et al., 2003a,b, 2005). Six of the seven heterozygous mutants (all but *nbb*) exhibit some degree of photoreceptor degeneration; however, the degree of degeneration varies dramatically between mutants. Interestingly, the homozygous mutant phenotypes for *nba, nbb, nbc*, and *nbd* is embryonic lethality, strongly suggesting these genes serve other functions in the central nervous system and are not retina-specific. Most dominant forms of retinal degeneration are associated with mutations in photoreceptor-specific genes, such as rhodopsin. Future identification of the mutated zebrafish genes should reveal novel pathways for dominant forms of late-onset retinal degeneration.

Posterior Segment Disorders—Retinal Pigment Epithelium (RPE)

The RPE is a single layer of pigmented cells that provides a variety of functions essential to the survival

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and physiology of the photoreceptors (reviewed in Strauss, 2005). The dark pigment absorbs excess light entering the retina, thus protecting the photoreceptors from excessive light damage. Located between the photoreceptors and the blood vessels of the choriocapillaris, the RPE transports nutrients from the blood to the retina, while also returning excess water, ions, and metabolic waste products from the retina back to the blood supply. Perhaps the most important role for the RPE in visual function is the regeneration of the visual pigment. Vertebrate photoreceptors cannot convert all-*trans*-retinal back to 11-*cis*-retinal following the absorption of photons. The RPE performs this critical function in a series of enzymatic steps known as the visual cycle. Following light absorption, all-*trans*-retinal is transported to the RPE and isomerized to 11-*cis*-retinal prior to being returned to the photoreceptors.

Each day, exposure to light generates photo-damaged proteins and lipids within photoreceptor outer segments. To maintain visual sensitivity, the outer segment material is constantly renewed at the base while the most damaged part of the outer segment is shed from the tip. The RPE maintains photoreceptor integrity by phagocytosing and digesting the shed disk membranes. As the survival and function of photoreceptors requires a coordinated interaction with the RPE, a number of genetic defects affecting RPE function lead to various forms of retinal degeneration, such as retinitis pigmentosa, Usher syndrome, and age-related macular degeneration.

The *fading vision* (*fdv*) mutant was originally identified in a screen for OKR mutants (Neuhauss et al., 1999) but was also found to affect melanosome biogenesis, as displayed by hypopigmentation of the RPE and the melanocytes on the body (Schonthaler et al., 2005). The *fdv* mutant is caused by a stop codon in the gene for *silva*, which is an ortholog to the mouse *silver* gene. During the evolution of teleosts, this gene underwent a duplication and zebrafish express two paralogs, *silver a* (*silva*) and *silver b* (*silvb*). The *silva* gene is expressed exclusively in melanogenic cells such as the RPE, but not photoreceptors. Analysis of the *fdv* phenotype revealed reduced melanosome density in the RPE and much shorter photoreceptor outer segments. The photoreceptor phenotype is likely a consequence of the RPE defects. Photoreceptors do not undergo apoptosis in *fdv* mutants, however, and eventually recover to wild-type size. Interestingly, approximately 60% of the mutants survive to adulthood and the appearance of the outer retina is indistinguishable from wild-type, with the exception of a mild hypopigmentation of the RPE. Although photoreceptor structure was affected in *fdv* mutants, the likely cause of the visual defect in the OKR assay was a block in the visual cycle. In *fdv* mutants, the amount of regenerated chromophore was significantly lower than sibling controls. As melanosomes may assist in detoxification of metabolic intermediates, the lack of functional melanosomes in *fdv* mutants could lead to a general breakdown of RPE metabolism and account for

the disruption in the visual cycle. The zebrafish *silva* and mouse *silver* genes are orthologs to the human PMEL17 gene and the dog *merle* locus. Dogs that are homozygous for the *merle* locus exhibit a merle coat pattern with decreased pigment, in addition to auditory and visual defects similar to that observed in Waardenburg Syndrome (OMIM: 193500) (Clark et al., 2006). While the auditory system was not examined in *fdv* mutants, future work may establish this mutant as a model for Waardenburg Syndrome.

The *gantenbein* (*gnn*), *bleached* (*blc*), and *fade out* (*fad*) mutants show signs of RPE degeneration and pigmentation defects but also exhibit various degrees of retinal degeneration (Biehlmaier et al., 2003; Neuhauss et al., 2003; Bahadori et al., 2006). The *blc* mutant presented the most severe phenotype, with almost complete hypopigmentation of the RPE, cell death occurring in all retinal layers, and complete absence of outer retina function, as assessed by ERG recordings. Cell death was restricted to the outer retina in *fad* mutants and photoreceptor morphology progressively worsened after 5 dpf. The RPE of *fad* mutants forms normally and was indistinguishable from wild-type at 3 dpf and did not become hypopigmented until 5 dpf, suggesting that photoreceptor degeneration did not occur until the RPE phenotype appears. The *gnn* mutant was originally identified by the presence of expanded melanophores and small eyes. The RPE phenotype is less severe in *gnn* than either the *fad* or *blc* mutants. RPE degeneration does not begin in *gnn* mutants until approximately 5 dpf. Melanin granules become abnormally distributed and the RPE microvilli project abnormally deep into the neuronal layer. Although phagocytosis of outer segment material was not affected, the RPE was clearly degenerating by 6 dpf. Cone photoreceptors formed normally in *gnn* mutants but started degenerating by 5 dpf, whereas rod photoreceptors were unaffected. Interestingly, the red cones degenerated first and were affected more severely than other cone types. The affected genes in *blc*, *fad*, and *gnn* have not yet been identified, so the mechanisms responsible for these phenotypes remain speculative. Nevertheless, these mutants share similarities to diseases such as retinitis pigmentosa, age-related macular degeneration, and Hermansky–Pudlak Syndrome.

Recently, the *rep1* mutant was characterized as a model for choroideremia (Krock et al., 2007). In humans, choroideremia is an X-linked form of retinal degeneration that causes night blindness in children and progresses to complete loss of vision in adults (OMIM: 303100). Choroideremia is caused by mutations in the gene for *R*ab *e*scort *p*rotein 1 (Rep1), a protein found in all tissues and highly expressed in the outer retina and RPE. Rep1 plays an essential role in the post-translational modification of Rab proteins, the small GTP-binding proteins that are necessary for many aspects of intracellular transport. Thus, the Rep1 protein plays an important role in intracellular trafficking within photoreceptors and RPE cells. Similar to humans with choroideremia, zebrafish *rep1* mutants

exhibited severe degeneration of both RPE and photo-receptors. The RPE of *rep1* mutants appeared highly irregular and patchy. In some regions the RPE was abnormally thickened and invaded the photoreceptor layer. A significant amount of outer segment debris was observed in the subretinal space around the RPE of *rep1* mutants, suggesting defects in phagocytosis or membrane degradation. As both photoreceptors and RPE express Rep1, a central question in choroideremia research is whether photoreceptor pathology reflects cell-autonomous photoreceptor degeneration, a secondary and noncell-autonomous effect caused by RPE dysfunction, or some combination of both. Genetic mosaic analysis revealed that the RPE of *rep1* mutant zebrafish was both necessary and sufficient to cause early photoreceptor degeneration. The results from zebrafish, therefore, suggest that therapies designed to correct the RPE may successfully rescue photoreceptor loss in choroideremia.

SUMMARY

Over the last 15 years, the zebrafish has provided an ideal animal model system to study human ocular disorders. Forward genetic screens have identified a number of mutants that serve as excellent models for coloboma, cataracts, and photoreceptor and RPE pathologies. Ongoing screens in several laboratories, utilizing transgenic strains and more advanced screening techniques, as well as the positional cloning of affected loci in many, as yet, unidentified mutants promises to continue this trend for many years to come.

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