Chapter 4

Animal Models for Retinal Degeneration

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Abstract

Retinal degeneration is often used to describe a category of human eye diseases, which are characterized by photoreceptor loss leading to severe visual impairment and blindness. An important, yet heterogeneous group of such diseases is called Retinitis Pigmentosa (RP). To understand the molecular mechanisms of disease induction and progression and to develop therapeutical strategies for the preservation of vision in RP patients, appropriate animal models are used in many research laboratories worldwide. The largest category of models consists of mutant (spontaneous and genetically engineered) mice. However, in recent years, zebrafish has been established as a highly valuable tool for the study of various biological problems, including retinal degeneration. In this review, we summarize the currently available mouse and zebrafish models to study retinal degeneration and give a short overview about recent developments in the field.

Key words: Mouse models, Zebrafish, Retinal degeneration, Retinitis Pigmentosa

1. Retinal Degeneration in Human Patients

Inherited retinal dystrophies are a heterogeneous group of diseases of which Retinitis Pigmentosa (RP) is the largest subgroup. RP is a genetically determined retinal degeneration affecting primarily the photoreceptor layer in both eyes.

With a prevalence of about 1:4,000 (1), more than 1 million people are affected worldwide.

The name “retinitis” is inaccurate, in that inflammation is not a prominent feature of the pathophysiology of this disease. Instead, progressive loss of retinal function and cell death leading to retinal atrophy are hallmarks of RP. The rod photoreceptors are first affected with a variable degree of subsequent degeneration of cones, therefore the disease is often also termed a rod–cone dystrophy.
Usually, visual impairment manifests itself initially by some degree of night blindness caused by rod photoreceptor dysfunction as well as visual field loss followed by the loss of central, cone-mediated vision. The age of disease onset depends mainly on the different types of RP and the underlying gene mutation and can vary from early childhood to late adulthood. The same is true for the severity of disease, ranging from mild unnoticed visual field loss with full vision to tunnel vision caused by profound field loss and markedly reduced central visual function. Complete blindness is often the final stage of the disease.

The most commonly used subclassification is based on the mode of inheritance. Typical RP can be inherited in an autosomal recessive, autosomal dominant, or X-linked recessive mode. Some cases occur in association with systemic disease such as Usher syndrome (autosomal recessive congenital deafness with RP) or Bardet–Biedl syndrome (RP, polydactyly, obesity, mental retardation, and hypogenitalism). Over the course of the last decade the knowledge about gene mutations associated with RP has quickly grown and many disease-associated genes and mutations have been described (http://www.sph.uth.tmc.edu/RetNet/sum-dis.htm). In a substantial proportion of patients, however, the causative mutation has not yet been identified, stressing the need for further research in this field. An accurate determination of the gene defect would facilitate an early and precise diagnosis as well as specific genetic counseling. Eventually, it might help to apply mutation-specific therapies in the future. Until now, no treatment is available for RP although there is some evidence that supplementation with oral vitamin A palmitate may slow the course of the disease (2). Fields of research aiming at the protection or restoration of useful vision for human patients include – but are not limited to – gene therapy, neuroprotection, stem cell transplantation or implantation of prosthetic devices.

2. Mouse Models to Study Retinal Degeneration and Photoreceptor Apoptosis

Mouse models used to study retinal degeneration can be classified into two basic categories: the induced and the inherited models. In the induced models, exposure of wild-type or genetically engineered animals (mostly mice and rats) to physical (e.g., light) or chemical (e.g., N-methyl-N-nitrosourea; MNU) treatments causes induction and progression of photoreceptor cell death leading to mild or severe retinal degeneration. The main advantage for using induced models lies in the controllable severity grade of the degeneration and the timing of disease onset.
The underlying cause of photoreceptor degeneration in the inherited group of animal models is a gene mutation or the expression of a transgene. Gene mutations can appear spontaneously as in the *rd1* (retinal degeneration 1) mouse which has a null mutation in the β subunit of the rod cGMP phosphodiesterase PDEβ or the RCS (Royal College of Surgeon) rat, which shows a functional deficiency in the MerTK gene (3). Larger animal models (dogs, cats) generally belong to this category. The vast majority of (mouse) models (Table 1), however, has been generated through various techniques of genetic modification. These models are highly useful to study the consequences of gene mutations on a molecular, cellular, tissue, or even systemic level.

Most of these models mimic the human pathology in many but certainly not all aspects. A large variety of toxic factors or stimuli have been used to induce photoreceptor damage. Prominent examples are the DNA alkylating agent MNU (4–6), iron overload (7), autoantibody treatments (8), excitotoxicity (9), laser treatment (10), and others. The most frequently used model, however, applies either white light or visible light of different wavelengths and intensities to induce photoreceptor cell death. Light-induced photoreceptor degeneration is mediated by the visual pigment rhodopsin (6, 11). This model can easily be controlled and induction and extension of retinal damage can be adjusted according to the needs of the particular experiment. The observation that light-damaged photoreceptors die via an apoptotic process similar to the cells in RP or AMD (age-related macular degeneration) patients (12–15) and the suggestion that light may be an important co-factor for these diseases (16, 17) further increase the relevance of the light damage model for the study of disease mechanisms important for human pathology.

Two fundamentally different light exposure protocols are used to study the molecular processes leading to blindness. A long-term exposure (days to months) of low-level light (18, 19) induces a rather slowly progressing degeneration, whereas exposure to high levels of light for minutes to a few hours (14) generates a synchronous burst of photoreceptor apoptosis, which can lead to an almost complete loss of photoreceptors within 10 days after treatment. The two protocols not only induce different kinetics of disease progression but also different molecular pathways for induction and execution of cell death (20). Whereas c-Fos/AP-1 is important for execution of photoreceptor death in the short-term exposure model (21–23), c-Fos seems without effect in the long-term, low-light-level model (20). Transducin (or phototransduction) on the other hand is dispensable for the short-term but not for the long-term exposure model (20).

Members of the Jak/STAT signaling pathway are also critically involved in the execution of photoreceptor death after exposure to
<table>
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<th>Gene</th>
<th>Enzyme/protein</th>
<th>Disease</th>
<th>OMIM</th>
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<td>arLCA, adRP</td>
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<td>KO, tg(L185P), KI(rd3-307), tg(R172W), tg(hIRBP)</td>
<td>(203–207)</td>
</tr>
<tr>
<td>69</td>
<td>PXMP3 (Pex2)</td>
<td>Peroxisomal membrane protein 3, 35 kDa</td>
<td>arCCRD, arCSNB</td>
<td>170993</td>
<td>KO</td>
<td>(208)</td>
</tr>
<tr>
<td>70</td>
<td>RB1</td>
<td>Retinoblastoma 1</td>
<td>adCAD, arRP</td>
<td>180200</td>
<td>KO, KI(R654W)</td>
<td>(209, 210)</td>
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<tr>
<td>71</td>
<td>RBP4</td>
<td>Retinol-binding protein 4, plasma</td>
<td>arOR</td>
<td>180250</td>
<td>KO</td>
<td>(211)</td>
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<tr>
<td>72</td>
<td>RD3</td>
<td>Retinal degeneration 3</td>
<td>arOR</td>
<td>180040</td>
<td>SM(rd3)</td>
<td>(212, 213)</td>
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(continued)
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<tr>
<th>Nr</th>
<th>Gene</th>
<th>Enzyme/protein</th>
<th>Disease</th>
<th>OMIM</th>
<th>Animal model</th>
<th>Ref</th>
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<tr>
<td>73</td>
<td>RDH12</td>
<td>Retinol dehydrogenase 12 (all-trans/9-cis/11-cis)</td>
<td>adCSNB, adRP, arRP</td>
<td>608830</td>
<td>KO, KO</td>
<td>(214, 215)</td>
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<td>74</td>
<td>RDH5 (11cRDH)</td>
<td>Retinol dehydrogenase 5 (11-cis/9-cis)</td>
<td>adCCRD</td>
<td>601617</td>
<td>KO, KO</td>
<td>(216, 217)</td>
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<td>75</td>
<td>RGR</td>
<td>Retinal G-protein-coupled receptor</td>
<td>arRP, arOR</td>
<td>600342</td>
<td>KO</td>
<td>(218)</td>
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<td>76</td>
<td>RGS9</td>
<td>Regulator of G-protein signaling 9</td>
<td>adRP</td>
<td>604067</td>
<td>KO</td>
<td>(219)</td>
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<td>77</td>
<td>RGS9BP</td>
<td>Regulator of G-protein signaling 9 binding protein</td>
<td>adRP, arRP</td>
<td>607814</td>
<td>KO</td>
<td>(220)</td>
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<tr>
<td>78</td>
<td>RHO</td>
<td>Rhodopsin</td>
<td>xRP</td>
<td>180380</td>
<td>tg(P23H), tgVPP, KO, KO, Palm(−/−), KI(E112Q), tg(P347S)</td>
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<td></td>
<td></td>
<td>Rhodopsin</td>
<td>xRP</td>
<td>180380</td>
<td>tg pig (P347L), tg rat (P23H), tg rat (S334ter), tg frog (P23H)</td>
<td>(228–231)</td>
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<tr>
<td>79</td>
<td>RIMS1</td>
<td>Regulating synaptic membrane exocytosis 1</td>
<td>arLCA, arRP</td>
<td>606629</td>
<td>KO</td>
<td>(232)</td>
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<tr>
<td>80</td>
<td>RLBP1 (CRALBP)</td>
<td>Cellular retinaldehyde-binding protein</td>
<td>xCCRD, xMD, xRP</td>
<td>180090</td>
<td>KO</td>
<td>(233)</td>
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<tr>
<td>81</td>
<td>ROM1</td>
<td>Rod outer segment membrane protein 1</td>
<td>arLCA</td>
<td>180721</td>
<td>KO</td>
<td>(234)</td>
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<tr>
<td>82</td>
<td>RP1</td>
<td>Retinitis pigmentosa 1 (autosomal dominant)</td>
<td>arSSDR</td>
<td>603937</td>
<td>KO, KO</td>
<td>(235, 236)</td>
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<tr>
<td>83</td>
<td>RP2</td>
<td>Retinitis pigmentosa 2 (X-linked recessive)</td>
<td>xOR</td>
<td>312600</td>
<td>Siberian Husky dog</td>
<td>(237)</td>
</tr>
<tr>
<td>84</td>
<td>RPE65</td>
<td>RPE:65</td>
<td>arCSNB, arRP</td>
<td>180069</td>
<td>KO, SM(rd12), KI(R91W), Briard dog</td>
<td>(238)</td>
</tr>
<tr>
<td>85</td>
<td>RPGR</td>
<td>Retinitis pigmentosa GTPase regulator</td>
<td>adCCRD, adRP</td>
<td>312610</td>
<td>KO, tg</td>
<td>(239, 240)</td>
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<td></td>
<td>Gene</td>
<td>Description</td>
<td>Model</td>
<td>Gene ID</td>
<td>Mutation</td>
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<tr>
<td>86</td>
<td>RPGRIP1</td>
<td>Retinitis pigmentosa GTPase regulator-interacting protein</td>
<td>adCAD</td>
<td>605446</td>
<td>KO, miniature longhaired dachshunds</td>
<td>(241, 242)</td>
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<td>87</td>
<td>RPGRIP1L</td>
<td>RPGRIP1L</td>
<td>adMD</td>
<td>610937</td>
<td>KO</td>
<td>(243)</td>
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<tr>
<td>88</td>
<td>RS1</td>
<td>Retinoschisin 1</td>
<td>arSSDR</td>
<td>312700</td>
<td>KO (lacZ), KO, CI</td>
<td>(244–246)</td>
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<tr>
<td>89</td>
<td>SAG (Arr)</td>
<td>S-antigen; retina and pineal gland (arrestin)</td>
<td>arLCA, arRP</td>
<td>181031</td>
<td>KO</td>
<td>(247)</td>
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<tr>
<td>90</td>
<td>SEMA4A</td>
<td>Semaphorin 4A</td>
<td>adCCRD</td>
<td>607292</td>
<td>GtI</td>
<td>(248)</td>
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<tr>
<td>91</td>
<td>TEAD1</td>
<td>TEA domain family member 1</td>
<td>arUS</td>
<td>189967</td>
<td>GtI, KO</td>
<td>(249, 250)</td>
</tr>
<tr>
<td>92</td>
<td>TTPA</td>
<td>Tocopherol (alpha) transfer protein</td>
<td>arRP, arUS</td>
<td>600415</td>
<td>KO</td>
<td>(253)</td>
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<tr>
<td>93</td>
<td>TULP1</td>
<td>Tubby like protein 1</td>
<td>adORDD, adSSDR</td>
<td>602280</td>
<td>KO</td>
<td>(254)</td>
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<tr>
<td>95</td>
<td>UNC119 (HRG4)</td>
<td>unc-119 homolog (Caenorhabditis elegans)</td>
<td>arSSDR</td>
<td>604011</td>
<td>tg, KO</td>
<td>(255, 256)</td>
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<td>96</td>
<td>USH1C (harmonin)</td>
<td>Usher syndrome 1C (autosomal recessive, severe)</td>
<td>arBBS</td>
<td>605242</td>
<td>SM, KO, KI(216G&gt;A)</td>
<td>(257–259)</td>
</tr>
<tr>
<td>97</td>
<td>USH1G (sans)</td>
<td>Usher syndrome 1G (autosomal recessive)</td>
<td>arBBS</td>
<td>607696</td>
<td>SM(shaker and seal)</td>
<td>(260, 261)</td>
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<tr>
<td>98</td>
<td>USH2A</td>
<td>Usher syndrome 2A (autosomal recessive, mild)</td>
<td>arBBS</td>
<td>608400</td>
<td>KO</td>
<td>(262)</td>
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<td>99</td>
<td>VCAN</td>
<td>Versican</td>
<td>adMD, adOR, arOR</td>
<td>118661</td>
<td>KO</td>
<td>(263)</td>
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<td>100</td>
<td>WFS1</td>
<td>Wolfram syndrome 1 (wolfarin)</td>
<td>adRP</td>
<td>606201</td>
<td>KO, floxed</td>
<td>(264, 265)</td>
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</table>

The table does not validate the listed models but merely gives an overview over the animal models which harbor a mutation in a gene identified to cause a particular retinal disease in human patients. Animal models other than mice (rat, dog, chicken, and frog) are indicated in italics. Zebrafish models are listed separately in Table 4.2. The search for animal models was done according to the 144 mapped and identified retinal disease genes published at RetNet (http://www.sph.uth.tmc.edu/RetNet/sum-dis.htm, last updated 20.11.08). Web pages used were: http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.informatics.jax.org/. Disease nomenclature was taken from RetNet.

Animal models: CI chemically induced, GTI gene trap insertion, KI knock-in, KO knock-out, SM spontaneous mutation, tg transgenic.
high levels of light and in models of inherited retinal degeneration (see below) (24, 25). Rhodopsin regeneration in the visual cycle is an important factor for the determination of light damage susceptibility and caspase-1 may be involved in not yet clearly defined mechanisms (26–28).

Despite their advantages, the induced models can only partially represent the mechanisms and molecular processes responsible for retinal degeneration in human patients. For a complete understanding of the human disease and for the development of therapeutic strategies, it is of high importance that findings from the induced models are evaluated in animal models with an inherited retinal degeneration. Since cell death in most inherited models stretches over a long period of time, only few cells die at any given time making it difficult to identify the biochemical and molecular events which are causative for cell death. However, the knowledge gained from the induced models allows a targeted approach and candidate molecules and signaling systems can be tested specifically. This facilitates the investigations and may allow finding common retinal mechanisms which are either involved in retinal cell death or in endogenous rescue systems evolved to protect cell viability and visual function.

2.2. Inherited Models of Retinal Degeneration – Focus on rd1 and VPP as Models for RP

More than 190 genes are mapped and more than 140 genes have been identified which, when mutated, cause retinal degeneration, photoreceptor apoptosis and/or retinal dysfunction (http://www.sph.uth.tmc.edu/Retnet/home.htm). Of those, more than 40 are associated with RP. Animal models exist for most of the identified genes. A comprehensive overview of these models, along with their genes, associated human disease, and respective references are given in Table 1. Due to space restrictions, this review cannot describe each individual model in detail. Instead, we will focus on the three models (rd1, VPP, and Rpe65_{R91W}) mainly used in our own laboratory and summarize the findings from our work, which may help to understand the consequences of the particular genetic defect.

The rd1 mouse is a model for autosomal recessive RP. Its nonsense mutation in the β-subunit of the phosphodiesterase causes a light-independent early onset rod–cone dystrophy (29) starting around postnatal day (PND) 10. At PND21, most rod photoreceptors have died and the debris has been cleared from the subretinal space with one row of cone cells remaining. With no support from rods, cones also die with almost no detectable photoreceptors left at 6 months of age.

VPP mice express a transgene in rods encoding a mutant rod opsin protein. The protein harbors three amino acid substitutions at the N-terminal end (V20G, P23H, and P27L). P23H accounts for approximately 10% of autosomal dominant RP in the USA. VPP-mediated retinal degeneration is influenced by light, has a later
onset (around PND 15) and a slower progression (several weeks) than the degeneration caused by the rd1 mutation (30, 31).

Both mouse lines have been studied to elucidate the molecular mechanisms for photoreceptor degeneration and to find therapeutical approaches to preserve vision. Although the rd1 mouse is one of the most frequently used model for retinal degeneration, the mechanisms of cell death are still not completely clear. Due to the nonfunctional phosphodiesterase, rd1 photoreceptors have elevated levels of cGMP and thus an increased influx of calcium (Ca$^{2+}$) leading to Ca$^{2+}$ overload (32, 33). This may induce Ca$^{2+}$-activated cysteine proteases (calpains), which may be at least partially causative for photoreceptor degeneration in the rd1 mouse (34). The reason for photoreceptor death in the VPP mouse is even less clear. Although most of the mutant protein may be correctly localized to rod outer segments and partially functional (35), some of the mutant rhodopsin molecules mislocalize, accumulate in intracellular inclusion bodies and form dimers (36, 37), which may lead to metabolic stress and death of photoreceptors.

Immune-related processes like the generation of auto-antibodies or the misregulation of the innate immune response have been implicated in various degenerative diseases of the neuronal system including RP and AMD (38–42). Similarly, in most of our models we found induced expression of several genes related to an immune response like the inflammatory protease caspase-1, interleukin-1β, monocyte chemoattractant protein 1 (MCP-1), and the complement components C1qα and CFH, among others (24, 28, 43, 44). However, we could not assign any specific role of these factors for the degenerative processes in photoreceptors after light damage or in the rd1 and VPP mice. One important exception may be caspase-1. Genetic inactivation of the Casp-1 gene increased survival of photoreceptors in the VPP mouse, but not in the rd1 mouse or the light-induced model of retinal degeneration (44). The significance of this observation is currently the focus of ongoing research.

Another molecular pathway which seems to be activated in many forms of retinal degeneration belongs to the Jak/STAT signaling system. In this signaling pathway, extracellular cytokines of the interleukin-6 (IL6) family of proteins interact with a bi- or tripartite transmembrane receptor causing the phosphorylation of intracellular members of the Janus kinase (Jak) family. Activated Jak kinases phosphorylate target proteins, among which signal transducer and activator of transcription (STAT) factors are prominent. Activation of STATs results in the differential regulation of target genes and the induction of a negative feedback loop involving the SOCS (suppressor of cytokine signaling) proteins (45). After light exposure as well as in the rd1 and the VPP mouse, we observed a strongly elevated expression of leukemia inhibitory factor (LIF) and of cardiotrophin like cytokine (CLC) in the
retina along with the activation of STAT3 and SOCS3 (25). Jak2 was prominently phosphorylated after light damage but not (rd1) or only weakly (VPP) in the inherited models (25). In addition, a strong activation of Jak3 gene expression was observed in all models analyzed (unpublished observation). The detailed analysis of this signaling system in the VPP mouse revealed that LIF controls an elaborate signaling system, which is activated during retinal degeneration. In this system, photoreceptor injury induces expression of LIF in a subset of Müller glia cells. Increased expression of LIF is required to induce endothelin-2 (Edn2) expression (presumably in photoreceptors (46)), which, in turn, may activate Müller cells via the Ednrb receptor. Müller cells respond with a gliotic reaction and the upregulation of glial fibrillary acidic protein (GFAP). This may activate the expression of the growth and survival factor FGF2 (fibroblast growth factor 2), which seems to support survival of injured photoreceptors (24). In the absence of LIF, none of these downstream factors is activated and photoreceptor death in the VPP mouse is strongly accelerated. Since similar observations were made in the light damage model (unpublished data), this molecular response may be a common reaction of the retina to injury with the goal to protect viable cells from further damage and to rescue visual function. Since application of recombinant LIF already successfully protected photoreceptors against light damage (47), the LIF-Jak/STAT signaling system may be a promising target for the development of therapeutic strategies.

In a series of other experiments we addressed the role of the visual pigment and its regeneration in the visual cycle in induced or inherited retinal degeneration. RPE65 is the isomerohydro-lase (48–50), which is required for the regeneration of 11-cis-retinal in the retinal pigment epithelium. Lack of functional RPE65 leads to a chromophore-deficient retina (48) and completely protects photoreceptor cells against light damage (6). This shows that light damage is mediated by the visual pigment as it has been suggested earlier (11). It may thus not be surprising that the kinetics of the regeneration of the visual pigment is a major determinant of the susceptibility to light-induced photoreceptor apoptosis and also influences the degenerative process in the VPP mouse (26, 27, 31, 51).

In addition to its role in modulating retinal degeneration, RPE65 is currently in the focus of the research community because of clinical trials aiming at the use of gene therapy to reconstitute vision in human RPE65 patients (52–55). Mutations in RPE65 lead to Leber Congenital Amaurosis (LCA) or to Early Onset Severe Retinal Degeneration (EOSRD) in affected patients. For a better understanding of disease induction and progression, we generated a knock-in mouse expressing the most commonly found mutation in RPE65 (Rpe65_{R91W}). Analysis of the retinal
pathology in this mouse model revealed that the R91W mutation reduces RPE65 protein levels and function, which leads to low levels of 11-cis-retinal and a progressive cone-rod dystrophy. In these conditions of limited chromophore supply, rods and cones compete for the available 11-cis-retinal. Since rods massively outnumber cones and because rhodopsin is thermo-dynamically more stable than cone opsin, rods incorporate most of the available chromophore leaving cones without sufficient 11-cis-retinal for proper opsin localization and function. This leads to cone opsin mislocalization and cone cell death (56, 57). These findings are not only relevant for the elucidation of the molecular cell death mechanisms but also for the design of treatment strategies using gene therapy to repair RPE65 deficiency. It seems important that such a treatment aims at the cone-rich region of the macula to increase local concentrations of 11-cis-retinal. Sufficiently high levels of 11-cis-retinal are required for cones to correctly localize opsin as a prerequisite to increase cell survival and prolong retinal function.

Neuroprotection is one possible approach to rescue photoreceptor cells from death to prolong the period of useful vision in patients. This approach is based on the observation that many patients have useful vision at younger ages, despite the presence of the disease causing gene mutation. This suggests that photoreceptor cells can function in such conditions but that a metabolic, cellular, or tissue-based stress induces the degeneration of the retina. Since it may not always be possible to prevent or reduce the underlying stress factor (e.g., the mutation) directly, increasing the resistance of photoreceptors is a valid approach to preserve vision. Recently, a phase I clinical trial using encapsulated cell therapy delivering ciliary neurotrophic factor (CNTF) to support survival of photoreceptor cells gave promising results (58). To establish a variety of effective therapies, however, it is essential to find and test additional neuroprotective treatments in animal models of retinal degeneration. Of particular interest are neuroprotective factors like lens epithelium-derived growth factor (LEDGF) (59), pigment epithelium-derived factor (PEDF) (60), glial cell-derived neurotrophic factor (GDNF) (61), rod-derived cone viability factor (RdCVF) (62), basic fibroblast growth factor (bFGF) (63), CNTF (64), and LIF (47) among others. Equally important are approaches to find and establish new factors which might be used to protect visual cells from degeneration. Recently, we showed that a short period of hypoxic preconditioning completely protected photoreceptors against light-induced degeneration (65). Part of the protective hypoxic response was mediated by erythropoietin (EPO) (65, 66) but additional factors are suspected to participate in the protection. The analysis of the retinal transcriptome after hypoxic preconditioning revealed that many
genes involved in an oxidative stress response, apoptosis (pro or anti) or cell cycle control were differentially regulated by the short exposure period to low oxygen \((67)\). The analysis of these candidate genes may lead to the identification of additional factors, which may be used to protect visual cells in a therapeutic approach.

While the mouse is a well-established model for ocular disease, the zebrafish is a more recent and increasingly popular addition to the animal models used for research on heritable eye diseases (see Table 2 for a collection of available Zebrafish models for the study of photoreceptor physiology and pathophysiology). Due to the rapid and external embryogenesis and high fecundity of the zebrafish, it is currently one of the best studied models for vertebrate development. Its superb genetics, in particular the feasibility to perform chemical induced mutagenesis screens, has led to an impressive number of mutations affecting all aspects of vertebrate development. More recently favorable features of visual system development have endeared the zebrafish to a growing number of vision researchers. The visual system of the zebrafish develops precociously, so that at 5 days postfertilization (dpf), a well-established retina that supports a number of visual behaviors has formed.

Another favorable feature of the zebrafish retina is the cone dominance. The zebrafish retina contains four different cone types, which absorb light maximally at 570 nm (red), 480 nm (green), 415 nm (blue), and 363 nm (ultraviolet) \((68, 69)\). Although some rods are already present at 5 dpf, their functional contribution only becomes apparent at about 15 dpf \((70)\). Hence all visual responses at 5 dpf, the typical age of analysis, can be considered purely cone driven. Therefore, research on the zebrafish retina nicely complements studies on the rod dominant retina of the nocturnal mouse.

Over the last few years, both the genetic tool kit to generate models of ocular diseases and to analyze them has been expanded dramatically.

Zebrafish can be efficiently mutagenized by simply adding the alkylating agent ethyl nitrosourea (ENU) to the water. The offspring of heterozygous parents can be screened for a variety of phenotypes of interest. The first two large-scale screens have isolated more than 6,000 mutant lines that cover all aspects of embryogenesis by simple visual inspection of the developing embryos \((71, 72)\). These initial screens revealed a number of mutants affecting the development of the retina \((73)\), which were
Table 2
Zebrafish photoreceptor mutants

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Disrupted gene</th>
<th>Ocular phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>bleached (blc)</td>
<td>Unknown</td>
<td>Hypopigmentation, general retinal degeneration</td>
<td>(266)</td>
</tr>
<tr>
<td>brudas (bru)</td>
<td>Unknown</td>
<td>Lack of outer segment, photoreceptor degeneration</td>
<td>(73)</td>
</tr>
<tr>
<td>elipsa (eli)</td>
<td>Unknown</td>
<td>Lack of outer segment, photoreceptor degeneration</td>
<td>(73, 93, 94)</td>
</tr>
<tr>
<td>fade out (fad)</td>
<td>Unknown</td>
<td>Photoreceptor degeneration</td>
<td>(91)</td>
</tr>
<tr>
<td>fading vision (fdv)</td>
<td>Silva</td>
<td>Hypopigmentation, shorter and misaligned photoreceptor outer segments</td>
<td>(99)</td>
</tr>
<tr>
<td>fleer (flr)</td>
<td>Unknown</td>
<td>Lack of outer segment, photoreceptor degeneration</td>
<td>(73, 267)</td>
</tr>
<tr>
<td>gantenbein (gnn)</td>
<td>Unknown</td>
<td>Photoreceptor degeneration</td>
<td>(268)</td>
</tr>
<tr>
<td>grumpy, laminin b1 (lamab1)</td>
<td>lamab1</td>
<td>Shorter photoreceptor outer segments</td>
<td>(269, 270)</td>
</tr>
<tr>
<td>ift57</td>
<td>ift57</td>
<td>Lack of outer segment, photoreceptor degeneration</td>
<td>(83, 96)</td>
</tr>
<tr>
<td>ift172</td>
<td>ift172</td>
<td>Lack of outer segment, photoreceptor degeneration</td>
<td>(83)</td>
</tr>
<tr>
<td>mikre oko (mok)</td>
<td>Dynactin 1</td>
<td>Photoreceptor degeneration</td>
<td>(73, 271, 272)</td>
</tr>
<tr>
<td>niezerka (nie)</td>
<td>Unknown</td>
<td>Photoreceptor degeneration</td>
<td>(73)</td>
</tr>
<tr>
<td>Night blindness (nba, nbb, nbc, nbd, nbe, nbf, nbg)</td>
<td>Unknown</td>
<td>Late onset night blindness (dominant)</td>
<td>(273–276)</td>
</tr>
<tr>
<td>no optokinetic response f (nof)</td>
<td>gnat2</td>
<td>Cone visual transduction</td>
<td>(277)</td>
</tr>
<tr>
<td>oval (ovl)</td>
<td>ift88</td>
<td>Lack of outer segment, photoreceptor degeneration</td>
<td>(73, 93–95)</td>
</tr>
<tr>
<td>partial optokinetic response a (pob)</td>
<td>pob</td>
<td>Specific red cone degeneration</td>
<td>(278)</td>
</tr>
<tr>
<td>Rab escort protein 1 (rep1)</td>
<td>rep1</td>
<td>Photoreceptor degeneration</td>
<td>(96)</td>
</tr>
<tr>
<td>sleppy, laminin g1 (lamac1)</td>
<td>lamac1</td>
<td>Shorter photoreceptor outer segments</td>
<td>(269, 270)</td>
</tr>
</tbody>
</table>

complemented by a number of subsequent screens (74). The strength of the zebrafish is the ease in which functional alterations of the visual system can be revealed. The most common approach
has been to test larvae for the optokinetic response, in which moving stripes in the surround – typically provided by a moving drum or a projection – elicit stereotyped eye movements (reviewed in (75)). Larvae that display spontaneous eye movements, but fail to elicit them in response to a moving grating in the surround, are likely defective in vision. The position of the defect in the visual pathway can subsequently be specified by histological analysis and electroretinography (ERG). The ERG, which records sum field potentials of the retina in response to light, can easily be measured in zebrafish larvae and is directly comparable to mouse and human ERG recordings (76–80).

Such chemically induced mutants may reveal interesting phenotypes, but the underlying molecular defect still has to be identified by genomic mapping techniques. An alternative is offered by viral insertion mutagenesis. Here the mutating agent is a pseudotyped virus that enables rapid cloning of the disrupted gene (81, 82). Although this treatment is, in orders of magnitude, less efficient in the generation of mutations, the effort is balanced by the ease of cloning any disrupted gene(s). A rescreen of such insertion mutagenized lines for defects in vision have yielded a collection of mutants with photoreceptor defects caused by known molecular defects (83). The rapid growth of transgenic technology in the fish may soon result in the efficient generation of mutations by transposon-mediated insertions (84).

Although the number of available zebrafish mutants with characterized gene defects is growing fast, it is still not possible to knock-out a gene of interest by homologous recombination. One alternative is the time consuming Targeting Local Lesions IN Genomes (TILLING) methodology for identifying ENU-induced mutations in specific genes of interest, that necessitates a huge sequencing capacity (85, 86). A recent alternative is the heritable disruption of target genes by the use of zinc finger nucleases (87, 88). The application of this technology is predicted to have a huge impact on the use of the zebrafish model, including its use as an eye disease model.

A widely used efficient alternative is the transient knock-down of a protein of choice by morpholino antisense technology. Here, modified (morpholino) antisense nucleotides are injected into the fertilized egg. These nucleotides are designed in such a way that they either base pair with the translational start site blocking translation initiation, or with splice sites, leading to aberrant splicing and hence nonfunctional mRNA (89). Morpholino anti-sense nucleotides are stable and nontoxic. Off target toxicity may be ameliorated by concomitant p53 downregulation (90). The knock-down by morpholino technology gets progressively less effective at later stages, likely due to dilution in the growing embryos. However, we and others had good success in knocking down proteins as late as 5 dpf, sufficient to quantitatively analyze visual behavior (91, 92).
In the following, we illustrate these different approaches and highlight the advantages of the zebrafish model by briefly discussing ciliopathies and pathologies of the visual cycle.

A number of mutants isolated in the large-scale chemical mutagenesis screens showed a combination of pronephric cysts and photoreceptor degeneration, an association observed in human ciliopathies such as Bardet–Biedl (BBS) and Senior–Loken syndrome. A detailed morphological analysis of the retina of these three mutants (oval, elipsa, fleer) revealed a block in outer segment formation (93, 94). Subsequent positional cloning of the oval locus identified a mutation in the ortholog of the intraflagellar transport protein 88 (IFT88) of Chlamydomonas (95). The IFT complex is involved in the generation and maintenance of ciliated structures, of which the outer segments of photoreceptors and the sensory cilia of auditory hair cells and olfactory sensory neurons are among many other homologous structures in the body. A careful analysis of the IFT88 mutant in combination with morpholino-mediated downregulation of other IFT proteins, revealed that members of the IFT complex B, such as IFT88, IFT52, and IFT57, are essential for the maintenance but not the initial assembly of sensory cell cilia. The loss of a member of the IFT complex A (IFT140) produced only a mild phenotype (95). This conclusion was corroborated by the isolation of two retroviral insertion mutants in genes coding for IFT complex B genes, IFT57 and IFT172, which also displayed disrupted photoreceptor outer segments (83). Subsequent elegant biochemical studies showed that IFT88 is essential for outer segment formation, while IFT57 is rather required for efficient intraflagellar transport. It mediates association of IFT20, another IFT complex A component, to enable joining the IFT particle (96). These sets of experiments delineated the importance of IFT complex B in the maintenance of photoreceptor outer segments and give important functional evidence for the causation of human vision impairments such as Bardet–Biedl syndrome.

In the first step of seeing, the visual chromophore 11-cis-retinal gets photoisomerized to all-trans-retinal. To ensure continuous vision, the visual pigment needs to be regenerated in the visual cycle. In vertebrates the canonical visual cycle is situated in two compartments, the photoreceptor cell and cells of the retinal pigment epithelium (RPE). Mutations in genes coding for any of the components of the visual cycle can lead to retinal degeneration (97, 98). In the zebrafish mutant fading vision, which harbors a mutation in the silver gene, the visual cycle is restricted due to a defect in melanosome biogenesis in the RPE. Photoreceptor outer segments in the mutant are much shorter and misaligned. Ultrastructural analysis of the RPE reveals large vacuoles, comparable to inclusion bodies observed in the RPE of retina dystrophy patients (99). Recently evidence has accumulated that the canonical visual cycle is not the only biochemical pathway to regenerate
visual pigment. Data from studies of the diurnal chicken and zebrafish retina support a parallel cone-specific visual cycle (100–102). One evidence was the surprising finding that cone vision in zebrafish is only marginally affected by the lack of RPE65 activity. RPE65-depleted zebrafish retinas display altered morphology of rod photoreceptors, similar to defects reported in the mouse knock-out mutant (103), while cone photoreceptors proved to be both morphologically and functionally intact (102). Cone photoreceptors are likely served by an alternative visual cycle, which involves Müller glia cells. So far, the only defined molecular component is the retinoid-binding protein CRALBP (cellular retinaldehyde-binding protein) (100). These studies provided evidence for a cone private visual pigment recycling pathway, while the canonical visual cycle serves both rod and cone photoreceptors. Since the human CRALBP gene is expressed in both Müller glia and RPE cells, the relative importance of a cone-specific visual cycle for human vision is still debated. The requirement of diurnal animals to keep cone vision functional at rod saturating light intensities may argue that at least diurnal animals must have evolved two visual cycles acting in parallel. The cone dominant zebrafish model is an ideal model to decipher the relative contribution of this alternative visual cycle for cone vision and its possible contribution to human visual impairments.

4. Conclusion

The study of retinal physiology and pathophysiology in a large number of different animal models has led to a good understanding of retinal development, maintenance, and function on a molecular, cellular, and tissue-specific level. This knowledge is used to identify causative mechanisms in degenerative diseases of the retina and to establish various therapeutical approaches to treat patients suffering from retinal degeneration, several of which are currently in clinical trials with promising results.

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