PERSPECTIVE

Molecular ophthalmology: an update on animal models for retinal degenerations and dystrophies

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For several decades, basic research on acquired and inherited retinal degeneration was substantially based on a variety of animal models, most of them originating from spontaneous mutations, others induced by damaging external agents. In the past few years, however, progress in genetic engineering has led to a rapidly growing number of transgenic animals, mostly mice, carrying constructs that lead to disruption or overexpression of candidate genes for retinal degenerations. On the one hand, these new models constitute a powerful and adaptable tool to investigate the role of specific gene mutations and the resulting cellular defects that finally lead to photoreceptor cell death. On the other hand, they extend the spectrum of animal models suitable for the newly arisen field of retinal somatic gene therapy.

To assist researchers and clinicians interested in the field, this article attempts to provide a structured overview on recently developed transgenic animal models as well as on models based on spontaneous mutations and induced degenerations. In this review the authors focus on animal models for photoreceptor degeneration since the rapidly growing field of models for ganglion cell death merits its own review and would be beyond the scope of this article. Even with this restriction, the abundance of information generated especially in the past few years makes the attempt of a complete overview almost illusory. Therefore, we apologise for omissions or shortfalls extant in this review. Furthermore, we want to point out that some of the model systems described have already been used extensively. We will therefore occasionally not cite original publications but rather reviews dealing with the specific model system.

Finally, we did not incorporate strategies using viral vectors and/or pharmacological substances. The specific dele-tion or overexpression of genes susceptible for the modulation of photoreceptor apoptosis, however, was included. The authors are aware that these two subjects may not be clearly separated in all cases.

Hereditary animal models

In 1923 Clyde E Keeler discovered a mouse strain lacking photoreceptors.[1] He speculated that these animals suffered from an inherited defect of retinal development and called these mice *r* (*rodless retina*) mice. These mice showed an early and rapid degeneration of the outer retina, which resulted in a single row of remaining photoreceptor nuclei in the central retina by postnatal day 21 (p21). After publishing several articles on his findings, the continuous lack of interest from the research community made him abandon the whole mouse strain in the early 1930s. In 1951, Brückner and colleagues reported severe retinal degeneration in a wild mouse strain found in the surroundings of the city of Basle that was soon named the *rd (retinal degeneration)* mouse.[2] This mouse model later became the most extensively studied animal model for human autosomal recessive retinitis pigmentosa (RP). However, it took four more decades to elucidate the genetic defect underlying photoreceptor degeneration in the *rd* mouse: a nonsense mutation in the rod photoreceptor cGMP phosphodiesterase β subunit gene.[3 4] In 1993, 70 years after Keeler's original observations on the *r* mouse, Pittler and co-workers demonstrated by polymerase chain reaction (PCR) analysis of *r* DNA isolated from old histological sections that the defects in the *r* mouse and the *rd* mouse were identical.[5]

Apart from the *rd* mouse, a number of hereditary retinal defects have been identified subsequently not only in

Table 1 Hereditary models for retinal degenerations

Strain	Trivial name	Gene	Mutation type	Cell layer	Degeneration time course	References
Mouse models:						
C57BL/6J	rd	β phosphodiesterase	null mutation	ONL	p8–p21	3, 4
C57BL/6	rds	Peripherin	null mutation	ONL	p21–1 year	19, 20
C57BL/6J	tubby (rd5)	tub	—	ONL	p14–9–12 months	21, 107, 108
C57BL/6J	vitiligo (mivit/mivit)	microphthalmia (mi) gene	—	ONL, OPL	8 weeks–8 months	109, 110
RBF/DnJ	rd3	rd3	—	ONL	p21–8 weeks	111
C57BL/6J	pcd	pcd (Chr 13)	—	ONL	initial degeneration p25, never complete	112
DBA/2J × C57BL/6J	rd4	rd4	inversion	ONL, OPL	p10–6 weeks	113
C57BL/6J × Krd/+	Krd	on Chr 19, including Pax2	deletion	entire retina	initial malformation at E 10.5	114
Others:						
cat	Abyssinian cat	rdy	—	ONL	p22–27 months	6, 7, 10
chicken	Rhode Island Red chicken	photoreceptor guanylate cyclase (GC1)	null mutation	ONL	starts at p7	15, 16
dog	labrador retriever	rdy	—	ONL	1–2 months–18 months	13
dog	Swedish briard	Rpe65	deletion	—	Manifest at 10 months	11, 48, 49, 51
dog	Irish setter	β phosphodiesterase	nonsense mutation	ONL	p25–1 year	11, 12, 14
rat	Fischer 344	rhodopsin	point mutatiuon	ONL	4–8 months–2 years	17, 18
rat	RCS	rdy	—	ONL	p21–7 weeks	115–117

Table 2 Transgenic models for retinal degenerations

Strain	Gene	Mutation type	Cell layer	Degeneration time-course	Ref
Mouse					
B6D2F1	*rhodopsin*	substitution (Pro23His)	ONL, RPE	starts at p20	34, 37
C57L/6J	*rhodopsin*	substitution (Pro347Ser)	ONL	p21–1 year	20, 35
C57BL/6 × SJL	*rhodopsin*	substitution (Val20Gly, Pro23His, Pro27Leu)	ONL	p20–sp250	36, 40
C57L/6J	*rhodopsin*	nonsense (Gln344ter = Q344ter)	ONL	starts at p14	41
FVBN × C57BL/6	*peripherin*	substitution (Pro216Leu)	ONL	1 month–7 months	42
C57BL/6J	*IRBP*	knockout (*IRBP$^{-/-}$*)	ONL	starts at p11	43
C57BL/6 × MF1	*γPDE*	knockout (*Pdeg$^{-/-}$*)	ONL	p 0–8 weeks	44
C57BL/6	*Rpe65*	knockout (*Rpe65$^{-/-}$*)	RPE, ONL	starts at 7 weeks	52
C57BL/6	—	insertion of diphtheria toxin A gene	ONL	p7–3 months	54
C57BL/6 × 129Sv	cyclic nucleotidegated channel (*CNG3*)	knockout (*CNG3$^{-/-}$*)	ONL (rods)	2 months–8 months	55
C57BL/6	*ABCR*	knockout (*RmP$^{-/-}$*)	ONL	—	56
C57BL6	*rhodopsin*	knockout (*Rho$^{-/-}$*)	ONL	p24–3 months	57
C57BL/6	*rhodopsin kinase (RK)*	knockout (*RK$^{-/-}$*)	ONL (rods)	depends on illumination state	59
C57BL/6 × 129Sv	*arrestin*	knockout (*arrestin$^{-/-}$*)	ONL (only rods)	3 months–12 months	60
Bax$^{-/-}$	*Bax*	knockout (*Bax$^{-/-}$*)	INL, GCL	reduced developmental cell death at p 7	71
C3H/Bax$^{-/-}$	*Bax/β PDE*	*Bax$^{-/-}$*, rd/rd	ONL	p 8–p 21	71
C3H × C57BL/6	*Pax2*	insertion (*Pax2 1Neu*)	OPL, INL, IPL, GCL	notable thinning in adult heterozygous mice	118
129SvJ	*GC-E*	knockout (*GC-E$^{-/-}$*)	ONL	Starts at 3 weeks	119
mouse (FVB/N × C57BL/6)	*SV40* large tumour antigen	overexpression	ONL, OPL	p 5–p 100	120
Others					
rat	*rhodopsin*	substitution (S344ter)	ONL	p 8–p20	30, 31
rat	*rhodopsin*	substitution (P23H)	ONL	p 15–1 year	30, 32
pig	*rhodopsin*	substitution (Pro347Leu)	ONL	p 14–20 months	33, 38, 39

rodents but also in other animals such as cats,[6–10] dogs,[11–14] and chickens[15 16] (Table 1). Some of these represent mutations that are also found in human autosomal recessive RP[11 12 14 17 18] or X linked congenital stationary night blindness (CSNB) while others carry mutations in rod structural proteins[19 20] or more complex syndromes such as Usher type I.[21] The *rds* (retinal degeneration slow) mouse, for instance, carries an insertional mutation in the *rds*/peripherin gene encoding for a photoreceptor structural protein expressed both in rods and cones.[22] Consequently, rod and cone photoreceptor outer segments in homozygous *rds* mice never develop and photoreceptor nuclei start to die by apoptosis as early as by p21 and at the age of 12 months, the entire outer nuclear layer (ONL) has disappeared.[19] Interestingly, the pattern of photoreceptor cell loss is peripheral to central in the *rds* mouse which is in marked contrast to the *rd* mouse where photoreceptor degeneration starts in the central retina progressing towards the periphery.[19]

Transgenic animal models

In the past two decades our understanding of the molecular events leading to human retinal dystrophy has improved markedly. The history of the identification of genetic loci for inherited retinal degeneration started in the early 1980s when Bhattacharya *et al* mapped the gene responsible for X linked retinitis pigmentosa to a subregion of the X chromosome.[23] In 1989, Humphries and his co-workers identified the first autosomal dominant RP locus[24] and only a year later, Dryja and his group described the first mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa[25 26] followed by numerous other groups (for review see Gal *et al*[27]). Consecutively, mutations in other photoreceptor specific genes such as the β subunit of the cGMP phosphodiesterase,[4] peripherin,[22] and the rod outer segment protein 1 (ROM-1)[28] were described.

The era of transgenic animals had begun just a few years earlier when in 1981 Wagner *et al* performed the first successful transgenesis by transplanting a rabbit β globin gene into a mouse embryo.[29] Therefore, the retinal research community was among the first to profit from the new possibility of designing specific transgenic animals mimicking genetically caused human disease; disruption or overexpression of the target gene allowed the investigation of the role of a single specific gene product on retinal function in vivo.

Transgenic mice and rats[30–32] are among the most commonly used animals to date; nevertheless, other species such as pig[33] may also be very useful models since they may show a cone distribution similar to the human eye (Table 2). Furthermore, in the transgenic pig, being a larger mammal, the time course of retinal degeneration may resemble the human disease more closely. Finally, the large size of the pig eye is very well suited to subretinal injections and somatic gene therapy.

Transgenic models for retinal degenerations and dystrophies may be divided into two major subgroups.

TRANSGENIC MODELS MIMICKING HUMAN RP AND/OR MODULATING PHOTORECEPTOR PHYSIOLOGY

Soon after the identification of the first gene mutations leading to RP in human, attempts were made to create transgenic animals carrying analogous mutations. To our knowledge, the earliest transgenic mouse generated for retinal research was the Pro23His rhodopsin mutant mouse created in 1992 by Olsson *et al*.[34] In the following years, a number of different animal models carrying rhodopsin mutations mimicking autosomal dominant RP were generated in various species.[20 30–41]

The *rds* mouse was used for the generation of double mutant mice carrying a peripherin mutation on an *rds* null background,[42] leading to an acceleration of the time course of retinal degeneration observed in the *rds* mouse.

Several groups investigated the role of gene products playing key parts in photoreceptor physiology. For example, the lack of interphotoreceptor retinoid binding protein (IRBP), responsible for the buffering of retinoids in the extracellular matrix, led to slow photoreceptor degeneration in *IRBP$^{-/-}$* mice.[43]

Among the proteins necessary for phototransduction, the role of the γ subunit of the rod cGMP phosphodiesterase was investigated through the generation of mice lacking a functional PDEγ gene.[44] These mice showed a rapid retinal degeneration starting as early as p5.

Another very promising recent finding does not primarily implicate photoreceptor cells but rather the

retinal pigment epithelium (RPE) where the RPE65 protein is an essential element for vitamin A metabolism. In humans, mutated RPE65 protein not only leads to autosomal recessive, childhood onset severe retinal dystrophy[45] but also to autosomal recessive RP[46] and Leber's congenital amaurosis.[46 47] In analogy to the human mutation, a four nucleotide deletion in the RPE65 gene was identified in Swedish briard dogs[48 49] as the cause of CSNB[50] but also for autosomal recessive, early onset and progressive retinal dystrophy.[50 51] Transgenic *Rpe65*$^{-/-}$ mice, because of a lack of rhodopsin in the outer segment discs, show no rod function as demonstrated by electroretinography and seem to be an excellent model for an all cone retina.[52] Furthermore, photoreceptors are completely protected from light induced degeneration indicating that rhodopsin is the key trigger in the initiation of the signalling pathway from light to photoreceptor death by apoptosis.[53]

McCall and co-workers generated a transgenic animal called the rdta mouse.[54] This animal expresses the gene for an attenuated diphtheria toxin—under control of a portion of the rhodopsin promoter—which inhibits G protein binding to rhodopsin. Expression of this transgene results in the elimination of rod photoreceptor cell bodies in the ONL and the elimination of any rod mediated ERG responses by p17.[54]

Recently, Biel *et al* demonstrated that deletion of the cyclic nucleotide gated channel 3 (CNG3) led to a selective loss of cone function. This all rod retina may serve as a model for human achromatopsia.[55]

The Rim protein (RmP) is an ABC transporter protein in rod photoreceptor outer segment discs. Recently, Weng and collaborators reported the generation of a mouse carrying a null mutation in the rim protein gene (ABCR).[56] *abcr* knockout mice show increased levels of all-*trans*-retinaldehyde following light exposure and a dramatic accumulation of the lipofuscin fluorophore A2-E. With increasing age, the loss of RPE cells leads to secondary photoreceptor degeneration. *abcr* knockout mice may represent a model for human recessive Stargardt's disease and may also provide insights into the pathogenesis of age related macular degeneration (AMD).

Humphries and collaborators designed the rhodopsin knockout mouse as a model serving two different purposes—firstly, to provide a genetic background on which other mutant opsin transgenes may be expressed and, secondly, to study the introduction of functional rhodopsin genes through somatic gene therapy. *rho*$^{-/-}$ mice do not develop rod outer segments (ROS) and photoreceptor degeneration starts as early as p24. At 8 weeks of age, no scotopic electroretinogram (ERG) response can be detected.[57]

Sieving and collaborators recently generated a mouse model for autosomal dominant CSNB by crossing rhodopsin G90D transgenic mice with rhodopsin knockout (*rho*$^{-/-}$) mice.[58] In addition, mice lacking rhodopsin kinase (RK)[59] as well as arrestin knockout mice[60] may both serve as models for Oguchi's disease, a human condition that causes autosomal recessive CSNB. *RK*$^{-/-}$ mice reared in cyclic light show a 50% shortening of ROS by 6 weeks of age whereas arrestin knockout mice show a progressive thinning of the ONL starting at p100.

TRANSGENIC MODELS MODULATING REGULATORY GENES OF APOPTOSIS

Apoptosis is a tightly regulated form of cell death that occurs physiologically during organ development in the retina[61] and other tissues but also in a variety of pathological conditions such as cancer and degenerative disorders. In the retina, apoptosis is the final common pathway of photoreceptor cell death not only in transgenic animal models for RP[20 41] and the model of light induced photoreceptor degeneration[62 63] but also in human RP.[64] Inhibition of apoptosis by modulating potential regulatory genes may therefore be a means to retard or even stop the time course of retinal degeneration.

THE BCL-2 FAMILY

The mammalian cell death suppressor gene Bcl-2[65] is a member of a group of proteins involved in the regulation of apoptosis.[66] Both death antagonists (for example, Bcl-2, Bcl-X$_L$, Bcl-w, Bfl-1, Bag-1, Mcl-1, A1) and agonists (for example Bax, Bak, Bcl-X$_S$, Bad, Bid, Bik, Hrk) belong to the Bcl-2 family. Data on the role of Bcl-2 in regulating photoreceptor apoptosis are ambiguous. Although overexpression of Bcl-2 delayed apoptotic photoreceptor death in several RP animal models, introduction of the transgene did not influence the final outcome of photoreceptor degeneration[67–69] and specific overexpression of human Bcl-2 in Müller cells led to early postnatal Müller cell apoptosis followed by photoreceptor degeneration in a transgenic mouse line.[70] Similarly, Bcl-X$_L$, a potent inhibitor of apoptosis in many cell types, was unable to efficiently inhibit photoreceptor apoptosis when overexpressed in the *rd* mouse.[67 68]

Little is known about the role of other Bcl-2 family members in the retina: the role of the pro-apoptotic Bcl-2 family member Bax on photoreceptor apoptosis was studied in Bax deficient mice. These studies indicated that Bax is involved in the control of developmental photoreceptor apoptosis in wild type mice but not of photoreceptor degeneration in the *rd* mouse.[71]

P53

The tumour suppressor gene p53, a sequence specific DNA binding transcription factor, is an important regulator of apoptosis in a variety of systems and tissues. However, both p53 dependent and p53 independent apoptosis has been described. In the retina, the lack of p53 did not protect from photoreceptor apoptosis in the model of light induced retinal degeneration[72] whereas it delayed photoreceptor death by 3 days in the *rd* mouse.[73]

AP-1

The proto-oncoprotein c-Fos is a member of the AP-1 transcription factor complex involved in the regulation of apoptosis in many systems. In contrast with p53, lack of c-Fos completely protected photoreceptors from light induced apoptosis.[63 74] but had no effect on the degeneration process in the *rd* mouse.[75]

AP-1 may be constituted of members of the Fos and Jun families of proteins. In the retina, AP-1 primarily consists of c-Fos and junD.[76] We therefore also investigated the role of JunD in light induced photoreceptor degeneration. However, no difference in the extent of light induced photoreceptor apoptosis was found between *junD*$^{-/-}$ and *junD*$^{+/+}$ mice.[77]

Inducible animal models

In the early 1960s Werner Noëll started to investigate the effect of light on inherited retinal degeneration and in 1965 he showed that photoreceptor degeneration in the Royal College of Surgeon (RCS) rat, a model for inherited retinal dystrophy, was dramatically enhanced when animals were exposed to light: "On the basis of this reasoning, I cannot help but be impressed by the fact that excessive light destroys the visual cells and the pigment epithelium albino as well as pigmented animals".[78] Since then, it has been postulated that light exposure may enhance inherited photoreceptor degeneration. In the past few years, a

Table 3   Inducible models for retinal degenerations

	Animal (strain)	Cell layer	Degeneration time course	References
Induction by light				
white fluorescent light, up to 2 hours	rat (ZUR-SIV)	ONL, RPE	immediately–24 hours after exposure	62, 87
white fluorescent light, up to 2 hours	mouse (C57BL/6 × 129Sv)	ONL	immediately–24 hours after exposure	63, 86
fluorescent light, 1 week, 2000 lux	rat (Sprague-Dawley)	ONL	full degeneration at end of exposure	88
intermittent green fluorescent light	albino rat (Lewis)	ONL, RPE	depending on stimulus onset	92
green fluorescent light, up to 24 hours	albino rat (Lewis)	ONL, RPE	starts at 6 hours after exposure	94
intraocular fibre optic light	owl monkey	ONL, RPE	1 hour–4 weeks after exposure	95
Others				
prenatal intraperitoneal injection of MNU	mouse (CD-1 albino)	ONL, OPL, IPL	time and dose dependent	96
intraperitoneal injection of MNU	rat (Brown-Norway)	ONL	time and dose dependent	97–99
intraperitoneal injection of MNU	mouse (C57BL6)	ONL	time and dose dependent	100
insertion of iron wire into vitreous	rabbit	ONL	24 hours–4 days after insertion	101
implantation of iron particle into vitreous	rat (Sprague-Dawley)	ONL	1–2 days after implantation	102
intravitreal injection of L-ornithine hydrochloride	rat (Brown-Norway)	RPE, ONL	immediately–14 days after injection	103, 121
murine coronavirus	mouse (BALB/c)	ONL, RPE	immediately–32 days after inoculation	122
vitamin E deficient diet for 3 months	rat (RCS-rdy+)	ONL, RPE	immediately after diet	123
intravitreal injection of LHP	rabbit (New Zealand white)	ONL, RPE	few hours–18 days after injection	124
hypoxia, beginning on p15	rat (Sprague-Dawley)	ONL, INL	initial degeneration at p 21	125

number of experimental studies performed in new transgenic models has further supported this hypothesis[56 59 60 79–81] and it was also hypothesised that light exposure may enhance the progression of RP in humans.[82 83]

It was conceivably based on his earlier findings in the RCS rat (D Organisciak, personal communication) that Noëll developed the first inducible animal model for retinal degeneration by damaging photoreceptors in wild type rats using bright light.[84] The advantages of an external damaging stimulus are evident. Animals can mature and develop a normal retina until the stimulus is provided; the latter being very flexible and adjustable for timing and intensity so that more or less severe photoreceptor damage can be obtained. Such a tightly tunable model therefore is an excellent tool to investigate the molecular stages of apoptotic photoreceptor death. Light induced photoreceptor degeneration is nowadays used as a model in its own right by a number of groups in a variety of different light conditions in wild type and transgenic animals[62 63 85–95] and it has been shown repetitively that photoreceptor death in these models occurs by apoptosis (see Table 3).[62 63 85–87 94]

Another well known method of inducing selective photoreceptor apoptosis is the intraperitoneal administration of N-methyl-N-nitrosourea (MNU). Depending on its dose, MNU leads to a rapid degeneration of photoreceptors leaving other cell layers unaffected.[96–100] However, the molecular mechanisms underlying MNU induced photoreceptor apoptosis are not known yet. Other systems of induced photoreceptor damage include the implantation of iron particles into the vitreous[101 102] or the intravitreal injection of L-ornithine hydrochloride.[103]

Conclusion

Currently, many of the animal models described in this review play a more important part than ever, serving a variety of different purposes: firstly, they are used for the study of the molecular mechanisms leading to photoreceptor degeneration. Secondly, they are the basis for therapeutic attempts to retard or even stop photoreceptor apoptosis using a multitude of different approaches. Finally, they serve as a platform for the establishment of experimental somatic gene therapy, probably the most promising approach to photoreceptor rescue (reviewed by Sharma and Ehinger,[104] Ali *et al*,[105] and Chong and Bird[106]).

Although still restricted and fragmentary, our knowledge about molecular mechanisms leading to photoreceptor degeneration increased markedly in the past two decades. It is therefore conceivable to speculate that ophthalmology may be among the first clinical specialties to benefit from "molecular medicine" once new therapeutic strategies evolve.

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