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 deletion 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* 1*Neu*)	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 of 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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1 Keeler CE. The inheritance of a retinal abnormality in white mice. *Proc Natl Acad Sci USA* 1924;**10**:329–33.
2 Brückner R. Spaltlampenmikroskopie und Ophthalmoskopie am Auge von Ratte und Maus. *DocOphthalmol* 1951;**5–6**:452–4.
3 Farber DB. From mice to men: the cyclic GMP phosphodiesterase gene in vision and disease. The Proctor lecture. *Invest Ophthalmol Vis Sci* 1995;**36**: 263–75.
4 Bowes C, Li T, Danciger M, *et al*. Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase. *Nature* 1990;**347**:677–80.
5 Pittler SJ, Keeler CE, Sidman RL, *et al*. PCR analysis of DNA from 70-year-old sections of rodless retina demonstrates identity with the mouse rd defect. *Proc Natl Acad Sci USA* 1993;**90**:9616–9.
6 Barnett KC, Curtis R. Autosomal dominant progressive retinal atrophy in Abyssinian cats. *J Hered* 1985;**76**:168–70.
7 Curtis R, Barnett KC, Leon A. An early-onset retinal dystrophy with dominant inheritance in the Abyssinian cat. Clinical and pathological findings. *Invest Ophthalmol Vis Sci* 1987;**28**:131–9.
8 Leon A, Curtis R. Autosomal dominant rod-cone dysplasia in the Rdy cat. 1. Light and electron microscopic findings. *Exp Eye Res* 1990;**51**:361–81.
9 Leon A, Hussain AA, Curtis R. Autosomal dominant rod-cone dysplasia in the Rdy cat. 2. Electrophysiological findings. *Exp Eye Res* 1991;**53**:489–502.
10 Voaden MJ, Curtis R, Barnett KC, *et al*. Dominant rod-cone dysplasia in the Abyssinian cat. *Prog Clin Biol Res* 1987;**247**:369–80.
11 Aquirre G, Farber D, Lolley R, *et al*. Rod-cone dysplasia in Irish setters: a defect in cyclic GMP metabolism in visual cells. *Science* 1978;**201**:1133–4.
12 Farber DB, Danciger JS, Aguirre G. The beta subunit of cyclic GMP phosphodiesterase mRNA is deficient in canine rod-cone dysplasia 1. *Neuron* 1992;**9**:349–56.
13 Kommonen B, Penn JS, Kylma T, *et al*. Early morphometry of a retinal dystrophy in Labrador retrievers. *Acta Ophthalmol Copenh* 1994;**72**:203–10.
14 Suber ML, Pittler SJ, Qin N, *et al*. Irish setter dogs affected with rod/cone dysplasia contain a nonsense mutation in the rod cGMP phosphodiesterase beta-subunit gene. *Proc Natl Acad Sci USA* 1993;**90**:3968–72.
15 Ulshafer RJ, Allen CB. Ultrastructural changes in the retinal pigment epithelium of congenitally blind chickens. *Curr Eye Res* 1985;**4**:1009–21.
16 Semple Rowland SL, Lee NR, Van Hooser JP, *et al*. A null mutation in the photoreceptor guanylate cyclase gene causes the retinal degeneration chicken phenotype. *Proc Natl Acad Sci USA* 1998;**95**:1271–6.
17 Lai YL, Jacoby RO, Jonas AM. Age-related and light-associated retinal changes in Fischer rats. *Invest Ophthalmol Vis Sci* 1978;**17**:634–8.
18 DiLoreto D Jr, Cox C, Grover DA, *et al*. The influences of age, retinal topography, and gender on retinal degeneration in the Fischer 344 rat. *Brain Res* 1994;**647**:181–91.
19 Sanyal S, De Ruiter A, Hawkins RK. Development and degeneration of retina in rds mutant mice: light microscopy. *J Comp Neurol* 1980;**194**:193–207.
20 Chang GQ, Hao Y, Wong F. Apoptosis: final common pathway of photoreceptor death in rd, rds, and rhodopsin mutant mice. *Neuron* 1993;**11**:595–605.
21 Heckenlively JR, Chang B, Erway LC, *et al*. Mouse model for Usher syndrome: linkage mapping suggests homology to Usher type I reported at human chromosome 11p15. *Proc Natl Acad Sci USA* 1995;**92**:11100–4.
22 Travis GH, Brennan MB, Danielson PE, *et al*. Identification of a photoreceptor-specific mRNA encoded by the gene responsible for retinal degeneration slow (rds). *Nature* 1989;**338**:70–3.

23 Bhattacharya SS, Wright AF, Clayton JF, *et al*. Close genetic linkage between X-linked retinitis pigmentosa and a restriction fragment length polymorphism identified by recombinant DNA probe L1.28. *Nature* 1984; **309**:253–5.
24 Farrar GJ, McWilliam P, Sharp EM, *et al*. Autosomal dominant retinitis pigmentosa: exclusion of a gene from extensive regions of chromosomes 6, 13, 20, and 21. *Genomics* 1989;**5**:612–8.
25 Dryja TP, McGee TL, Hahn LB, *et al*. Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. *N Engl J Med* 1990;**323**:1302–7.
26 Dryja TP, McGee TL, Reichel E, *et al*. A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. *Nature* 1990;**343**:364–6.
27 Gal A, Apfelstedt-Sylla E, Janecke AR, *et al*. Rhodopsin mutations in inherited retinal dystrophies and dysfunctions. *Prog Retin Eye Res* 1997;**16**:51–79.
28 Kajiwara K, Berson EL, Dryja TP. Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. *Science* 1994;**264**:1604–8.
29 Wagner TE, Hoppe PC, Jollick JD, *et al*. Microinjection of a rabbit beta-globin gene into zygotes and its subsequent expression in adult mice and their offspring. *Proc Natl Acad Sci USA* 1981;**78**:6376–80.
30 Steinberg RH, Flannery JG, Naash M, *et al*. Transgenic rat models of inherited retinal degeneration caused by mutant opsin genes. *Invest Ophthalmol Vis Sci* 1996;**37**:S698 Abstract no 3190.
31 Liu C, Li Y, Peng M, *et al*. Activation of caspase-3 in the retina of transgenic rats with the rhodopsin mutation s334ter during photoreceptor degeneration. *J Neurosci* 1999;**19**:4778–85.
32 Lewin AS, Drenser KA, Hauswirth WW, *et al*. Ribozyme rescue of photoreceptor cells in a transgenic rat model of autosomal dominant retinitis pigmentosa. *Nat Med* 1998;**4**:967–71.
33 Li ZY, Wong F, Chang JH, *et al*. Rhodopsin transgenic pigs as a model for human retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 1998;**39**:808–19.
34 Olsson JE, Gordon JW, Pawlyk BS, *et al*. Transgenic mice with a rhodopsin mutation (Pro23His): a mouse model of autosomal dominant retinitis pigmentosa. *Neuron* 1992;**9**:815–30.
35 Huang PC, Gaitan AE, Hao Y, *et al*. Cellular interactions implicated in the mechanism of photoreceptor degeneration in transgenic mice expressing a mutant rhodopsin gene. *Proc Natl Acad Sci USA* 1993;**90**:8484–8.
36 Naash MI, Hollyfield JG, al Ubaidi MR, *et al*. Simulation of human autosomal dominant retinitis pigmentosa in transgenic mice expressing a mutated murine opsin gene. *Proc Natl Acad Sci USA* 1993;**90**:5499–503.
37 Roof DJ, Adamian M, Hayes A. Rhodopsin accumulation at abnormal sites in retinas of mice with a human P23H rhodopsin transgene. *Invest Ophthalmol Vis Sci* 1994;**35**:4049–62.
38 Petters RM, Alexander CA, Wells KD, *et al*. Genetically engineered large animal model for studying cone photoreceptor survival and degeneration in retinitis pigmentosa. *Nat Biotechnol* 1997;**15**:965–70.
39 Tso MO, Li WW, Zhang C, *et al*. A pathologic study of degeneration of the rod and cone populations of the rhodopsin Pro347Leu transgenic pigs. *Trans Am Ophthalmol Soc* 1997;**95**:467–79; discussion 79–83.
40 Goto Y, Peachey NS, Ripps H, *et al*. Functional abnormalities in transgenic mice expressing a mutant rhodopsin gene. *Invest Ophthalmol Vis Sci* 1995; **36**:62–71.
41 Portera Cailliau C, Sung CH, Nathans J, *et al*. Apoptotic photoreceptor cell death in mouse models of retinitis pigmentosa. *Proc Natl Acad Sci USA* 1994;**91**:974–8.
42 Kedzierski W, Lloyd M, Birch DG, *et al*. Generation and analysis of transgenic mice expressing P216L-substituted rds/peripherin in rod photoreceptors. *Invest Ophthalmol Vis Sci* 1997;**38**:498–509.
43 Liou GI, Fei Y, Peachey NS, *et al*. Early onset photoreceptor abnormalities induced by targeted disruption of the interphotoreceptor retinoid-binding protein gene. *J Neurosci* 1998;**18**:4511–20.
44 Tsang SH, Gouras P, Yamashita CK, *et al*. Retinal degeneration in mice lacking the gamma subunit of the rod cGMP phosphodiesterase. *Science* 1996;**272**:1026–9.
45 Gu SM, Thompson DA, Srikumari CR, *et al*. Mutations in RPE65 cause autosomal recessive childhood-onset severe retinal dystrophy. *Nat Genet* 1997;**17**:194–7.
46 Morimura H, Fishman GA, Grover SA, *et al*. Mutations in the RPE65 gene in patients with autosomal recessive retinitis pigmentosa or leber congenital amaurosis. *Proc Natl Acad Sci USA* 1998;**95**:3088–93.
47 Marlhens F, Bareil C, Griffoin JM, *et al*. Mutations in RPE65 cause Leber's congenital amaurosis. *Nat Genet* 1997;**17**:139–41.
48 Narfstrom K, Wrigstad A, Nilsson, SE. The Briard dog: a new animal model of congenital stationary night blindness. *Br J Ophthalmol* 1989;**73**:750–6.
49 Wrigstad A, Narfstrom K, Nilsson SE. Slowly progressive changes of the retina and retinal pigment epithelium in briard dogs with hereditary retinal dystrophy. A morphological study. *Doc Ophthalmol* 1994;**87**:337–54.
50 Aguirre GD, Baldwin V, Pearce-Kelling S, *et al*. Congenital stationary night blindness in the dog: common mutation in the RPE65 gene indicates founder effect. *Mol Vis* 1998;**4**:23.
51 Veske A, Nilsson SE, Narfstrom K, *et al*. Retinal dystrophy of Swedish briard/briard-beagle dogs is due to a 4-bp deletion in RPE65. *Genomics* 1999;**57**:57–61.
52 Redmond TM, Yu S, Lee E, *et al*. Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. *Nat Genet* 1998;**20**:344–51.
53 Grimm C, Wenzel A, Hafezi F, *et al*. Protection of Rpe65-deficient mice identifies rhodopsin as mediator of light-induced retinal degeneration. *Nat Genet* 2000 (in press).
54 McCall MA, Gregg RG, Merriman K, *et al*. Morphological and physiological consequences of the selective elimination of rod photoreceptors in transgenic mice. *Exp Eye Res* 1996;**63**:35–50.
55 Biel M, Seeliger M, Pfeifer A, *et al*. Selective loss of cone function in mice lacking the cyclic nucleotide-gated channel CNG3. *Proc Natl Acad Sci* 1999;**96**:7553–7.
56 Weng J, Mata NL, Azarian SM, *et al*. Insights into the function of Rim protein in photoreceptors and etiology of Stargardt's disease from the phenotype in abcr knockout mice. *Cell* 1999;**98**:13–23.
57 Humphries MM, Rancourt D, Farrar GJ, *et al*. Retinopathy induced in mice by targeted disruption of the rhodopsin gene. *Nat Genet* 1997;**15**:216–9.
58 Sieving PA, Bush RA, Fowler M, *et al*. Crossing the rhodopsin G90D transgenic mouse onto a rho-knockout background recreates autosomal dominant human congenital nightblindness. *Invest Ophthalmol Vis Sci* 1998;**39**:S 101, Abstract no 474.
59 Chen CK, Burns ME, Spencer M, *et al*. Abnormal photoresponses and light-induced apoptosis in rods lacking rhodopsin kinase. *Proc Natl Acad Sci* 1999;**96**:3718–22.
60 Chen J, Simon MI, Matthes MT, *et al*. Increased susceptibility to light damage in an arrestin knockout mouse model of Oguchi disease (stationary night blindness). *Invest Ophthalmol Vis Sci* 1999;**40**:2978–82.
61 Young RW. Cell death during differentiation of the retina in the mouse. *J Comp Neurol* 1984;**229**:362–73.
62 Hafezi F, Marti A, Munz K, *et al*. Light-induced apoptosis: differential timing in the retina and pigment epithelium. *Exp Eye Res* 1997;**64**:963–70.
63 Hafezi F, Steinbach JP, Marti A, *et al*. The absence of c-fos prevents light-induced apoptotic cell death of photoreceptors in retinal degeneration in vivo. *Nat Med* 1997;**3**:346–9.
64 Li Z-Y, Milam AH. Apoptosis in retinitis pigmentosa. In: Anderson RE, LaVail MM, Hollyfield JG, eds. *Retinal degeneration*. New York: Plenum Press, 1995:1–8
65 Hengartner MO, Horvitz HR. Programmed cell death in Caenorhabditis elegans. *Curr Opin Genetics Develop* 1994;**4**:581–6.
66 Kroemer G. The proto-oncogene Bcl-2 and its role in regulating apoptosis. *Nat Med* 1997;**3**:614–260.
67 Joseph RM, Li T. Overexpression of Bcl-2 or Bcl-XL transgenes and photoreceptor degeneration. *Invest Ophthalmol Vis Sci* 1996;**37**:2434–46.
68 Chen J, Flannery JG, LaVail MM, *et al*. bcl-2 overexpression reduces apoptotic photoreceptor cell death in three different retinal degenerations. *Proc Natl Acad Sci USA* 1996;**93**:7042–7.
69 Tsang SH, Chen J, Kjeldbye H, *et al*. Retarding photoreceptor degeneration in Pdegtm1/Pdegtml mice by an apoptosis suppressor gene. *Invest Ophthalmol Vis Sci* 1997;**38**:943–50.
70 Dubois-Dauphin M, Poitry-Yamate C, de Bilbao F, *et al*. Early postnatal Muller cell death leads to retinal but not optic nerve degeneration in NSE-Hu-Bcl-2 transgenic mice. *Neuroscience* 2000;**95**:9–21.
71 Mosinger Ogilvie J, Deckwerth TL, Knudson CM, *et al*. Suppression of developmental retinal cell death but not of photoreceptor degeneration in Bax-deficient mice. *Invest Ophthalmol Vis Sci* 1998;**39**:1713–20.
72 Marti A, Hafezi F, Lansel N, *et al*. Light-induced cell death of retinal photoreceptors in the absence of p53. *Invest Ophthalmol Vis Sci* 1998;**39**:846–9.
73 Ali RR, Reichel MB, Kanuga N, *et al*. Absence of p53 delays apoptotic photoreceptor cell death in the rds mouse. *Curr Eye Res* 1998;**17**:917–23.
74 Wenzel A, Grimm C, Marti A, *et al*. c-fos controls the "private pathway" of light-induced apoptosis of retinal photoreceptors. *J Neurosci* 2000;**20**:81–8.
75 Hafezi F, Abegg M, Grimm C, *et al*. Retinal degeneration in the rd mouse in the absence of c-fos. *Invest Ophthalmol Vis Sci* 1998;**39**:2239–44.
76 Hafezi F, Marti A, Grimm C, *et al*. Differential DNA binding activities of the transcription factors AP-1 and Oct-1 during light-induced apoptosis of photoreceptors. *Vis Res* 1999;**39**:2511–8.
77 Hafezi F, Grimm C, Wenzel A, *et al*. Retinal photoreceptors are apoptosis-competent in the absence of JunD/AP-1. *Cell Death Differ* 1999;**6**:934–6.
78 Noëll WK. Aspects of experimental and hereditary retinal degeneration. In: Graymore CN, ed. *Biochemistry of the retina*. London: Academic Press, 1965:51–72.
79 Sanyal S, Hawkins RK. Development and degeneration of retina in rds mutant mice: effects of light on the rate of degeneration in albino and pigmented homozygous and heterozygous mutant and normal mice. *Vis Res* 1986;**26**:1177–85.
80 Wang M, Lam TT, Tso MO, *et al*. Expression of a mutant opsin gene increases the susceptibility of the retina to light damage. *Vis Neurosci* 1997; **14**:55–62.
81 LaVail MM, Gorrin GM, Yasumura D, *et al*. Increased susceptibility to constant light in nr and pcd mice with inherited retinal degenerations. *Invest Ophthalmol Vis Sci* 1999;**40**:1020–4.
82 Li ZY, Jacobson SG, Milam AH. Autosomal dominant retinitis pigmentosa caused by the threonine-17-methionine rhodopsin mutation: retinal histopathology and immunocytochemistry. *Exp Eye Res* 1994;**58**:397–408.
83 Heckenlively JR, Rodriguez JA, Daiger SP. Autosomal dominant sectoral retinitis pigmentosa. Two families with transversion mutation in codon 23 of rhodopsin. *Arch Ophthalmol* 1991;**109**:84–91.
84 Noëll WK, Walker VS, Kang BS, *et al*. Retinal damage by light in rats. *Invest Ophthalmol Vis Sci* 1966;**5**:450–73.
85 Abler AS, Chang CJ, Ful J, *et al*. Photic injury triggers apoptosis of photoreceptor cells. *Res Commun Mol Pathol Pharmacol* 1996;**92**:177–89.
86 Reme CE, Grimm C, Hafezi F, *et al*. Apoptotic cell death in retinal degenerations. *Prog Retin Eye Res* 1998;**17**:443–64.
87 Remé CE, Weller M, Szczesny P, *et al*. Light-induced apoptosis in the rat retina in vivo: Morphological features, threshold and time course. In: Anderson RE, LaVail MM, Hollyfield JG, eds. *Retinal degeneration*. New York: Plenum Press, 1995.
88 LaVail MM, Unoki K, Yasumura D, *et al*. Multiple growth factors, cytokines, and neurotrophins rescue photoreceptors from the damaging effects of constant light. *Proc Natl Acad Sci USA* 1992;**89**:11249–53.
89 Williams TP, Howell WL. Action spectrum of retinal light-damage in albino rats. *Invest Ophthalmol Vis Sci* 1983;**24**:285–7.
90 Li ZY, Tso MO, Wang HM, *et al*. Amelioration of photic injury in rat retina by ascorbic acid: a histopathologic study. *Invest Ophthalmol Vis Sci* 1985;**26**:1589–98.
91 Rapp LM, Williams TP. Rhodopsin content and electroretinographic sensitivity in light-damaged rat retina. *Nature* 1977;**267**:835–6.
92 Organisciak DT, Jiang YL, Wang HM, *et al*. Retinal light damage in rats exposed to intermittent light. Comparison with continuous light exposure. *Invest Ophthalmol Vis Sci* 1989;**30**:795–805.
93 Organisciak DT, Winkler BS. Retinal light damage: practical and theoretical considerations. In: Osborne NN, Chader GJ, eds. *Progress in retinal and eye research*. Oxford, New York, Tokyo: Pergamon, 1994:1–29.
94 Shahinfar S, Edward DP, Tso MO. A pathologic study of photoreceptor cell death in retinal photic injury. *Curr Eye Res* 1991;**10**:47–59.
95 Fuller D, Machemer R, Knighton RW. Retinal damage produced by intraocular fiber optic light. *Am J Ophthalmol* 1978;**85**:519–37.
96 Smith SB, Hashimi W, Yielding KL. Retinal degeneration in the mouse induced transplacentally by N-methyl-N-nitrosourea: effects of constant illumination or total darkness. *Exp Eye Res* 1988;**47**:347–59.
97 Nakajima M, Nambu H, Shikata N, *et al*. Pigmentary degeneration induced by N-methyl-N-nitrosourea and the fate of pigment epithelial cells in the rat retina. *Pathol Int* 1996;**46**:874–82.

98 Ogino H, Ito M, Matsumoto K, *et al*. Retinal degeneration induced by N-methyl-N-nitrosourea and detection of 7-methyldeoxyguanosine in the rat retina. *Toxicol Pathol* 1993;**21**:21–5.
99 Herrold KM. Pigmentary degeneration of the retina induced by N-methyl-N-nitrosourea. An experimental study in syrian hamsters. *Arch Ophthalmol* 1967;**78**:650–3.
100 Nambu H, Yuge K Nakajima M, *et al*. Morphologic characteristics of N-methyl-N-nitrosourea-induced retinal degeneration in C57BL mice. *Pathol Int* 1997;**47**:377–83.
101 Burger PC, Klintworth GK. Experimental retinal degeneration in the rabbit produced by intraocular iron. *Lab Invest* 1974;**30**:9–19.
102 Wang ZJ, Lam KW, Lam TT, *et al*. Iron-induced apoptosis in the photoreceptor cells of rats. *Invest Ophthalmol Vis Sci* 1998;**39**:631–3.
103 Maeda H, Ogata N, Yi X, *et al*. Apoptosis of photoreceptor cells in ornithine-induced retinopathy. *Graefes Arch Clin Exp Ophthalmol* 1998;**236**:207–12.
104 Sharma RK, Ehinger B. Management of hereditary retinal degenerations: present status and future directions. *Surv Ophthalmol* 1999;**43**:427–44.
105 Ali RR, Reichel MB, Hunt DM, *et al*. Gene therapy for inherited retinal degeneration. *Br J Ophthalmol* 1997;**81**:795–801.
106 Chong NH, Bird AC. Management of inherited outer retinal dystrophies: present and future. *Br J Ophthalmol* 1999;**83**:120–2.
107 Ohlemiller KK, Hughes RM, Lett JM, *et al*. Progression of cochlear and retinal degeneration in the tubby (rd5) mouse. *Audiol Neurootol* 1997;**2**:175–85.
108 Ohlemiller KK, Hughes RM, Mosinger Ogilvie J, *et al*. Cochlear and retinal degeneration in the tubby mouse. *Neuroreport* 1995;**6**:845–9.
109 Smith SB. C57BL/6J-vit/vit mouse model of retinal degeneration: light microscopic analysis and evaluation of rhodopsin levels. *Exp Eye Res* 1992;**55**:903–10.
110 Smith SB, Cope BK, McCoy JR. Effects of dark-rearing on the retinal degeneration of the C57BL/6-mivit/mivit mouse. *Exp Eye Res* 1994;**58**:77–84.
111 Chang B, Heckenlively JR, Hawes NL, *et al*. New mouse primary retinal degeneration (rd-3). *Genomics* 1993;**16**:45–9.
112 LaVail MM, Blanks JC, Mullen RJ. Retinal degeneration in the pcd cerebellar mutant mouse. I. Light microscopic and autoradiographic analysis. *J Comp Neurol* 1982;**212**:217–30.
113 Roderick TH, Chang B, Hawes NL, *et al*. A new dominant retinal degeneration (Rd4) associated with a chromosomal inversion in the mouse. *Genomics* 1997;**42**:393–6.
114 Otteson DC, Shelden E, Jones JM, *et al*. Pax2 expression and retinal morphogenesis in the normal and Krd mouse. *Dev Biol* 1998;**193**:209–24.
115 Bourne MC, Campbell DA, Tansley K. Hereditary degeneration of the rat retina. *Br J Ophthalmol* 1938;**22**:613–23.
116 Davidorf FH, Mendlovic DB, Bowyer DW, *et al*. Pathogenesis of retinal dystrophy in the Royal College of Surgeons rat. *Ann Ophthalmol* 1991;**23**:87–94.
117 Mullen RJ, LaVail MM. Inherited retinal dystrophy: primary defect in pigment epithelium determined with experimental rat chimeras. *Science* 1976;**192**:799–801.
118 Favor J, Sandulache R, Neuhauser Klaus A, *et al*. The mouse Pax2 (1Neu) mutation is identical to a human PAX2 mutation in a family with renal-coloboma syndrome and results in developmental defects of the brain, ear, eye, and kidney. *Proc Natl Acad Sci USA* 1996;**93**:13870–5.
119 Yang RB, Robinson SW, Xiong WH, *et al*. Disruption of a retinal guanylyl cyclase gene leads to cone-specific dystrophy and paradoxical rod behavior. *J Neurosci* 1999;**19**:5889–97.
120 al Ubaidi MR, Hollyfield JG, Overbeek PA, *et al*. Photoreceptor degeneration induced by the expression of simian virus 40 large tumor antigen in the retina of transgenic mice. *Proc Natl Acad Sci USA* 1992;**89**:1194–8.
121 Kuwabara T, Ishikawa Y, Kaiser Kupfer, MI. Experimental model of gyrate atrophy in animals. *Ophthalmology* 1981;**88**:331–5.
122 Wang Y, Burnier M, Detrick B, *et al*. Genetic predisposition to coronavirus-induced retinal disease. *Invest Ophthalmol Vis Sci* 1996;**37**:250–4.
123 el Hifnawi S, Lincoln DT, Dashti H. Nutritionally induced retinal degeneration in rats. *Nutrition* 1995;**11**(5 Suppl):705–7.
124 Armstrong D, Hiramitsu T. Studies of experimentally induced retinal degeneration: 2. Early morphological changes produced by lipid peroxides in the albino rabbit. *Jpn J Ophthalmol* 1990;**34**:158–73.
125 Maslim J, Valter K, Egensperger R, *et al*. Tissue oxygen during a critical developmental period controls the death and survival of photoreceptors. *Invest Ophthalmol Vis Sci* 1997;**38**:1667–77.

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