Review

Mouse mutants as models for congenital retinal disorders

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Abstract

Animal models provide a valuable tool for investigating the genetic basis and the pathophysiology of human diseases, and to evaluate therapeutic treatments. To study congenital retinal disorders, mouse mutants have become the most important model organism. Here we review some mouse models, which are related to hereditary disorders (mostly congenital) including retinitis pigmentosa, Leber’s congenital amaurosis, macular disorders and optic atrophy.

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1. Introduction

Mice suffering from hereditary eye defects (and in particular from retinal degenerations) have been collected since decades (Keeler, 1924). They allow the study of molecular and histological development of retinal degenerations and to characterize the genetic basis underlying retinal dysfunction and degeneration. The recent progress of genomic approaches has added increasing numbers of such models.

In recent years systematic phenotype-driven approaches have been developed to screen for mice harboring chemically induced mutations, mainly by use of N-ethyl-N-nitrosourea (ENU), which predominantly causes point mutations (Justice et al., 1999). Moreover, many transgenic and knockout animal models were created to investigate the role of specific genes on retinal function. Finally, the genetrapping method was developed for the systematic generation of knockout mice (Skarnes et al., 2004).

Although mouse models are a good tool to investigate retinal disorders, one should keep in mind that the mouse retina is somehow different from a human retina, particularly with respect to the number and distribution of the photoreceptor cells. The mouse as a nocturnal animal has a retina dominated by rods; in contrast, cones are small in size and represent only 3–5% of the photoreceptors. Mice do not form cone-rich areas like the human fovea. Instead of three cone pigments present in the human retina, mice express only two distinct pigments with absorption maxima near 350 and 510 nm (Lyubarsky et al., 1999).

In this review we discuss important mouse mutants for retinal degenerations (for cross information on their mutated genes and chromosomal localization see Table 1 and Fig. 1). Concerning the nomenclature of genes and mutations, we follow the mouse genetic nomenclature as outlined by the Jackson Laboratory (http://www.informatics.jax.org).

1.1. Retinal disorders including degeneration of photoreceptor cells

1.1.1. Models for retinitis pigmentosa (RP)

One of the first mouse mutants described in the field of vision research was the rodless mouse (r, Keeler, 1924), which carries a nonsense mutation in the Pde6b gene coding for the β-subunit of phosphodiesterase. The gene mutation was later discovered in the retinal degeneration mouse (actual gene symbol Pde6b<sup>rd1</sup>, formerly referred to as rd1 or rd; Pittler and Baehr, 1991). A viral insertion in intron 1 of the Pde6b<sup>rd1</sup> allele (Bowes et al., 1993) coding for...
<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Chr. (cM)</th>
<th>Defect alleles</th>
<th>Mutation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crb1 crumbs homolog 1 (Drosophila)</td>
<td>1 (73.0)</td>
<td>Crb1&lt;sup&gt;rd8&lt;/sup&gt;</td>
<td>1 bp deletion causing a frame shift and premature stop codon</td>
<td>Mehalow et al., 2003</td>
</tr>
<tr>
<td>Cnga3 cyclic nucleotide gated channel alpha 3 Vsx1 visual system homeobox 1 homolog (zebrafish)</td>
<td>1 cyto-band B</td>
<td>Cnga3&lt;sup&gt;mb1Wij&lt;/sup&gt;</td>
<td>Knockout, insertion of a hygromycin resistance cassette the promoter region, exon 1 and part of intron 1</td>
<td>van de Pavert et al., 2004</td>
</tr>
<tr>
<td></td>
<td>2 (83.9)</td>
<td>Vsx1&lt;sup&gt;ml1Bhr&lt;/sup&gt;</td>
<td>Knockout, a neo cassette replacing the coding region for the entire homeodomain and CVC domain</td>
<td>Ohtoshi et al., 2004</td>
</tr>
<tr>
<td>Abca4 ATP-binding cassette, sub-family A (ABC1), member 4</td>
<td>3 (61.8)</td>
<td>Abca4&lt;sup&gt;tm1Gle&lt;/sup&gt;</td>
<td>Knockout, replacement of a 4 kb genomic fragment containing the promoter and first exon with a neomycin cassette</td>
<td>Weng et al., 1999</td>
</tr>
<tr>
<td></td>
<td>3 (87.6)</td>
<td>Rpe65&lt;sup&gt;tm1Tmr&lt;/sup&gt;</td>
<td>Knockout, exons 1–3 of the gene were replaced with a PGK-neo cassette</td>
<td>Redmond et al., 1998</td>
</tr>
<tr>
<td>Pde6b rod phosphodiesterase, beta subunit (r,rodless; rd, retinal degeneration)</td>
<td>5 (57.0)</td>
<td>Pde6b&lt;sup&gt;rd10&lt;/sup&gt;</td>
<td>Nonsense mutation, base substitution (C to T) in codon 44</td>
<td>Chang et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pde6b&lt;sup&gt;rd12&lt;/sup&gt;</td>
<td>Nonsense mutation, base substitution (C to T) in codon 44</td>
<td></td>
</tr>
<tr>
<td>Mitf microphthalmia-associated transcription factor</td>
<td>6 (40.0)</td>
<td>Mitf&lt;sup&gt;mi-sp&lt;/sup&gt;</td>
<td>Insertion of an extra C residue in the poly-pyrimidine tract located upstream of an 18 bp alternative exon</td>
<td>Steingrimsson et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitf&lt;sup&gt;mi-vit&lt;/sup&gt;</td>
<td>G to A transition at bp 793 that leads to an aspartate to asparagine substitution</td>
<td>Steingrimsson et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitf&lt;sup&gt;mi-wb&lt;/sup&gt; microphthalmia white</td>
<td>T to A transition at bp 764, which leads to an isoleucine to asparagine substitution</td>
<td>Steingrimsson et al., 1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rho&lt;sup&gt;mi-hom&lt;/sup&gt;</td>
<td>Knockout, a PGK-neo cassette was inserted into the first coding exon</td>
<td>Lem et al., 1999</td>
</tr>
<tr>
<td>Rho rhodopsin</td>
<td>6 (51.5)</td>
<td>Rho&lt;sup&gt;mi-tmm&lt;/sup&gt;</td>
<td>Knockout, a neomycin cassette under the control of a polymerase II promoter was inserted at exon 135 in exon 2</td>
<td>Humphries et al., 1997</td>
</tr>
<tr>
<td>Crx cone-rod homeobox containing gene</td>
<td>7 (8.5)</td>
<td>Crx&lt;sup&gt;n1Clc&lt;/sup&gt;</td>
<td>Knockout, the homeodomain coding region containing exon 3 and a portion of exon 4 was replaced by a neomycin selection cassette</td>
<td>Furukawa et al., 1999</td>
</tr>
<tr>
<td>Tub tubby candidate gene</td>
<td>7 (51.4)</td>
<td>Tub&lt;sup&gt;nr-nd5&lt;/sup&gt;</td>
<td>Knockout, a neomycin cassette replaced 16 kb of sequence spanning exons 1–8</td>
<td>Noben-Trauth et al., 1996</td>
</tr>
<tr>
<td>Cln8 ceroid-lipofuscinosis, neuronal 8 nr nervous Bbs2 Bardet-Biedl syndrome 2 homolog (human)</td>
<td>8 (6.0)</td>
<td>Cln8&lt;sup&gt;mdn&lt;/sup&gt; (motor neuron degeneration)</td>
<td>A single nucleotide insertion (267-268C, codon 90) predicts a frameshift and a truncated protein</td>
<td>Ranta et al., 1999</td>
</tr>
<tr>
<td></td>
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<td>Cln8&lt;sup&gt;mdn&lt;/sup&gt; (motor neuron degeneration)</td>
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<td>Ranta et al., 1999</td>
</tr>
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<td></td>
<td></td>
<td>Bbs2&lt;sup&gt;nr-1Vc&lt;/sup&gt;</td>
<td>Knockout, exons 5–13 were replaced with a neo cassette</td>
<td>De Jager et al., 1996</td>
</tr>
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<td></td>
<td></td>
<td>Mfrp&lt;sup&gt;nt&lt;/sup&gt;</td>
<td>4 bp deletion in the splice donor sequence of intron 4 - skipping of exon 4 (no frame shift)</td>
<td>Kameya et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mfrp&lt;sup&gt;nt&lt;/sup&gt;</td>
<td>4 bp deletion in the splice donor sequence of intron 4 - skipping of exon 4 (no frame shift)</td>
<td>Kameya et al., 2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bbs4&lt;sup&gt;Glt1Nk&lt;/sup&gt;</td>
<td>A gene trap vector was inserted into intron 1, causing aberrant splicing</td>
<td>Kulaga et al., 2004</td>
</tr>
<tr>
<td>Bbs4 Bardet-Biedl syndrome 4 homolog (human)</td>
<td>9 (25.5)</td>
<td>Bbs4&lt;sup&gt;n1Vc&lt;/sup&gt;</td>
<td>Exons 6–11 were replaced with a neo cassette</td>
<td>Mykytyn et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bbs4&lt;sup&gt;n1Vc&lt;/sup&gt;</td>
<td>Exons 6–11 were replaced with a neo cassette</td>
<td>Mykytyn et al., 2004</td>
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(continued on next page)
the β-subunit of Pde, allows the genotyping by PCR analysis (Fig. 2a). Due to the early degeneration of the outer retina and the complete loss of rods by 35 days (Fig. 2c), mice homozygous for the Pde6brd1 allele completely lack an ERG response (Fig. 2b). Moreover, vessel attenuation and pigment patches can be seen in the fundus. This mouse mutant became the most extensively studied model for human autosomal recessive retinitis pigmentosa (RP). Several common mouse inbred strains, like C3H, SWR and FVB, are homozygous carriers of the Pde6brd1 allele (Jax Notes, 2002). Recently, the presence of the Pde6brd1 allele also was reported in a colony of 129Sv mice (Dalke et al., 2004).

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Nr2e3</td>
<td>9 (33.5)</td>
<td>Nr2e3&lt;sup&gt;rd7&lt;/sup&gt;</td>
<td>Deletion of 380 bp (exon 4 and 5) - frame shift resulting in a premature stop codon</td>
<td>Akhmedov et al., 2000</td>
</tr>
<tr>
<td>Cltn6</td>
<td>9 (35.0)</td>
<td>Cltn&lt;sup&gt;ndf&lt;/sup&gt; (neural ceroid lipofuscinosis)</td>
<td>1 bp insertion of a cysteine, located within a run of cysteines in exon 4, producing a frameshift at amino acid 103, followed by a premature stop codon</td>
<td>Wheeler et al., 2002; Gao et al., 2002</td>
</tr>
<tr>
<td>Elo14</td>
<td>9 syntenic</td>
<td></td>
<td>Transgene, 5-bp deletion corresponding to the human mutation (delAACTT at 790–794)</td>
<td>Karan et al., 2005</td>
</tr>
<tr>
<td>Pde6g</td>
<td>11 (75.0)</td>
<td>Pde6&lt;sup&gt;rd1&lt;/sup&gt;</td>
<td>Knockout, a neomycin selection cassette replaced genomic sequences including the third exon</td>
<td>Tsang et al., 1996</td>
</tr>
<tr>
<td>Aipl1</td>
<td>11 syntenic</td>
<td>Aipl&lt;sup&gt;rd1&lt;/sup&gt;</td>
<td>Knockout, a neo replaced exons 1 and 2</td>
<td>Dyer et al., 2004</td>
</tr>
<tr>
<td>Chx10</td>
<td>12 (38.0)</td>
<td>Chx10&lt;sup&gt;rd1&lt;/sup&gt;</td>
<td>Knockout, exons 2–5 were replaced with a neomycin resistance gene</td>
<td>Ramamurthy et al., 2004</td>
</tr>
<tr>
<td>Agtbp1</td>
<td>13 (37.5)</td>
<td>Agtbp1&lt;sup&gt;rd1&lt;/sup&gt;</td>
<td>Premature stop codon (Y176stop) mutation is likely in a regulatory region of the gene</td>
<td>Theiler et al., 1976</td>
</tr>
<tr>
<td>Nrl</td>
<td>14 (19.5)</td>
<td>Nrl&lt;sup&gt;rd1&lt;/sup&gt;</td>
<td>Knockout, a PKG-neomycin resistance cassette replaced the entire coding region (exons 2 and 3)</td>
<td>Swain et al., 2001</td>
</tr>
<tr>
<td>Rpgrip1</td>
<td>14 syntenic</td>
<td>Rpgrip1&lt;sup&gt;rd1&lt;/sup&gt;</td>
<td>Knockout, the gene was disrupted by insertion of a large cassette containing 3 duplicated exons and a neomycin resistance gene. Insertion of ~10kb, disrupting the coding sequence in exon 2</td>
<td>Zhao et al., 2003</td>
</tr>
<tr>
<td>Rds</td>
<td>17 (18.8)</td>
<td>Rds&lt;sup&gt;rd1&lt;/sup&gt;</td>
<td>Premature stop codon (Y176stop) mutation is likely in a regulatory region of the gene</td>
<td>Swain et al., 2001</td>
</tr>
<tr>
<td>Ndpf</td>
<td>X (5.3)</td>
<td>Ndpf&lt;sup&gt;rd1&lt;/sup&gt;</td>
<td>Knockout, the coding portion of exon 2 was replaced with a neomycin cassette</td>
<td>McNally et al., 2002</td>
</tr>
<tr>
<td>Rs1h</td>
<td>X (70.0)</td>
<td>Rs1h&lt;sup&gt;rd1&lt;/sup&gt;</td>
<td>1 bp deletion at codon 307</td>
<td>Berger et al., 1996</td>
</tr>
<tr>
<td>Rpgr</td>
<td>X syntenic</td>
<td>Rpgr&lt;sup&gt;rd1&lt;/sup&gt;</td>
<td>Knockout, exon 3 was disrupted by insertion of a lacZ-neo cassette via homologous recombination. Knockout, a neo replaced exon 1, including 9 bp upstream of the ATG. Part of exon 4 through part of exon 6 was replaced with a promoter-less lacZ-neo cassette via homologous recombination.</td>
<td>Weber et al., 2002; Zeng et al., 2004; Hong et al., 2000</td>
</tr>
</tbody>
</table>
Analysis of differentially expressed genes in the Pde6b<sup>rd1</sup> mouse results in diverse patterns during rod and cone degeneration, depending on the age (Hackam et al., 2004). So far, ten phenotypic alleles of the Pde6b gene were discovered, eight of them in ENU screens. All mutants show a recessive inheritance, but in contrast to Pde6b<sup>rd1</sup>, a slower onset of retinal degeneration was observed in three Pde6b mutants (Thaung et al., 2002; Chang et al., 2002).

Fig. 1. Overview of genes involved in retinal disorders. Some mutations are mentioned in the text; for others additional information can be found in previously published reviews (Hafezi et al., 2000; Chang et al., 2002; Peachey and Ball, 2003) or in the MGI database (Mouse Genome Informatics, http://www.informatics.jax.org).

Fig. 2. C3H mice: well-known carrier strain of the Pde6b<sup>rd1</sup> allele. (A) PCR analysis for Pde6b<sup>rd1</sup> (modified according to Gimenez and Montoliu, 2001). Lanes 1–3 controls of a C3H, C57BL/6 and a SWR mouse, lanes 4–9 DNA of different mice to be tested for the Pde6b<sup>rd1</sup> allele. (B) A typical ERG response of a C3H mouse. (C). Histology of the retina of a C3H mouse. The arrow indicates the missing retinal layers, especially the photoreceptor cells. OFL, outer fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; RPE, retinal pigment epithelium.
The knockout of the \(\gamma\)-subunit of Pde (\(Pde6g^{-/-}\)) leads to rapid photoreceptor degeneration and reduced ERG amplitudes. However, this disorder cannot be rescued by the expression of a transgene containing a mutant \(Pde6g\) gene (Tsang et al., 2002), although in humans no \(PDE6G\) mutations are known to be associated with retinal disorders.

Mutations in the rhodopsin (\(RHO\)) gene account for approximately 15% of all inherited retinal degenerations in humans. \(Rho\) knockout mice do not develop rod outer segments and lose their photoreceptor cells within 3 months. At the age of 8 weeks, the visual function in \(Rho\) knockout mice is mediated by cones only. However, not only the loss-of-function mutation lead to retinal degeneration, but also the additional activity of \(Rho\). Transgenic mice expressing different levels of opsin were generated to analyze the consequences of opsin overexpression. Even an opsin expression level of 123% of normal leads to a reduction of a-wave amplitudes in ERG to less than 30% of normal values, and ERG responses of the highest expressing line (222% of normal opsin level) were indistinguishable from noise. The overexpression of normal opsin induces photoreceptor degeneration that is similar to that seen in many mouse models of RP (Tan et al., 2001).

\(NRL\), a basic neural retina motif-leucine zipper transcription factor of the \(Maf\) subfamily, synergistically interacts with the homeodomain protein CRX to regulate rhodopsin transcription. Missense mutations in the \(NRL\) gene cause RP in humans, a similar phenotype is observed in \(Nrl^{-/-}\) mice, exhibiting the complete loss of rod function and super-normal cone function, mediated by S-cones (short-wavelength sensitive). Functional transformation of rods into S-cones was demonstrated in the \(Nrl^{-/-}\) retina, suggesting that \(Nrl\) acts as ‘molecular switch’ during rod cell development (Mears et al., 2001).

A late onset of photoreceptor cell degeneration is observed in mice with a mutant \(Nr2e3\) gene, a member of the nuclear receptor transcription factor family. After one year of relatively stable a- and b-wave ERG responses a progressive loss of their amplitudes follows. The retinal fundus of one month old \(Nr2e3\) mutant mice displays discrete white spots due to retinal folds, whorls and rosettes in the outer nuclear layer. However, a higher quantity of cone photoreceptors leads to the suggestion that \(Nr2e3\) mice could be a model for the human enhanced S-cone syndrome (ESCS), but the obtained ERG results are quite different. Recent results demonstrate that \(Nr2e3\) is involved in regulating the expression of rod photoreceptor-specific genes (Cheng et al., 2004).

1.1.2. Models for a combined phenotype of retinitis pigmentosa and other photoreceptor degeneration diseases

Two mouse models have been reported with mutation in the \(Crb1\) gene coding for the crumbs homolog 1. The human patient mutations in the \(CRB1\) gene lead to various forms of hereditable retinal disorders like retinitis pigmentosa (RP) and Leber’s congenital amaurosis (LCA). Similarly, the spontaneous mutant (\(Crb1^{rd8}\)) shows irregular white spots from the age of 3 weeks, which are caused by retinal folds and pseudorosettes. Shortened photoreceptor inner and outer segments are observed as early as 2 weeks after birth, suggesting a developmental defect. Interestingly, in \(Crb1^{rd8}\) mutants, retinal dysplasia and spotting strongly varies with genetic background (Mehalow et al., 2003). The recently described \(Crb^{-/-}\) knockout mice initially develop normal retinas, but by 3 to 9 months the integrity of the outer limiting membrane is lost and giant half rosettes are formed (van de Pavert et al., 2004).

Defects in genes coding for retinitis pigmentosa GTPase regulator (\(Rpgr\)) and RPGR-interacting protein (\(Rpgrip1\)) are known to be involved in the development of RP and LCA, respectively. Both gene products are localized in the photoreceptor connecting cilium, the connection between cell body and the light-sensing outer segment. \(Rpgrip1^{/-}\) mice have grossly oversized outer segment disks and show a more severe disease than \(Rpgr^{-/-}\) mice (Zhao et al., 2003; Hong et al., 2004).

Mutations in the \(Rds\) gene (retinal degeneration slow) coding for the photoreceptor-specific membrane glycoprotein (peripherin) are associated with multiple retinal diseases like macular dystrophy and autosomal dominant RP. Peripherin is essential for outer segment disc morphogenesis. Mice homozygous for the spontaneous mutation \(Rds^{Prph2-rd2}\) do not develop outer segments of rods and cones, and at the age of 12 months all photoreceptor cells have disappeared. At first, the mutation was considered to be recessive, but progressive abnormalities were also observed in heterozygotes. Another \(Rds\) allele (\(Rds^{sm1Nmc}\)) with a targeted single base deletion at codon 307 is identical to a human mutation associated with an autosomal dominant form of RP. The \(Rds^{sm1Nmc}\) mutation leads to a more rapid development of the retinopathy than observed in the naturally occurring null mutant, suggesting a dominant negative phenotype in combination with haploinsufficiency (McNally et al., 2002). A positive correlation was observed between \(Rds\) expression levels and the structural and functional integrity of photoreceptor outer segments, about 60% of wild type \(Rds\) is nessecary for the functional integrity of the retina; whereas overexpression of \(Rds\) caused no detectable adverse effects on rod or cone structure and function (Nour et al., 2004).

1.1.3. Mouse mutants as models for macular degeneration

Macular degeneration is a heterogeneous group of human disorders characterized by photoreceptor degeneration and atrophy of the retinal pigment epithelium in the central retina. An example of a congenital macular degeneration is the Stargardt’s macular dystrophy (STGD). One gene found to be mutated in patients suffering from STGD is the \(ABCA4\) gene, coding for an ATP-binding cassette transporter, which is expressed in the rim of rod outer segment disks. Mice lacking both alleles of the \(Abca4\) gene accumulate toxic lipofuscin pigments in ocular tissues, similar to affected
humans, and showed delayed dark adaptation. The major fluorophore of lipofuscin is the bis-retinoid, N-retinylidene-N-retinylethanolamine (A2E), which was thought to increase following light exposure. Nevertheless, increased retinal illuminance was not correlated with higher A2E levels in the mouse model (Radu et al., 2004). However, Abca4 knockout mice develop progressive photoreceptor degeneration and an enhanced delay in dark adaptation with increasing age.

A dominant form of human congenital STGD is caused by mutations in the ELOVL4 gene (elongation of very long chain fatty acids-like 4). Transgenic mice expressing a mutant form of human ELOVL4 show photoreceptor degeneration in the central retina in a pattern closely resembling that of human STGD and AMD (age-related macular degeneration), therefore these mice provide a good model for both disorders (Karan et al., 2005).

A common form of macular degeneration in males is X-linked juvenile retinoschisis (RS) caused by mutations in the RS1 gene. For a corresponding mouse model Rs1h deficient mice were generated; Rs1h is the homolog to the human RS1 gene. The pathologic changes in hemizygous mice are evenly distributed across the retina, contrasting with the macular-dominated features in humans. However, similar functional anomalies were observed in man and mouse, suggesting that both conditions are a disease of the entire retina, which affects the organization of the retinal cell layers as well as structural properties of the retinal synapse (Weber et al., 2002).

1.1.4. Models for Leber’s congenital amaurosis (LCA)

LCA describes a group of autosomal recessive blinding retinal dystrophies in early childhood. Beside Crb1, Rprgr and Rpgrip1 (described above), the conductive gene for this disorder is RPE65 gene (retinal pigment epithelium 65). Corresponding knockout mice (Rpe65−/−) show disorganized outer segment discs of their rod photoreceptors. Residual ERG responses of Rpe65−/− mice were attributed to rods, enabled to mimic cone function by responding under normally cone-isolating lighting conditions (Seeliger et al., 2001). Studies with Rpe65+/− mice clearly showed that activation of sensory transduction by unliganded opsins induce light-independent retinal degeneration in LCA; the accumulation of retinal esters is not causative (Woodruff et al., 2003). To quantify the impairment of the transient pupillary light reflex due to severe retinal dysfunction and degeneration in a murine model and in human patients with LCA, pupillometry was used (Aleman et al., 2004).

Additionally, a new, spontaneous Rpe65 mutant mouse (Rpe65el12) was detected. Homozygous Rpe65el12 mice exhibit small punctate white spots in the fundus at the age of 5 months. The first signs of retinal degeneration were seen at the age of 3 weeks, when occasional small lipid-like droplets were detected in the retinal pigment epithelium (RPE). At the age of 3 months voids were detected in the outer nuclear layer. The ERG phenotype is similar to that reported in Rpe65−/− mice in which ERG responses were profoundly diminished to all but the brightest stimuli (Pang et al., 2005).

Mutations in the AIPL1 gene, coding for aryl hydrocarbon receptor-interacting protein-like 1, in humans lead to LCA. Several knockout mice were generated resulting in a total loss of the protein. Therefore, similar phenotypes were reported. Rapid retinal degeneration and lack of both rod and cone ERG responses were observed in Aipl1−/− mice. However, no gross abnormalities of proliferation during retinal development were detected (Ramamurthy et al., 2004; Dyer et al., 2004).

Another model for LCA was recently created in which the cone-rod homeobox containing gene (Crx) was disrupted using homologous recombination. Crx−/− mice display abnormal development of photoreceptors followed by their degeneration (Pignatelli et al., 2004). Further, Crx−/− outer segment morphogenesis was found to be blocked in development, leading to a failure in the production of the phototransduction apparatus. Highly disorganized synapses of photoreceptors were observed in the outer plexiform layer (Morrow et al., 2005).

1.1.5. Other retinopathies

For some genes an expression in the retina is known. However, if no human mutation is described, frequently a corresponding mouse model is generated by gene targeting approaches.

The Chx10 homeobox gene (C. elegans ceh-10 homeo domain containing homolog) is expressed in neural progenitor cells during retinal development. The absence of Chx10 in spontaneously occurring mutant mice causes mutant ocular retardation and microphthalmia. In Chx10−/− mutants rod and cone outer segments are not correctly formed (Rutherford et al., 2004), additionally retinal cells transdifferentiate into pigmented cells (Rowan et al., 2004).

This deflection of the neuroretina towards an RPE-like identity is caused by the ectopic expression of Mitf in Chx10−/− mice, which is repressed by Chx10 in wild-type mice. The antagonistic interaction between Chx10 and Mitf in regulating retinal cell identity was confirmed, using Chx10 and Mitf transgenic and mutant mice (Horsford et al., 2005).

The Vsx1, visual system homeobox 1 gene, is expressed in differentiating and mature cone bipolar cells in mice. Vsx1−/− deficient mice showed altered ERG responses, demonstrating defects in their cone visual pathway, whereas the rod visual pathway was unaffected. A disturbed differentiation of cone bipolar cells was assumed in these mice (Chow et al., 2004; Ohtoshi et al., 2004).

Retinal degeneration frequently is associated with alterations in the fundus, reflecting structural aberrations of the retinal cell layers. In spontaneous Mfrp mutant mice, which represent a model for human retinitis punctata albescens, the appearance of white spots in the retinal fundus, at the age of 8–10 weeks, corresponds to large cells, juxtaposed to the retinal pigment epithelium and a progressive photoreceptor cell loss. Beginning even at
the age of one month, ERG responses show a slow progressive retinal dysfunction of both rods and cones; ERG is extinguished at the age of 16 months (Hawes et al., 2000). Mfrp, coding for a membrane-type frizzled-related protein, was found to be expressed specifically in the retinal pigment epithelium and ciliary epithelium of the eye (Kameya et al., 2002).

Cnga3 (cyclic nucleotide gated channel alpha 3) knockout mice lack any cone-mediated photoreponse, but have a completely intact rod pathway (Biel et al., 1999). To assess the structural changes in the retina caused by functional block of rods and cones, double knockout mice were bred with Cnga3<sup>−/−</sup> and Rho<sup>−/−</sup> mice. Retinal layers of these double knockouts showed normal structural organization until the age of 4 weeks, but photoreceptors are almost completely lost at 3 months (Claes et al., 2004).

1.2. Combined retinal and neuronal degeneration phenotype

In some human diseases and the corresponding mouse models a combined phenotype of retinal and neuronal degenerations is observed.

Retinal degeneration combined with the loss of cerebellar Purkinje cells is observed in both, nervous (nr) and Agtphp1 mutant mice. The recessive nervous (nr) mutation is associated with hyperactivity at the age of 3 weeks, retinal disorganization of the outer segment membranes and thinning of the inner nuclear and plexiform layers. In Agtphp1 (ATP/GTP binding protein 1) mutant retinae, the outer nuclear layer and the mitral neurons of the olfactory bulb were degenerated. The extensive loss of photoreceptor cells starts around weaning and proceeds slowly for about one year. Two additional spontaneous Agtphp1 alleles (pcd<sup>A</sup> and pcd<sup>B</sup>) were detected, but no major differences in the retinal degeneration phenotype were reported (Fernandez – Gonzalez et al., 2002).

Two spontaneous mouse mutants for neuronal ceroid lipofuscinosis, Cln6 and Cln8, are associated with progressive retinal degeneration, ataxia and neurodegeneration. Mutations in the human orthologues have been shown to underlie neurological syndromes including retinal disorders, seizures and mental retardation. Homozygous mice of both lines die, at around 9 months of age. Progressive photoreceptor cell loss starts early in life and by 6 to 9 months the entire retina is atrophied (Bronson et al., 1998). In Cln8 mice, oxidation and apoptotic processes are involved in the retinopathy (Guarneri et al., 2004). Both genes, Cln6 and Cln8, are active in similar biochemical pathways; therefore, it is not surprising that mutation in both genes lead to very similar phenotypes.

1.3. Retinal degeneration as part of a multisyndromic defect

The Bardet-Biedl syndrome (BBS) is characterized by RP as a major defect, but obesity, polydactyly, renal and cardiac malformations, learning disabilities, and hypogenitalism have been also reported in the affected patients. Two knockout mouse models, lacking expression of Bbs2 or Bbs4, respectively, display major components of the human phenotype including retinal degeneration and other defects associated with cilia dysfunction. Retinal degeneration in Bbs2<sup>−/−</sup> mice starts, after normal retinal development, by apoptotic death of photoreceptors, the primary ciliated cells of the retina. Molecularily, photoreceptor cell death is preceded by mislocalization of rhodopsin, indicating a defect in transport (Mykytyn et al., 2004; Nishimura et al., 2004).

Another model for BBS, the Tubby mouse (Tub; Noben-Trauth et al., 1996), exhibit retinal disorders and progressive hearing loss, combined with obesity. The early onset of photoreceptor cell loss leads to reduced ERG amplitudes, which extinguish by 6 months of age. Mutations in other members of the Tub gene family, the tubby-like proteins (Tulp1, 2, 3, 4), are also involved in retinal degeneration, but with distinct phenotypes (Ikeda et al., 2000).

Multiple phenotypes are associated also with mutations in the Mitf gene, a microphthalmia associated transcription factor. Common to the allelic series of up to now 26 different mutations are defects in neural-crest-derived melanocytes, resulting in reduction or loss of pigmentation in the eye, inner ear, skin and coat. In addition to the reduced eye size, early-onset deafness and osteoporosis are observed. The allelic nature of some phenotypes was confirmed by complementation tests of certain Mitf alleles, e.g. Mitf<sup>Mic-wh</sup>/Mitf<sup>mut</sup> animals display normal eye size while homozygous littermates exhibit severe (Mitf<sup>Mic-wh</sup>/Mitf<sup>Mic-wh</sup>) or intermediate (Mitf<sup>mut</sup>/Mitf<sup>mut</sup>) microphthalmia (Steingrimsson et al., 2003). Mice homozygous for the vitiligo mutation (Mitf<sup>pos-vit</sup>) show gradual depigmentation and progressive loss of photoreceptor cells. The outer plexiform layer is significantly thinner at the age of 4 months, and by 8 months, photoreceptor cell nuclei have diminished to 2 or 3 rows (Smith, 1995).

A syndrome including congenital blindness, sensori-neural deafness and mental retardation is displayed in the X-linked Norrie disease. Hemizygous Ndph (Norrie disease homolog) knockout mice develop retrolental structures in the vitreous body and an overall disorganization of the retinal ganglion cell layer, with focal absence of the outer plexiform layer. These ocular findings are consistent with observations in human patients (Berger et al., 1996). Mice deficient in norrin, the gene product of Ndph, additionally show a distinct failure in retinal angiogenesis, and completely lack the deep capillay layers of the retina. Ohllmann et al. (2005) showed that the transgenic expression of ectopic norrin restores the formation of a normal retinal vasular network in Ndph (y/-) mutant mice, suggesting that pharmacologic modulation of norrin might be used for treatment of Norrie disease.
2. Conclusions and perspectives

At present, 158 genes are known to be involved in the formation of retinal diseases in humans (RetNet, http://www.sph.uth.tmc.edu/Retnet), but mouse models are available for only a few of them. Therefore, more mouse models are needed to cover the diversity of the clinical situations. Studies can be improved using knockout and transgene techniques. Moreover screening efforts in ENU mutagenesis should be intensified because the phenotyping in these screens is done according to clinical features. A system for inducible photoreceptor-specific gene expression in transgenic mice was developed to control gene expression at the temporal and spatial level to check for a reversal point (Chang et al., 2000; Angeletti et al., 2003). A rapid method for functional studies is the transfection of RNA interference vectors into retinal cells leading to similar phenotypes known from corresponding knockout mice (Chang et al., 2000; Angeletti et al., 2003). A rapid method for functional studies is the transfection of RNA interference vectors into retinal cells leading to similar phenotypes known from corresponding knockout mice (Matsuda and Cepko, 2004), but the medical relevance seems to be unclear.

One of the major challenges is to develop new therapies for retinal disorders. The benefit of mouse models was shown recently as treatment with bone marrow-derived hematopoietic stem cells prevents cone loss in rd1 and rd10 mice (Smith, 2004). Moreover, some other successful approaches in retinal gene therapies were reported. In a model of LCA the vision of blind Rpe65<sup>−/−</sup> mice was restored (Jacobson et al., 2005), and in rs1h deficient mice, a model for retinoschisis, significant improvement in the ERGs, retinal morphology and preservation of photoreceptors was demonstrated after subretinal injection of an adeno-associated virus construct containing human R<sub>SI</sub> gene (Weber et al., 2005). Mouse models are one of the few test systems to develop new therapies for human patients.

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