

Mechanisms of Apoptosis in Retinitis Pigmentosa

Sandra Cottet^{*1,2} and Daniel F. Schorderet^{1,2,3}

¹*IRO, Institute for Research in Ophthalmology, Sion, Switzerland;* ²*Department of Ophthalmology, University of Lausanne;* ³*Ecole Polytechnique Fédérale of Lausanne (EPFL), Lausanne, Switzerland*

Abstract: Mutations in humans are associated with several forms of inherited retinal dystrophies, such as Retinitis Pigmentosa which lead to retinal cell death and irreversible loss of vision. Genes involved in affected patients mainly encode proteins related to vision physiology including visual cycle and light-dependent phototransduction cascade. As reported in spontaneous and genetically engineered mouse models, apoptosis is a common fate in retinal degeneration, although the triggered signals to retinal apoptosis remain largely unraveled. Several studies highlighted that many of the molecular pathways involved in ocular diseases rely on caspase-dependent or -independent apoptotic mitochondrial pathway involving the Bcl-2 family of proteins. Anti- and pro-apoptotic Bcl-2 members are present in retinal tissues and are thought to play a role in the pathogenesis of several retinal disorders. Since almost no efficient treatments are available so far, it remains a great challenge to decipher the molecular pathways involved in retinal dystrophies and to develop alternative therapies to prevent or inhibit eye defect. Toward this goal, mutation-independent strategies such as molecular therapy provides promising and exciting approaches to deliver anti-apoptotic molecules targeting the Bcl-2 pathway through the use of cell permeable transport peptides. Modulation of common apoptotic signaling pathways may be of outstanding potential to target multiple retinal dystrophies regardless of the primary genetic defect.

Keywords: Retinal disease, retinitis pigmentosa, apoptosis, Bcl-2, Bax, therapy.

1. RETINAL DEGENERATIVE DISEASES

1.1. Retinitis Pigmentosa (RP)

Retinal dystrophies encompass a heterogeneous group of inherited conditions, with more than 100 genes or loci implicated so far. Retinitis Pigmentosa (RP) is characteristic of mutations leading to progressive death of retinal rod and cone photoreceptors with subsequent loss of vision. Typical symptoms include night blindness and decrease in visual field leading to tunnel vision and eventually complete blindness. Retinal degeneration starts during childhood or in the early teenage followed by severe visual defects by ages 40 to 50 years. Leber's Congenital Amaurosis (LCA) is a rare, although important, juvenile retinal dystrophy characterized by the appearance of severe visual defect early in life, within the first two years after birth. The pathological outcome, onset and progression of the disease, are subjected to considerable variations and range from complete congenital blindness to partially impaired vision [1]. Prevalence of all RP diseases is around 1 per 4000 persons for a total of more than 1 million affected individuals worldwide. Among all RP cases, about 30-40% are autosomal dominant (adRP), 50-60% autosomal recessive, and 5-15% X-linked RP [2]. Molecular diagnosis progressed rapidly during the last decades, and several recent reviews reported on the genetic and biochemical features of inherited eye diseases [2-6]. By studying patients and their families, many genes were localized to a chromosomal locus by linkage analysis, whereas others were identified by direct sequencing of candidate genes. According to the

known or predicted functions of the mutated proteins, essential biochemical pathways related to vision physiology are affected in diseased retina, including visual cycle and phototransduction cascade (Fig. (1)). Following light-induced activation of chromophore (11-*cis*-retinal)-bound rhodopsin, activated rhodopsin (RHO) triggers activation of transducin (T), a heterotrimeric G-protein constituted of α , β and γ subunits. T α activates cyclic GMP (cGMP) phosphodiesterase (PDE) which, in turn, promotes the hydrolysis of cGMP causing the closure of cGMP-gated cation channel (CNG) of the photoreceptor plasma membrane, the decrease in intracellular Ca²⁺ level in outer segments (OS), and the hyperpolarization of rod plasma membrane. Inactivation of the phototransduction cascade is mediated by rhodopsin kinase (RK)-dependent phosphorylation (P) of bleached rhodopsin, followed by specific binding of phosphorylated rhodopsin by arrestin (A). After dissociation of rhodopsin into opsin and all-*trans*-retinal (AT-RAL), the latter is reduced to all-*trans*-retinol (AT-ROL) by retinol dehydrogenase (RDH) enzymes and transported to the retinal pigment epithelium (RPE) to be regenerated through the visual cycle. Within the RPE, AT-ROL is esterified to all-*trans*-retinyl ester (AT-RE) by lecithin:retinol acyl transferase (LRAT) and subsequently used as substrate by the RPE-specific 65 kDa isomerohydrolase (RPE65) to form 11-*cis*-retinol (11CIS-ROL). Following oxidation by several RDHs, 11-*cis*-retinal (11CIS-RAL) is released by the RPE and the regenerated chromophore recombines with opsin to form rhodopsin in the photoreceptor (PR).

Phototransduction-related genes implicated in RP include phosphodiesterase catalytic subunits α (*PDE6A*) [7] and β (*PDE6B*) [8-10], rod cGMP-gated channel (*CNGA1*) [11], retinal guanylate cyclase (*RE-TGC1*) [12], arrestin (*SAG*) [13] and rhodopsin (*RHO*)

*Address correspondence to this author at the IRO, Institute for Research in Ophthalmology, Avenue Grand-Champsec 64; 1950 Sion, Switzerland; Fax: +41 27 205 79 01; E-mail: sandra.cottet@irovision.ch

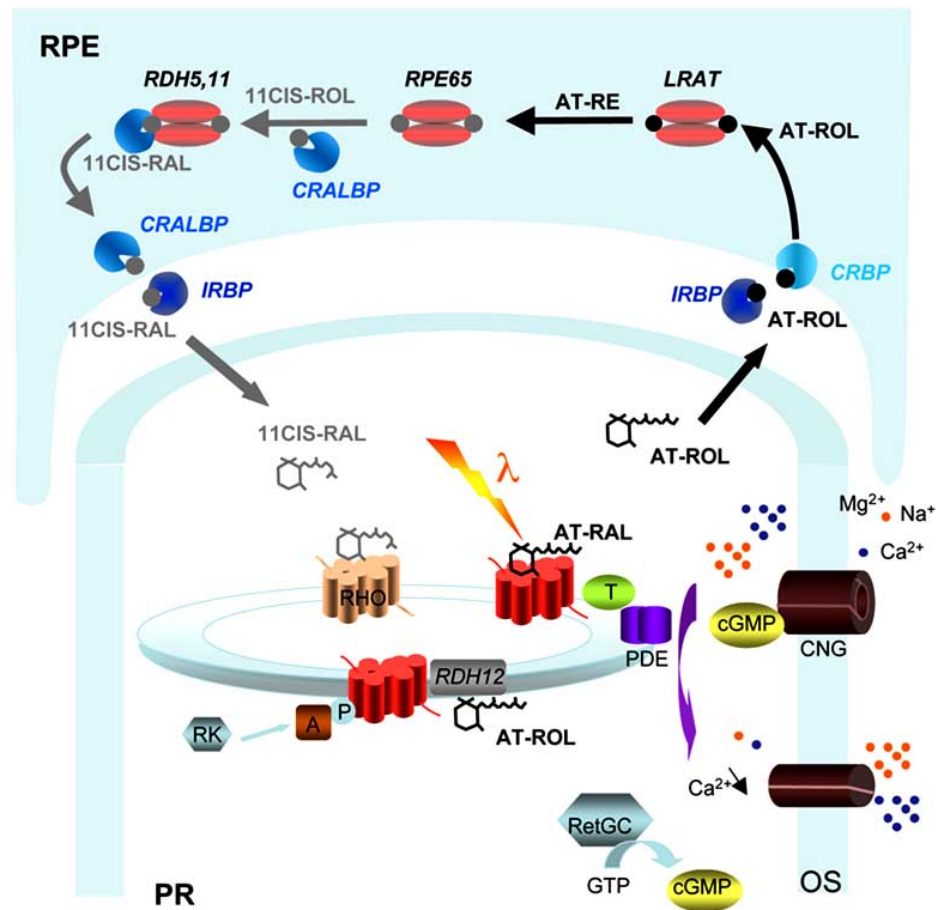


Fig. (1). Visual cycle and phototransduction in the retina.

In photoreceptor (PR), phototransduction cascade starts with the absorption of light, which causes photoisomerization of rhodopsin (RHO)-bound chromophore 11-*cis*-retinal (11CIS-RAL) to all-*trans*-retinal (AT-RAL) and conformational changes of the visual pigment. This permits the binding and activation of the G-protein transducin (T), which in turn activates the enzyme cGMP phosphodiesterase (PDE) and the hydrolysis of cGMP. The resulting decrease in cGMP levels and the closure of cGMP-gated cation channels (CNG) on the plasma membrane of the rod outer segment (OS) lead to fall in intracellular Ca^{2+} level and hyperpolarization of the rod cell plasma membrane. Termination of the photoresponse requires inactivation of photoactivated rhodopsin by rhodopsin kinase (RK) and arrestin (A), as well as stimulation of retina-specific guanylate cyclase (RetGC) to reverse intracellular cGMP and Ca^{2+} levels. Following dissociation of rhodopsin into opsin and AT-RAL, the isomerized chromophore is reduced to all-*trans*-retinol (AT-ROL) by retinol dehydrogenase (RDH) enzymes, such as RDH12, and taken up by interphotoreceptor retinoid-binding protein (IRBP) to be regenerated through the visual cycle within the retinal pigment epithelium (RPE). Following transport by another retinoid binding protein, cellular retinoid-binding protein (CRBP), AT-ROL is esterified to all-*trans*-retinyl ester (AT-RE) by lecithin:retinol acyl transferase (LRAT) and subsequently used as substrate by the RPE-specific 65 kDa isomerohydrolase (RPE65) to form 11-*cis*-retinol (11CIS-ROL). Before to be released from RPE, 11CIS-ROL binds to cellular retinaldehyde-binding protein (CRALBP) to be oxidized to 11CIS-RAL by several RDHs, including RDH5 and RDH11. Upon release by the RPE, the regenerated chromophore recombines with opsin to form rhodopsin in the PR.

[14]. Mutations in rhodopsin are the most common cause of autosomal dominant (ad) RP and, to date, more than 120 mutations have been described in affected patients. Mutations in peripherin/*rds* gene (*RDS*), a structural component of the OS disks of the photoreceptors, are also responsible for RP [15]. Genes involved in visual cycle (*RPE65*, *LRAT*) have been identified, that are thought to account for LCA. Among the different genes identified, mutations in *RPE65* seem to be the most frequent in this disease [16, 17]. Other more basic functions related to retina-specific transcription factors, cytoskeleton organization, intracellular trafficking, synaptic interaction and mRNA processing are

also targets of disease-causing mutations (reviewed in [4-6]). Additional candidate genes may be those involved in retinal disease or known to cause retinal degeneration in animal models. 181 genes causing inherited retinal dystrophies have been mapped to a specific chromosomal site and the sequence of 129 of them has been identified (see Retina International <http://www.retina-international.org/sci-news/longa.htm> and RetNet www.sph.uth.tmc.edu/RetNet). To date, mutations in 17 genes have been reported to cause adRP while mutations in 25 genes are responsible for recessive RP [18]. Thanks to the significant progress in molecular genetics coupled to efficient clinical diagno-

sis, it is nowadays possible to detect mutations in 56% of patients with adRP and 30% of patients with recessive RP. However, even if this allows for the detection of mutations in 25% to 90% of patients with inherited retinopathies, more disease-causing genes are yet to be found. Mutation analysis in candidate genes is one of the most active areas of research in this domain, along with new and powerful screening methods such as genotyping microarray.

1.2. RP Murine Models

Identification of inherited mutations in murine models and development of gene-deficient mice proved to be of outstanding importance to study hereditary eye diseases at the cellular and molecular levels. It is noteworthy that many of the genes genetically engineered or found to be mutated in animals closely mimic the human pathology, showing retinal degeneration and similar pathophysiological defects. Mutations in β subunit of rod *Pde* cause retinal degeneration in the naturally occurring retinal degeneration (rd) mouse, a fast degenerating model of RP with death of all photoreceptors by 4 weeks of age [19, 20]. Degeneration is correlated with deficient PDE enzymatic activity, associated with accumulation of cGMP and increased intracellular Ca^{2+} level within photoreceptor OS [21]. Mutations in the gene coding for *Peripherin/Rds* are responsible for retinal cell death in the retinal degeneration slow (rds) mouse, another widely used natural RP model. PERIPHERIN/RDS is a membrane glycoprotein whose expression is restricted to OS disks of the photoreceptors. This structural protein is essential for morphogenesis and stabilization of the OS of cones and rods. Pathogenesis in rds mouse is characterized by the absence of OS followed by loss of cellular bodies of photoreceptors [22-24]. Rds mutant mice undergo a relatively slow degeneration, similar to many forms of human retinal degenerations. In these mice, photoreceptor cell death starts around the third postnatal week to be finished at one year of age.

The collection of spontaneous models of RP is now being completed with the increasing list of genetically modified transgenic, knock-out and knock-in mice targeting gene defects that resemble the characteristic features of human retinal dystrophies (reviewed in [25-27]). Several transgenic mouse strains reproducing human mutations found in rhodopsin gene have been generated. As reviewed by Mendes and colleagues, these mutations may affect rhodopsin posttranslational modifications, protein folding, stability and trafficking, as well as phototransduction activation [28]. Knock-out mice to study LCA have also been generated, that are considered as good models for the human disease. Deficient LCA genes in *Crx*^{-/-} [29] and *Rpe65*^{-/-} [30] mice present similar cellular and biochemical dysfunctions and progressive retinal degeneration as observed in human disease [31]. Despite this increasing number of spontaneous and genetically engineered murine models of human RP, only a few of them have been described regarding the triggered intracellular signals leading to retinal cell apoptosis. A lot of work remains

to be undertaken, beyond mutational analysis, to unravel the molecular events and signal transduction pathways involved in retinal degeneration.

2. APOPTOSIS IN RP DISEASES

2.1. Apoptotic Cell Death Pathways in RP

Apoptosis is a tightly regulated, programmed cell death process. Cells participate in their own demise, in which fine regulation between pro- and anti-apoptotic molecules shift cell fate toward survival or death. In apoptotic intracellular signaling, mitochondria are considered as the commitment step, "point of no return", triggering the release of downstream apoptogenic factors including cytochrome C, apoptosis-inducing factor (Aif), endonuclease G (EndoG) and serine protease Omi/HtrA2 [32]. Some of them act in a caspase-dependent or caspase-independent manner.

Photoreceptors are specialized cells directing energy-consuming and light-dependent phototransduction cascade essential to promote vision. This makes them particularly fragile to any long-term perturbation in either component of visual transduction and visual cycle, energy metabolism and structural integrity, and prompt to cell death. It is obvious that the functional defect and disease mechanism for each protein involved in retinal degeneration may be different, nevertheless a common fate consists in the loss of retinal cells by apoptosis. Chang and colleagues demonstrated for the first time in 1993, that heterogeneous genetic defects presented a common final death feature, namely apoptosis, as reflected by internucleosomal cleavage and fragmented retinal DNA in rd, rds and rhodopsin mutant mice [33]. To date, apoptosis commitment was reported in all types of RP analyzed, while only a limited number of molecular pathways have been described yet. On one hand, it is tempting to speculate that a limited number of initiating pre-apoptotic signaling molecules, shared by several RP genes, may converge toward common committed apoptotic pathways. On the other hand, we may imagine that the disease-causing mutations each initiate individual and unique death pathway. Identification and deciphering of the intracellular apoptotic signals appear of crucial interest to provide substantial insight into retinal cell pathogenesis and to target them to prevent retinal degeneration.

2.2. Caspase-Dependent and -Independent Apoptotic Pathways in RP

Caspase action in retinal degeneration was observed in several models of RP. Increased activity of caspase-3 was correlated with a rapid degeneration of photoreceptors in transgenic rats carrying rhodopsin mutation S334ter, which was partially rescued by administration of caspase-3 inhibitor [34]. Intravitreal injection of caspase-3 inhibitor provided a significant decrease in apoptotic photoreceptor cells in tubby mice, a model system for human Usher syndrome type 1 and autosomal recessive RP [35]. In genetically inherited rds mouse retina, caspase-1 upregulation [36], along

with caspase-3 activation [37], was associated with photoreceptor apoptosis and paralleled the observed peak of degenerating cells. Whereas retinal cell death in RP was first depicted as a caspase-dependent mechanism, it becomes now widely admitted that photoreceptor apoptosis may be governed by either caspase-dependent or -independent ways. Evidences highlighted alternative and paralleled death pathways, other than caspase activation, that may account for apoptosis occurring in murine models of retinal dystrophies.

Contradictory results regarding the role of caspase-3 in retinal cell death were observed in rd mouse. Doonan and collaborators reported caspase-independent apoptosis, that did neither require caspase-9, -8, -7, -3 and -2, nor cytochrome C release from the mitochondria [38]. This is in direct opposition with the work of Jomary *et al.* where they observed activation of caspase-8 and -3 at the peak of photoreceptor apoptosis, along with mitochondrial release of cytochrome C in diseased retina [39]. In other reports using the same RP model, only delayed photoreceptor degeneration was observed following caspase-3 inhibitor administration *in vivo* in rd mice [40], whereas the absence of caspase-3 only provided transient protection in these mice [41]. The light-induced model of retinal degeneration, in which apoptosis of photoreceptors did not involve caspases, highlighted the role of Ca^{2+} -dependent protease calpains as an alternative death pathway in retinal dystrophy [42, 43]. Activation of calpains have been further reported in apoptotic cells in RP [44-46], in retinal ischemia-reperfusion [47] and following hypoxia-induced retinal damage [48]. The role of Ca^{2+} and calpain was further investigated in mouse photoreceptor cells, in which both calpain and caspase-3 were shown to be implicated in Ca^{2+} -dependent apoptosis, suggesting a death pathway mediated by calpain activation and apoptosis execution *via* mitochondria-dependent process and caspase-3 activity [49]. Besides caspases and calpains, another family of proteases, namely the cathepsins, may be implicated in retinal degeneration. In addition to the activation of cathepsin D in the degenerating retina in rd mouse [44], cathepsin D knock-out mice showed retinal defect including shortened OS and photoreceptor degeneration, suggesting its potential role in lysosomal digestion of phagocytosed photoreceptor OS [50]. The lysosome-associated membrane protein-2 (*LAMP2*) was also reported to be associated with human retinopathy [51].

It is now evident that, according to the different RP models involving phototransduction (rd), structural (rds) or oxidative stress (light-induced) defects, photoreceptor degeneration may share a common but multiple repertoire of pro-apoptotic mechanisms leading to retinal dystrophies [36].

3. ROLE OF THE BCL-2 FAMILY OF PROTEINS IN PHOTORECEPTOR APOPTOSIS

3.1. The Bcl-2 Family of Proteins

The mitochondrial pathway of apoptosis is dependent upon the Bcl-2 proteins for the efficient release of

mitochondrial apoptogenic factors, which in turn trigger caspase-dependent or caspase-independent irreversible apoptotic cascade [32, 52] (Fig. (2A)). The proteins of the Bcl-2 family are described as key players in the execution of the apoptotic program; indeed they can either promote cell survival or cell death depending on the equilibrium between their pro- and anti-apoptotic members (reviewed in [52, 53]). The Bcl-2 family of proteins is divided into three classes, based on the presence of Bcl-2 homology (BH) domains (Fig. (2B)). Anti-apoptotic Bcl-2 proteins contain BH1 to BH4 domains and are generally present at the mitochondria and ER membranes. They participate in the apoptotic pathway by directly binding and inhibiting pro-apoptotic effector BH-multidomain proteins. The pro-apoptotic members are divided into two groups, including the effector BH-multidomain (BH1 to BH3) molecules Bax and Bak, which permeabilize the outer mitochondrial membrane and trigger irreversible commitment to apoptotic fate [54]. The third group, the pro-apoptotic BH3-only proteins, function as sensitizers of diverse upstream signals and interact, *via* the BH3 domain, with other Bcl-2 family members following stress stimuli.

3.2. Expression of Bcl-2 Proteins in Retinal Tissue

Numerous studies reported on the expression of Bcl-2 members in the retina of rodents. In the adult rat retina, anti-apoptotic proteins BCL-X_L [55] and BCL-2 [56] are expressed in nearly all retinal cell layers, in contrast to pro-death BAX found only in retinal ganglion cell layer (GCL) [56]. These data indicate that Bcl-2-mediated signaling is a default pathway that needs to be kept under fine control to allow for retinal neuron survival or differentiation. Main proteins of this pathway, including BH3-only members BAD and BID, are also expressed in murine retinal tissue [57-60]. The heterogeneous level of mRNA and protein expression of the various Bcl-2 members depicted in these studies mainly reflect strain- and age-specific variations, as well as the different detection procedures used. BCL-2 mRNA and protein seem to be localized to the inner nuclear (INL) and outer nuclear (ONL) layers of the mouse retina [58, 61], while BAX is mainly observed in the GCL [58].

3.3. Role of Bcl-2 Proteins in Retinitis Pigmentosa

As regulators dictating survival or apoptosis cascades, the Bcl-2 family of proteins are also involved in retinal dystrophies.

In *Rpe65*^{-/-} murine model of LCA, altered expression of Bcl-2 family members was observed at the onset and during progression of the retinal disease. Decreased expression of BCL-2 was paralleled with increased level of BAX and BH3-only proteins BAD and BMF, indicating that altered ratio of the different Bcl-2 members may impact the balance between anti- and pro-apoptotic signals toward cell death of photoreceptors in LCA disease [62]. Activation and mitochondrial translo-

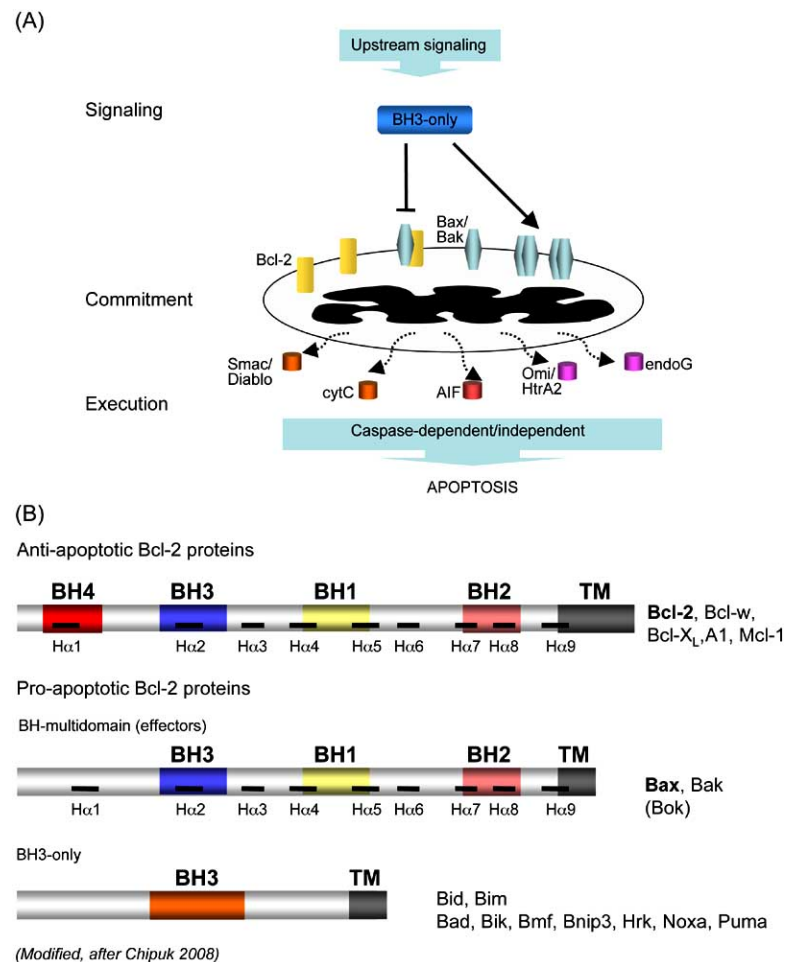


Fig. (2). The Bcl-2 family of proteins.

(A) The Bcl-2-dependent mitochondrial pathway of apoptosis. Following upstream stress signals, BH3-only proteins trigger Bcl-2-mediated intracellular pathways by direct activation of the BH-multidomain effectors Bax and Bak, or by indirect activation through inhibition of the anti-apoptotic functions of the pro-survival members such as Bcl-2. Bax/Bak activation and oligomerization in turn promote mitochondrial outer membrane permeabilization (MOMP) resulting in committed, irreversible apoptotic cascade. MOMP is responsible for the release of apoptogenic factors including cytochrome C (cytC), Smac/Diablo, endoG, Omi/HtrA2 and AIF, which trigger caspase-dependent or caspase-independent execution phase of the apoptotic program. **(B) Domain structure of the Bcl-2 proteins.** Anti-apoptotic and pro-apoptotic members of the Bcl-2 protein family are shown schematically, with approximate position of the Bcl-2 homology (BH) domains indicated. The anti-apoptotic Bcl-2 members, which include Bcl-2, Bcl-X_L, Bcl-w, A1 and Mcl-1, contain four BH domains (BH1 to BH4). The pro-apoptotic BH-multidomain subfamily encompasses Bax, Bak and Bok, which are constituted of domains BH1 to BH3. As designed, the BH3-only group only retains BH3 domain and either includes direct activators (Bid, Bim) or de-repressors/sensitizers (Bad, Bik, Bmf, Bnip3, Hrk, Noxa, Puma). Many family members have a carboxy-terminal hydrophobic transmembrane domain (TM), based on *in silico* predictions and/or structural data, that aids targeting to intracellular membranes. Known α -helical regions are numbered (α 1 to α 9) and indicated as a bold line.

cation of BAX associated with apoptosis of photoreceptors was further observed in *Rpe65*-deficient retina, suggesting that Bcl-2-mediated intracellular pathway plays a crucial role in LCA-dependent retinal degeneration [63]. In rd mice, while BAD [64] and BIM [65] were not involved in photoreceptor apoptosis, detection of cleaved BID at the peak of retinal cell death was paralleled with the activation of caspases and mitogen-activated protein kinase p38 [39]. These data suggest that Bid-dependent triggering of apoptotic mitochondrial pathway occurs during photoreceptor degeneration in the rd model of recessive RP. In contrast, pro-apoptotic BAX did not seem to be involved since rd mice defi-

cient for the latter protein were not rescued from photoreceptor degeneration. This may be explained by additional role of other effector molecules of the Bcl-2 family such as BAK [66]. In support of this view, Hahn and colleagues provided *in vivo* evidences that combined deficiency of BAX and BAK can rescue photoreceptor cells from light-induced injury more efficiently than BAX deficiency [67] alone.

To assess the protective role of BCL-2 in retinal diseases, photoreceptor-specific ectopic expression of the protein has been evaluated in mice with inherited or engineered mutations leading to retinal cell death. In

Pdeg^{tm1/tm1} mutant mice carrying a targeted disruption of the gene encoding the γ subunit of cGMP phosphodiesterase, enhanced expression of BCL-2 was retarding photoreceptor apoptosis [68]. As observed by Nir and colleagues [69], overexpression of BCL-2 caused a significant and long-term inhibition of photoreceptor apoptosis in rds mutant mice. The rescue was partial, with 60% protection against retinal cell death in transgenic rds mice. While the protective effect of BCL-2 was observed during the early phase of cell death, the photoreceptors continued to die as the disease progressed, although at a slower rate. These results most likely reflect a delayed loss of photoreceptors rather than a full protection, which may require higher level of heterologous BCL-2 or enhanced expression of other anti-apoptotic proteins such as BCL-X_L. Indeed, a marked synergistic effect of ectopic co-expression of the anti-apoptotic molecules BCL-2 and BAG-1, a Bcl-2-binding protein acting as a chaperone regulator, was efficient in preventing photoreceptor loss in opsin S334ter mutants showing impaired sorting of C-terminus truncated rhodopsin [70]. In this model, photoreceptor-driven over-expression of BCL-2 was accompanied by a reduction of apoptotic cell death in the retina by more than 80%, early during the onset of retinal degeneration. Photoreceptor loss was further delayed and showed 2-3 times longer survival than in non transgenic opsin S334ter mutants [71]. In contrast, in the study of Joseph and Li, no anti-apoptotic effect of Bcl-2 was observed in mutant mice with opsin K296E missense mutation [72]. Other studies showed discrepant results regarding the protective role of BCL-2 in rd mutant retina, or in photoreceptors exposed to light injury. While Chen and colleagues [71] observed that photoreceptors over-expressing BCL-2 survive genetic or environmental insult longer than those with normal endogenous level of BCL-2, the report by Joseph and Li [72] did not show any beneficial effect of ectopic expression of BCL-2 or BCL-X_L in mutant rd mice or following light-induced damage. These discrepant data might rely on differences in experimental design and transgene expression level between the two studies. Of note, it was demonstrated that dose-dependent, excessive expression of BCL-2 in transgenic mice could decrease photoreceptor survival [71], and that in high BCL-2-expressing retina photoreceptor-targeted localization of the transgene was toxic and triggered retinal cell death [73]. In favor of a protective role of BCL-X_L in photoreceptor susceptibility to light-induced stress, Zheng and colleagues reported increased cell death in rod-specific BCL-X_L-deficient retina following light damage [74]. Again, these results are contradictory with the observations of Joseph and Li [72], and the differences observed might arise from the different genetic background of the mice which show different resistance/sensitivity to low intensity light damage.

The transient pro-survival effect of BCL-2 as displayed in RP murine models may be due to negative impact of the overexpressed protein by itself that may counteract its endogenous survival promoting functions. Or, it may be explained by modulated regulation of pro-apoptotic Bcl-2 members and compensatory ef-

fects toward forced expression of BCL-2, thus limiting the physiological protective function of BCL-2. Alternatively, inherited mutations and light damage may activate multiple intracellular death pathways during the course of retinal degeneration, some of which may trigger Bcl-2-independent apoptosis. The kinetics of opsin promoter-driven expression of the transgene by around postnatal day 10, simultaneous with the onset of massive photoreceptor degeneration in rd retina may not prove to be optimal to assess the protective function of BCL-2. All these data are in favor of a complex death regulatory network in retinal cells in which Bcl-2-mediated signaling may act in conjunction with diverse apoptosis-promoting and -inhibiting gene products. Moreover, careful regulation of BCL-2 expression, not too low or too high, may be a prerequisite for its biological function.

4. TOWARD NEW MOLECULAR THERAPEUTIC STRATEGIES TO PREVENT RETINAL DYSTROPHIES

4.1. Gene Therapy

Retinal degeneration represents the most prevalent cause of visual handicap among the human population, for which no cure actually exists. Gene transfer targets primary genetic defect and aims at curing the disease by restoring a correct gene function. In mutations leading to loss-of-function, as autosomal recessive retinal degeneration, gene replacement allows to correct the mutated gene and the most conclusive results in animal RP models were observed for *Rpe65*-mediated LCA disease. 2001 was the year of the first successful gene therapy trial in LCA by replacement of the mutated *Rpe65* gene in Briard dog. Several groups are now developing gene therapy approaches to rescue RPE65 function in murine models of retinal degeneration [75-78]. However, in many forms of retinal dystrophies, as autosomal dominant inherited defects, gene-specific therapeutic approach is less suited for gene replacement. In this case, gene transfer strategy is based in silencing of the deleterious mutation by using several strategies including antisense oligonucleotides, ribozymes, or the recently developed RNAi interference technique.

RP is one of the most genetically heterogeneous hereditary diseases. This represents a major impediment to the development of gene-specific therapies since, considering this huge genetic diversity, gene replacement will not be applicable to all patients suffering from RP. A crucial need toward the development of mutation-independent therapies is expected. Targeting apoptosis through its triggered signaling pathways is one method to achieve this goal, since apoptosis appears as a common fate in the pathological process of RP. Gene transfer can also be suitable to focus on intracellular targets involved in the activation of apoptosis or in neuroprotection. Delivery to the eye of protective genes was successfully achieved by means of viral vectors, either lentiviral, adenoviral or adeno-associated vectors. Somatic delivery of rhodopsin-

promoted BCL-2 by subretinal injection of recombinant adenoviruses resulted in partial rescue of retinal degeneration [79]. Successful recombinant virus-mediated delivery of neuroprotective factors was also achieved in retinal tissues (reviewed in [80]). While this approach will not abrogate the disease, it will extend the survival of retinal cells through modulation of intracellular signaling molecules and may prove valuable to target multiple retinal dystrophies caused by diverse genetic defects. However, viral-based strategies need additional developments for suitable gene delivery since they still encounter safety concerns, such as risks related to virus transmission and inflammatory response.

4.2. Molecular Therapy to Target Bcl-2 Proteins in Retinal Degeneration

Various methods have been developed to transport genes into ocular tissues and correct cells, such as delivery of the transgene by viral vectors (see above), *in vivo* electroporation of naked DNA and retinal cell transplantation. All of them, although offering promising potentials, still present limitations. Special issues dealing with activity, duration of expression, inability to reach the target tissue, and safety concerns have prevented the general use of these delivery systems. The need is real for the development of additional delivery systems. One of them consists in a carrier system based on cell-penetrating peptide. Transcriptional factor *trans*-activator protein (TAT) from human immunodeficiency virus type 1 (HIV-1) has been shown to transduce most of the retinal cell layers upon subretinal or intravitreal injections, and thus represents an efficient transport system for the delivery of peptides and proteins in ocular tissues [81]. Following intravitreal injection, TAT-BCL-X_L fusion protein was shown to be efficiently transduced into the retinal ganglion cell layer and to rescue axotomized retinal ganglion cells (RGCs) from apoptosis [82]. Peptides can also present transduction properties within ocular compartments. It was indeed observed that BCL-2 peptide administration increased RGC survival following retinal ischemia induced by elevation of intraocular pressure [83]. Furthermore, attempts to interfere with Bax-dependent apoptosis in axotomized mice by using cell-permeable BAX-inhibiting peptide (BIP) resulted in significant increase in RGC survival after intravitreal injection [84]. Similarly, intravitreal treatment with Bax-directed antisense oligonucleotide inhibited BAX upregulation as well as RGC cell death locally and temporarily following optic nerve transection [85]. However, the use of this strategy is limited due to limited transfection efficacy and temporal restriction of the protective effect. Altogether, these encouraging findings indicate that proteins from the Bcl-2 family can be administered extracellularly into the eye and cross cell membrane to exert their antiapoptotic action intracellularly.

5. CONCLUSIONS

Retinal dystrophies, including inherited RP diseases, remain a great challenge to the researchers and

clinicians involved in the ophthalmology field, since an always increasing number of disease-causing genes remains to be found, and almost no efficient cure for the disease are available so far.

Whereas there is no doubt of apoptotic events in retinal degeneration, it seems clear nowadays that complex crosstalk and interconnected signals may link upstream stress, non obligate apoptotic regulated pathways, with committed executor apoptotic events. Important clues remain to be unraveled regarding which pathogenic factors and signaling pathways may trigger the decision to die according to the primary genetic defect. There is now an increased body of evidence that the intracellular molecular pathways involved in RP rely on caspase-dependent or -independent apoptotic mitochondrial pathway involving the Bcl-2 family of proteins.

Mutation-independent approaches such as molecular therapy provide a tool to target common intracellular pro-apoptotic signaling molecules and pathways implicated in retinal degeneration. Proof-of-principle has been demonstrated toward the efficient extracellular delivery of cell-permeable peptide-based compounds targeting Bcl-2-dependent apoptotic pathways. Such a strategy shows promising and exciting perspectives to delay or even stop photoreceptor degeneration in medium-term, with the advantage to be applicable to most patients regardless of their genetic defects.

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ABBREVIATIONS

RP	= Retinitis Pigmentosa
LCA	= Leber's Congenital Amaurosis
RHO	= rhodopsin
T	= transducin
PDE	= phosphodiesterase
CNG	= cGMP-gated cation channel
OS	= outer segment
RK	= rhodopsin kinase
P	= phosphorylation
A	= arrestin
AT-RAL	= all- <i>trans</i> -retinal
AT-ROL	= all- <i>trans</i> -retinol
RDH	= retinol dehydrogenase
RPE	= retinal pigment epithelium
AT-RE	= all- <i>trans</i> -retinyl ester
LRAT	= lecithin:retinol acyl transferase

RPE65 = RPE-specific 65 kDa isomerohydrolase
 11CIS-ROL = 11-*cis*-retinol
 11CIS-RAL = 11-*cis*-retinal
 PR = photoreceptor
 adRP = autosomal dominant RP
 rd = retinal degeneration
 rds = retinal degeneration slow
 BH = Bcl-2 homology domain
 ER = endoplasmic reticulum
 GCL = ganglion cell layer
 INL = inner nuclear layer
 ONL = outer nuclear layer
 TAT = *trans*-activator protein
 HIV-1 = human immunodeficiency virus type 1
 RGCs = retinal ganglion cells
 BIP = BAX -inhibiting peptide

REFERENCES

- [1] Preising, M. and Heegard, S. (2004) *Trends Mol. Med.*, **10**, 51-54.
- [2] Hartong, D. Berson and E. Dryja, T. (2006) *Lancet*, **368**, 1795-809.
- [3] Koenekoop, R., Lopez, I., den Hollander, A., Allikmets, R. and Cremers, F. (2007) *Clin. Exp. Ophthalmol.*, **35**, 473-85.
- [4] Pacione, L.R., Szego, M.J., Ikeda, S., Nishina, P.M. and McInnes, R.R. (2003) *Annu. Rev. Neurosci.*, **26**, 657-700.
- [5] Farrar, G., Kenna, P. and Humphries, P. (2002) *EMBO J.*, **21**, 857-64.
- [6] Clarke, G., Héon, E. and McInnes, R. (2000) *Clin. Genet.*, **57**, 313-29.
- [7] Huang, S.H., Pittler, S.J., Huang, X., Oliveira, L., Berson, E.L. and Dryja, T.P. (1995) *Nat. Genet.*, **4**, 468-71.
- [8] McLaughlin, M., Sandberg, M., Berson, E. and Dryja, T. (1993) *Nat. Genet.*, **4**, 130-34.
- [9] McLaughlin, M., Ehrhart, T., Berson, E. and Dryja, T. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 3249-53.
- [10] Danciger, M., Blaney, J., Gao, Y., Zhao, D., Heckenlively, J., Jacobson, S. and Farber, D. (1995) *Genomics*, **30**, 1-7.
- [11] Dryja, T., Finn, J., Peng, Y., McGee, T., Berson, E. and Yau, K. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 10177-81.
- [12] Perrault, I., Rozet, J.M., Calvas, P., Gerber, S., Camuzat, A., Dollfus, H., Chatelin, S., Souied, E., Ghazi, I., Leowski, C., Bonnemaïson, M., Paslier, D.L., Frezal, J., Dufier, J.-L., Pittler, S., Munnich, A. and Kaplan, J., (1996) *Nat. Genet.*, **14**, 461-64.
- [13] Nakamachi, Y., Nakamura, M., Fujii, S., Yamamoto, M. and Okubo, K. (1998) *Am. J. Ophthalmol.*, **125**, 249-51.
- [14] Dryja, T., McGee, T., Hahn, L., Cowley, G., Olsson, J., Reichel, E., Sandberg, M. and Berson, E. (1990) *N. Engl. J. Med.*, **323**, 1302-07.
- [15] Goldberg, A., Loewen, C. and Molday, R. (1998) *Biochemistry*, **37**, 680-85.
- [16] El Matri, L., Ambresin, A., Schorderet, D.F., Kawasaki, A., Seeliger, M.W., Wenzel, A., Arsenijevic, Y., Borruat, F.-X. and Munier, F.L. (2006) *Graefes Arch. Clin. Exp. Ophthalmol.*, **244**, 1104-12.
- [17] Thompson, D., Gyürüs, P., Fleischer, L., Bingham, E., McHenry, C., Apfelstedt-Sylla, E., Zrenner, E., Lorenz, B., Richards, J., Jacobson, S., Sieving, P. and Gal, A. (2000) *Invest. Ophthalmol. Vis. Sci.*, **41**, 4293-99.
- [18] Daiger, S., Bowne, S. and Sullivan, L. (2007) *Arch. Ophthalmol.*, **125**, 151-58.
- [19] Bowes, C., Li, T., Danciger, M., Baxter, L., Applebury, M. and Farber, D. (1990) *Nature*, **347**, 677-80.
- [20] Pittler, S. and Baehr, W. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 8322-26.
- [21] Farber, D. (1995) *Invest. Ophthalmol. Vis. Sci.*, **36**, 263-75.
- [22] Travis, G., Brennan, M., Danielson, P., Kozak, C. and Sutcliffe, J. (1989) *Nature*, **338**, 70-73.
- [23] Connell, G., Bascom, R., Molday, L., Reid, D., McInnes, R. and Molday, R. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 723-26.
- [24] Travis, G., Sutcliffe, J. and Bok, D. (1991) *Neuron*, **6**, 61-70.
- [25] Chader, G. (2002) *Vision Res.*, **42**, 393-99.
- [26] Chang, B., Hawes, N., Hurd, R., Davisson, M., Nusinowitz, S. and Heckenlively, J. (2002) *Vision Res.*, **42**, 517-25.
- [27] Fauser, S., Lubrichs, J. and Schüttauf, F. (2002) *Surv. Ophthalmol.*, **47**, 357-67.
- [28] Mendes, H., van der Spuy, J., Chapple, J. and Cheetham, M. (2005) *Trends Mol. Med.*, **11**, 177-85.
- [29] Furukawa, T., Morrow, E., Li, T., Davis, F. and Cepko, C. (1999) *Nat. Genet.*, **23**, 466-70.
- [30] Redmond, T.M., Yu, S., Lee, E., Bok, D., Hamasaki, D., Chen, N., Goletz, P., Ma, J.X., Crouch, R.K. and Pfeifer, A. (1998) *Nat. Genet.*, **20**, 344-51.
- [31] Koenekoop, R. (2004) *Surv. Ophthalmol.*, **49**, 379-98.
- [32] Donovan, M. and Cotter, T. (2004) *Biochim. Biophys. Acta*, **1644**, 133-47.
- [33] Chang, G., Hao, Y. and Wong, F. (1993) *Neuron*, **11**, 595-605.
- [34] Liu, C., Li, Y., Peng, M., Laties, A. and Wen, R. (1999) *J. Neurosci.*, **19**, 4778-85.
- [35] Bode, C. and Wolfgram, U. (2003) *Mol. Vis.*, **9**, 144-50.
- [36] Lohr, H., Kuntchithapatham, K., Sharma, A. and Rohrer, B. (2006) *Exp. Eye Res.*, **83**, 380-89.
- [37] Hughes, E., Schlichtenbrede, F., Murphy, C., Broderick, C., van Rooijen, N., Ali, R. and Dick, A. (2004) *Exp. Eye Res.*, **78**, 1077-84.
- [38] Doonan, F., Donovan, M. and Cotter, T. (2003) *J. Neurosci.*, **23**, 5723-31.
- [39] Jomary, C., Neal, M. and Jones, S. (2001) *Mol. Cell Neurosci.*, **18**, 335-46.
- [40] Yoshizawa, K., Kiuchi, K., Nambu, H., Yang, J., Senzaki, H., Kiyozuka, Y., Shikata, N. and Tsubura, A. (2002) *Graefes Arch. Clin. Exp. Ophthalmol.*, **240**, 214-19.
- [41] Zeiss, C., Neal, J. and Johnson, E. (2004) *Invest. Ophthalmol. Vis. Sci.*, **45**, 964-70.
- [42] Donovan, M., Carmody, R. and Cotter, T. (2001) *J. Biol. Chem.*, **276**, 23000-08.
- [43] Donovan, M. and Cotter, T. (2002) *Cell Death Differ.*, **9**, 1220-31.
- [44] Doonan, F., Donovan, M. and Cotter, T. (2005) *Invest. Ophthalmol. Vis. Sci.*, **46**, 3530-38.
- [45] Paquet-Durand, F., Azadi, S., Hauck, S., Ueffing, M., van Veen, T. and Ekström, P. (2006) *J. Neurochem.*, **96**, 802-14.
- [46] Sanges, D., Comitato, A., Tammaro, R. and Marigo, V. (2006) *Proc. Natl. Acad. Sci. USA*, **103**, 17366-71.
- [47] Sakamoto, Y., Nakajima, T., Fukiage, C., Sakai, O., Yoshida, Y., Azuma, M. and Shearer, T. (2000) *Curr. Eye Res.*, **21**, 571-80.
- [48] Tamada, Y., Nakajima, E., Nakajima, T., Shearer, T. and Azuma, M. (2005) *Brain Res.*, **1050**, 148-55.
- [49] Sharma, A. and Rohrer, B. (2004) *J. Biol. Chem.*, **279**, 35564-72.
- [50] Rakoczy, P., Zhang, D., Robertson, T., Barnett, N., Papadimitriou, J., Constable, I. and Lai, C.-M. (2002) *Am. J. Pathol.*, **161**, 1515-24.
- [51] Schorderet, D.F., Cottet, S., Loblunus, J., Borruat, F.-X., Balmer, A. and Munier, F. (2007) *Arch. Ophthalmol.*, **125**, 231-36.
- [52] Youle, R. and Strasser, A. (2008) *Mol. Cell Biol.*, **9**, 47-59.
- [53] Chipuk, J.E. and Green, D.R. (2008) *Trends Cell Biol.*, **18**, 157-64.

- [54] Wei, M.C., Zong, W.X., Cheng, E.H., Lindsten, T., Panoutsakopoulou, V., Ross, A.J., Roth, K.A., MacGregor, G.R., Thompson, C.B. and Korsmeyer, S.J. (2001) *Science*, **292**, 727-30.
- [55] Levin, L., Schlamp, C., Spielfoch, R., Geszvain, K. and Nickells, R., (1997) *Invest. Ophthalmol. Vis. Sci.*, **38**, 2545-53.
- [56] Shin, D., Lee, H., Kim, H., Lee, E., Cho, S., Baik, S. and Lee, K. (1999) *Neuroreport*, **10**, 2165-67.
- [57] Grimm, C., Wenzel, A., Hafezi, F. and Remé, C.E. (2000) *Mol. Vis.*, **6**, 252-60.
- [58] Chen, S., Hsu, J., Hsu, P. and Chuang, J. (2003) *Neurochem. Res.*, **28**, 805-14.
- [59] Ji, J., Chang, P., Pennesi, M.E., Yang, Z., Zhang, J., Li, D., Wu, S.M. and Gross, R.L. (2005) *Vision Res.*, **45**, 169-79.
- [60] Donovan, M., Doonan, F. and Cotter, T. (2006) *Dev. Biol.*, **291**, 154-69.
- [61] Péquignot, M., Provost, A., Sallé, S., Taupin, P., Sainton, K., Marchant, D., Martinou, J., Ameisen, J., Jais, J.P. and Abitbol, M. (2003) *Dev. Dyn.*, **228**, 231-38.
- [62] Cottet, S., Michaut, L., Boisset, G., Schlecht, U., Gehring, W. and Schorderet, D.F. (2006) *FASEB J.*, **20**, 2036-49.
- [63] Cottet, S. and Schorderet, D.F. (2008) *Apoptosis*, **13**, 329-42.
- [64] Jomary, C., Cullen, J. and Jones, S.E. (2006) *Invest. Ophthalmol. Vis. Sci.*, **47**, 1620-29.
- [65] Doonan, F., Donovan, M., Gomez-Vicente, V., Bouillet, P. and Cotter, T.G. (2007) *J. Neurosci.*, **27**, 10887-94.
- [66] Mosinger Ogilvie, J., Deckwerth, T.L., Knudson, C.M. and Korsmeyer, S.J. (1998) *Invest. Ophthalmol. Vis. Sci.*, **39**, 1713-20.
- [67] Hahn, P., Lindsten, T., Lyubarsky, A., Ying, G.S., Pugh, J.E.N., Thompson, C.B. and Dunaief, J.L. (2004) *Cell Death Differ.*, **11**, 1192-97.
- [68] Tsang, S.H., Chen, J., Kjeldbye, H., Li, W.S., Simon, M.I., Gouras, P. and Goff, S.P. (1997) *Invest. Ophthalmol. Vis. Sci.*, **38**, 943-50.
- [69] Nir, I., Kedziarski, W., Chen, J. and Travis, G.H. (2000) *J. Neurosci.*, **20**, 2150-54.
- [70] Eversole-Cire, P., Concepcion, F.A., Simon, M.I., Takayama, S., Reed, J.C. and Chen, J. (2000) *Invest. Ophthalmol. Vis. Sci.*, **41**, 1953-61.
- [71] Chen, J., Flannery, J.G., LaVail, M.M., Steinberg, R.H., Xu, J. and Simon, M.I. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 7042-47.
- [72] Joseph, R.M. and Li, T. (1996) *Invest. Ophthalmol. Vis. Sci.*, **37**, 2434-46.
- [73] Quiambao, A.B., Tan, E., Chang, S., Komori, N., Naash, M.I., Peachey, N.S., Matsumoto, H., Ucker, D.S. and Al-Ubaidi, M.R. (2001) *Exp. Eye Res.*, **73**, 711-21.
- [74] Zheng, L., Anderson, R.E., Agbaga, M.-P., Rucker, E.B. and Le, Y.-Z. (2006) *Invest. Ophthalmol. Vis. Sci.*, **47**, 5583-89.
- [75] Bemelmans, A.-P., Kostic, C., Crippa, S., Hauswirth, W., Lem, J., Munier, F., Seeliger, M., Wenzel, A. and Arsenijevic, A. (2006) *PLoS Med.*, **3**, e347.
- [76] Bemelmans, A.-P., Kostic, C., Hornfeld, D., Jaquet, M., Crippa, S., Hauswirth, W., Lem, J., Wang, Z., Schorderet, D., Munier, F., Wenzel, A. and Arsenijevic, Y. (2006) *Adv. Exp. Med. Biol.*, **572**, 247-53.
- [77] Stoddart, C.W., Yu, M.J., Martin-Iverson, M.T., Daniels, D.M., Lai, C.M., Barnett, N.L., Redmond, T.M., Narfstrom, K. and Rakoczyt, P.E. (2006) *Adv. Exp. Med. Biol.*, **572**, 239-45.
- [78] Pang, J.J., Chang, B., Kumar, A., Nusinowitz, S., Noorwez, S.M., Li, J., Rani, A., Foster, T.C., Chiodo, V.A., Doyle, T., Li, H., Malhotra, R., Teusner, J.T., McDowell, J.H., Min, S.H., Li, Q., Kaushal, S. and Hauswirth, W.W. (2006) *Mol. Ther.*, **13**, 565-72.
- [79] Bennett, J., Zeng, Y., Bajwa, R., Klatt, L., Li, Y. and Maguire, A.M. (1998) *Gene Ther.*, **5**, 1156-64.
- [80] Chaum, E. (2003) *J. Cell Biochem.*, **88**, 57-75.
- [81] Schorderet, D.F., Manzi, V., Canola, K., Bonny, C., Arsenijevic, Y., Munier, F.L. and Maurer, F. (2005) *Clin. Exp. Ophthalmol.*, **33**, 628-35.
- [82] Dietz, G.P., Kilic, E. and Bähr, M. (2002) *Mol. Cell Neurosci.*, **21**, 29-37.
- [83] Vidal-Sanz, M., Lafuente, M., Sobrado-Calvo, P., Selles-Navarro, I., Rodriguez, E., Mayor-Torroglosa, S. and Villegas-Perez, M.P. (2000) *Neurotox. Res.*, **2**, 215-27.
- [84] Qin, Q., Patil, K. and Sharma, S.C. (2004) *Neurosci. Lett.*, **372**, 17-21.
- [85] Isenmann, S., Engel, S., Gillardon, F. and Bähr, M. (1999) *Cell Death Differ.*, **6**, 673-82.