Gene Therapy for Rhodopsin-associated Autosomal Dominant Retinitis Pigmentosa

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Introduction

Retinitis pigmentosa (RP) affects between 1 in 3000 and 1 in 4000 people and between 1.77 and 2.35 million people worldwide (https://rarediseases.org/rare-diseases/retinitis-pigmentosa/). The disease is characterized by the death of rod photoreceptor cells leading to defects in dark adaptation and night blindness. RP typically progresses to loss of peripheral vision and eventually to loss of central vision over a period of decades. Half of the cases occur in people with no family history, but 25% to 30% of cases are inherited in an autosomal dominant fashion.1 Mutations in RHO, the gene for rhodopsin, affect one quarter of the patients.

Rhodopsin is a G protein–coupled receptor with 7 membrane-spanning domains that initiates the phototransduction cascade in rod photoreceptor cells. It is also the most abundant protein in photoreceptors, comprising 10% of the total protein and requiring a tremendous flux in protein synthesis and transport from the inner segment to the outer segment of photoreceptors. Rhodopsin is tightly packed as dimers in the disc membranes of outer segments.2 Consequently, it is not surprising that mutations affecting rhodopsin synthesis, transport, folding, and catalytic function lead to the demise of photoreceptor cells and to loss of vision. Indeed, over 150 mutations in RHO lead to autosomal dominant retinitis pigmentosa (adRP) (www.ncbi.nlm.nih.gov/clinvar), and several others result in congenital stationary night blindness. Only 2 missense mutations in RHO are associated with autosomal recessive RP.3,4
The genotype-phenotype relationship of \textit{RHO} mutations has been examined biochemically in animal models and cell lines,\textsuperscript{5} and clinically in RP patients.\textsuperscript{6} Identifying the consequences of \textit{RHO} defects is particularly relevant to therapeutic approaches designed to sustain photoreceptor viability without correcting the underlying mutation. Mutations resulting in adRP have been separated into 2 classes based on whether they are synthesized at wild-type levels and reconstitute with 11-cis retinal in cell culture (class I) or whether they accumulate in the endoplasmic reticulum (ER) and reconstitute poorly (or not at all) with the chromophore (class II).\textsuperscript{7,8} With the discovery of additional \textit{RHO} mutations, the Cheetham group has categorized mutations with greater granularity, depending on whether the mutations lead to protein misfolding, defects in post-Golgi protein trafficking, alterations in posttranslational modification, constitutive activation, disrupted vesicular traffic, and endocytosis and alteration in protein dimerization.\textsuperscript{9,10} Despite this attention to detail, the cellular consequences of over half of known \textit{RHO} mutations remains undefined or unexamined.

The clinical phenotype of \textit{RHO} mutations is particularly relevant to gene therapy because the gene delivery approach depends on having viable cells to which genes can be delivered. Based on the clinical description, there are 2 main classes of adRP patients.\textsuperscript{11} Class A patients report loss of night vision at a younger age and exhibit death of rod photoreceptors throughout the retina. Any residual visual function comes from surviving cone photoreceptors. Class B patients experience a milder disease. The length of outer segments is better preserved, and the activation kinetics of rhodopsin is normal. In class B patients, the defects in rod visual cycle depends on the particular mutation. The death of rod cells is restricted to sectors of the retina, but photoreceptors are retained in adjacent regions.\textsuperscript{12,13} The slow progression of disease in patients with class B adRP poses a challenge for determining the efficacy of gene therapy, but serial measurement of photoreceptor retention by spectral-domain optical coherence tomography may provide a suitable outcome measure.\textsuperscript{14}

\section*{Animal and Organoid Models of adRP}

The availability of animal models of adRP has been key to testing gene therapy vectors for this disease. The only naturally occurring \textit{RHO} mutation leading to adRP is the T4R \textit{RHO} dog, originally found in English Mastiffs.\textsuperscript{15,16} This mutation renders the dogs exquisitely sensitive to retinal injury by illumination.\textsuperscript{17,18} The most common models of \textit{RHO} adRP have been generated in rodents. These include transgenic models of adRP caused by the P23H, T17M, P347S, and S334Ter mutations.\textsuperscript{19-22} Mutagenesis of mice has led to the construction of mouse lines bearing
mutations at the \textit{Rho} locus.\textsuperscript{23} Sakami et al\textsuperscript{24} produced a knock-in line bearing P23H \textit{Rho}, and Sancho-Pelluz et al\textsuperscript{25} constructed a knock-in mouse line with the D190N mutation. Both resulted in good phenocopies of human adRP. Porcine models of adRP have also been generated,\textsuperscript{26} but the large size of domestic swine make them difficult to work with as adults. Ross et al\textsuperscript{27} described an inbred line of miniature pigs bearing a human P23H transgene. These pigs are easier to handle than domestic swine, but they do not have rod outer segments or a rod electroretinogram (ERG) response at birth.\textsuperscript{28}

In the absence of a faithful animal model of retinal disease, induced pluripotent stem cell–derived retinal organoids provide an alternative platform for testing gene therapy vectors.\textsuperscript{29–35} Retinal organoids derived from human-induced pluripotent stem cells have the advantage of producing \textit{human} retinal cells. For nucleic acid–based therapies, such as RNA interference (RNAi) and CRISPR/Cas9, the use of human gene sequences is essential to assess both effectiveness and off-target effects. Organoids are especially valuable if they are generated from patient cells and recapitulate a retinal degeneration phenotype.\textsuperscript{36} Retinal organoids express rhodopsin; however, only rudimentary rod outer segments are formed, and the interaction between photoreceptors and the retinal pigment epithelium cannot be studied using current experimental systems. These limitations will undoubtedly be overcome by continued research. In the United States, the Food and Drug Administration (FDA) is pursuing alternative methods for the validation of drugs and biologics (www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda), so that proof of efficacy in retinal organoids may soon be sufficient to advance a gene therapy to clinical trials.

\section*{Gene Delivery to Photoreceptor Cells}

Adeno-associated virus (AAV) vectors are currently the best choice for gene delivery to photoreceptors.\textsuperscript{37} Adenoviral vectors lead only to transient expression of delivered genes and stimulate an inflammatory response. Lentiviral vectors based on human immunodeficiency virus 1 or equine infectious anemia virus transduce the retinal pigment epithelium but transduce photoreceptors only near the injection site. AAV2/5 and AAV2/8 lead to efficient transduction of photoreceptor cells following subretinal injections.\textsuperscript{38} (The terminology indicates that the packaging signals of the vector genome are derived from AAV2 but that the genomes have been packaged in the capsid proteins of AAV5 or AAV8.) Replacement of certain capsid residues with other amino acids (eg, phenylalanine in place of tyrosine) prevents degradation of viral particles by the proteasome within the cell and increases the efficiency of productive infection (transduction).\textsuperscript{39} The use of these capsid-modified
vectors increases the transduction of photoreceptors.\textsuperscript{40-45} Nondividing cells transduced by AAV continue to express delivered genes for the lifetime of the cell. Therefore, treatment of photoreceptors with AAV should be required only once per lifetime for each eye.

Despite efforts to modify AAV capsid by rounds of selection,\textsuperscript{44,45} it is not currently possible to efficiently access photoreceptors by injecting AAV in the vitreous, and subretinal injections are typically used. This is a difficult procedure in rodents and a surgical procedure in larger animals including humans. The area of the retina that is detached by subretinal injection is termed the bleb, and infection of photoreceptors with AAV2, 2/5, or 2/8 is confined to that region. Recently, however, a new AAV serotype of AAV, AAV44.9, was tested by subretinal injection in rodents and macaques.\textsuperscript{46} Genes delivered with AAV2/44.9 spread widely outside the bleb so that larger regions of the retina could be accessed with less recombinant virus.

One limitation of AAV is that most humans have been naturally exposed to 1 or more AAV serotypes, and previous exposure can reduce transduction in the context of gene therapy.\textsuperscript{47,48} Another drawback of AAV is the size limitation of genes that it can deliver (no > 4.7 kbp). The use of dual vectors has overcome the size limitation for the delivery of some large genes.\textsuperscript{49-51} Size is not a problem for the 1 kbp $RHO$ cDNA, but precise regulation of rhodopsin expression requires sequence elements in introns of the $RHO$ gene and in flanking sequences. This has led Han and colleagues to develop nanoparticle strategies to deliver large regions of the $RHO$ genomic sequence.\textsuperscript{52-54} They found that delivery of genomic $RHO$ was superior to $RHO$ cDNA in preserving photoreceptors of $RHO$ knockout mice. The longevity of the response remains an issue with nanoparticle delivery, and the technology is still under development.

\section*{Rhodopsin-directed Therapy}

The most direct route to treating $RHO$ adRP is to overcome the expression of mutant rhodopsin. For recessive mutations, which are relatively uncommon, supplementation of the missing gene may be sufficient, but for dominant mutations in rhodopsin, it may also be necessary to silence the mutated allele. Wilson and colleagues, however, demonstrated that 1 class B mutation, P23H, causes a dominant-negative rather than a toxic gain-of-function effect on rod photoreceptors, implying that it may interfere with the assembly or function of disc membranes.\textsuperscript{55,56} Following their lead, we used AAV2/5 to overexpress murine rhodopsin in P23H $RHO$ transgenic mice on the mouse $Rho^{+/+}$ background.\textsuperscript{57} We observed protection of both retinal function based on ERG and structure based on histology. More recently, Orlans et al\textsuperscript{58} used an AAV expressing the human $RHO$ cDNA to treat the P23H $Rho$ knock-in mice but observed no preservation of
retinal structure based on OCT or function based on ERG, despite robust expression of human rhodopsin. Therefore, it remains controversial whether supplementation will suffice to treat RHO mutations resulting in adRP, including P23H RHO. In addition, overexpression of rhodopsin can be toxic to rod cells; thus precise control of rhodopsin expression is likely required.57,59

As an alternative to simple supplementation, gene delivery of RHO can be paired with inhibition of the synthesis of endogenous rhodopsin. Suppression of synthesis can occur at the DNA level by gene disruption, at the transcriptional level by inhibition of mRNA synthesis, or at the posttranscriptional level by antisense techniques, such as RNAi or antisense oligonucleotides (ASO). Because of the heterogeneity of adRP mutations, allele-specific gene or RNA knockdown is not appropriate because one would have to validate a new guide RNA (gRNA), short hairpin RNA (shRNA), or ASO for each affected family.

**Gene Editing of RHO**

Gene editing employs several technologies, including zinc-finger nuclease, transcription activator-like effector nucleases, and clustered regularly interspaced short palindromic repeats (CRISPR) paired with Cas9 or similar nucleases. The CRISPR/cas9 system is the most versatile and has the largest community support.60 Tsai and colleagues61 used employed CRISPR/Cas9 technology using a 2 AAV vector gene therapy strategy in 2 different mouse models of RHO adRP. One AAV vector delivered the Cas9 gene from *Streptococcus pyogenes* (*SpCas9*), while the second vector encoded 2 gRNAs designed to direct cleavage of mouse Rho and a copy of a human RHO gene. Mismatches between the human and mouse DNA sequences rendered the replacement gene resistant to CRISPR/Cas9 cleavage. Delivering the gRNAs in the same vector as the replacement RHO gene meant that DNA cleavage of endogenous Rho did not occur in cells that did not also receive the replacement gene. Subretinal injection of both vectors led to a 25% decrease in Rho mRNA in wild-type mice. When they treated a P23H Rho adRP mouse model by subretinal injection, histology revealed retention of 6 to 8 rows of nuclei in the outer nuclear layer (ONL) when compared with 3 to 4 rows in phosphate-buffered saline–injected control eyes at 3 months postinjection. ERG responses were also significantly improved by the 2 vector ablation and replacement system. A group from EDITAS medicine also reported the efficacy of CRISPR/Cas9-mediated ablation of Rho and concomitant replacement with a wild-type gene at the 2020 Annual Meeting of the American Society for Gene and Cell Therapy (reviewed by Meng et al62). Several groups have reported editing of specific mutations of rhodopsin using CRISPR technology,63,64 but, as noted, clinical
translation of this approach may be limited by the number of patients carrying the mutant allele.

**Transcriptional Repression**

Design principles for DNA binding zinc-finger domains are well established. Mussolino and colleagues produced a series of transcriptional repressors that block rhodopsin expression by designing zinc-finger domains targeting various 18-nucleotide segments of the upstream region of RHO. These domains were fused with the repressive KRAB (Krüppel-associated box repressor) domain and subsequently screened in tissue culture and then in mice. Their most active repressor was encoded on and delivered by subretinal injection of AAV2/8 into mice bearing the P347S human RHO transgene on a Rho+/Rho+ background. At 60 days postinjection, transcription of RHO mRNA was reduced by 26%, and ERG b-wave amplitudes were increased by almost 30% in treated eyes. The transcriptional inhibitor had no effect on the production of mouse rhodopsin. In a similar methodology, AAV-delivered CRISPR/Cas9 fused with the KRAB domain has been used to suppress transcription in the mouse retina, though this approach was not specifically applied to the gene for rhodopsin. For therapy of adRP, suppression of endogenous RHO would be coupled with the delivery of a RHO gene whose promoter is modified to be resistant to the transcriptional inhibitor. In the case of toxic gain-of-function mutants of RHO, however, it is possible that reducing the level of both mutant and wild-type rhodopsin would preserve photoreceptor viability.

**ASO**

Murray et al described the design and screening of a series of chimeric 20 residue oligonucleotides with a phosphorothioate backbone. These oligonucleotides were further modified by 2′-O-methoxyethyl ribose at positions 1 to 5 and 15 to 20 and unmodified deoxyribonucleotides at positions 6 to 14. Ribose modifications serve to stabilize the ASO, while the formation of RNA:DNA hybrids in the center of the ASO leads to RNA degradation mediated by cellular RNase H. When tested in mice, a single intravitreal injection of their most active ASO led to a 70% reduction in rhodopsin mRNA that persisted at least 60 days thereafter. Treatment of P23H rats, which express a murine P23H Rho transgene, led to a significant improvement in the rod ERG response (a-wave) and a slight increase in the thickness of the ONL when compared with eyes treated with a control ASO. Clinical application of allele-specific ASOs have the same disadvantage of the limited patient population as allele-specific CRISPR/cas9 approaches. However, designing, testing, and producing ASOs is much faster and simpler than designing and
producing other forms of gene therapy. Furthermore, therapy is easily aborted if adverse effects arise. A clinical trial of one such ASO (QR-1123) to treat adRP caused by the P23H RHO mutation is currently in progress (ClinicalTrials.gov Identifier: NCT04123626). ASOs to promote exon-skipping are also being tested for Usher syndrome and recessive Stargardt Macular Dystrophy. Exon-skipping could be used to decrease the expression of RHO via nonsense-mediated decay in the setting of adRP.

**RNA Replacement**

RNA knockdown can be achieved by several methods, including RNA tools such as ribozymes, small interfering RNAs (siRNAs), artificial microRNAs (a-miRNAs), and nucleases such as RNA-directed CRISPR/Cas9 or Cas13a. The RNA tools are small (<75 nucleotides) and can easily be combined with a replacement rhodopsin gene (1047 nucleotides) on a vectors like AAV with a limited coding capacity. AAV-vectored hairpin and hammerhead ribozymes have been successfully tested for both allele-specific and allele-independent knockdown of rhodopsin mRNA. Nevertheless, siRNAs delivered by AAV as shRNAs are more effective than ribozymes in diminishing rhodopsin mRNA in animal models. siRNAs are also easier to design and can be purchased commercially for screening purposes. Taking advantage of this technology, investigators at Trinity College Dublin utilized 2 AAV vectors to deliver an shRNA gene driven by the RNA polymerase II H1 promoter and a replacement rhodopsin cDNA driven by a hybrid mouse Rho promoter. Using fluorescent marker genes, they first established that coinjection of 2 AAV vectors led to the dual infection of most photoreceptors. Subretinal injection of the shRNA and Rho replacement vectors led to an improved ERG b-wave response in treated eyes 5 months postinjection, compared with a baseline response in control-treated eyes. In contrast to the dual vector approach, we used a single AAV delivery vector (AAV-RS301) to deliver an RHO-specific shRNA under the control of the H1 promoter and a resistant Rho gene under the control of the mouse opsin proximal promoter. This strategy ensures the codelivery of the shRNA and RHO cDNA elements. The resistant Rho gene contained silent mutations at the siRNA target site to inhibit siRNA binding while maintaining the amino acid sequence. The shRNA in this vector bound to both mouse and human rhodopsin mRNA and led to a 50% reduction in total rhodopsin in human P23H RHO transgenic mice. Eyes treated with AAV-RS301 had a 2-fold increase in total rhodopsin content relative to untreated eyes and had a sustained protective effect on the retina (80% of the normal ERG response) up to 9 months postinjection.
Based on success in mice, we constructed a single vector RNA replacement vector to treat the rapid retinal degeneration caused by light exposure in the T4R rhodopsin dog.86 Because of sequence differences between the human and canine RHO genes, shRNA301 used in AAV-RS301 was not suitable for this purpose, and a new set of shRNAs was screened for level of knockdown. An shRNA that leads to cleavage of the human and canine RHO mRNA at position 820 caused 95% degradation of human RHO mRNA in cultured cells and was incorporated into a new combination vector, cloned in self-complementary AAV and designated scAAV2/5-hOP-RHO820-H1-shRNA820. Treatment of T4R dogs with this vector led to protection from the retinal degeneration associated with light exposure over a period of >30 weeks and 4 repeated light exposures after injection. This vector has been licensed for clinical development by Iveric Bio Inc.

An alternative approach to deliver a siRNA using a viral or plasmid vector is to embed the siRNA sequence within the processing signal (flanking sequences and loop sequence) of a-miRNA.87 The greatest advantage of this approach is that the a-miRNA can be produced under the control of an RNA polymerase II promoter, meaning that a cell type-specific or a regulated promoter can be used, as opposed to the highly active but nonspecific pol III promoters used to produce shRNAs. This approach has been effective in treating the dominantly inherited liver degeneration caused by mutations in α-1-antitrypsin.88,89

The coding sequence of the a-miRNA is typically inserted within the intron or the 3′-UTR of a protein-coding gene. In comparison with shRNAs targeting the same sequences, a-miRNAs appear to stimulate less inflammatory and nonspecific knockdown (off-target effects).73,90 Greenwald and colleagues have used this approach to treat mice transgenic for the human P347S RHO transgene with a dual AAV2/9 vector system to express an RHO-specific a-miRNA and a resistant RHO cDNA. Mice treated with this dual vector system showed an improved ERG response, despite the severe degeneration seen in the P347S RHO model.91

Any of the nucleic acid–based technologies have the potential for nonspecific (off-target) effects. ASOs, gRNAs, ribozymes, microRNAs (miRNAs), or shRNAs can have a target sequences that are sufficiently complementarity with an unintended target to lead to destruction or editing of unintended mRNA or DNA sequences. The problem is particularly acute for shRNAs or a-miRNAs that can interfere with the processing and transport of endogenous miRNA by saturating the endogenous RNAi machinery or by stimulating an inflammatory response if overexpressed. siRNAs, whether supplied as shRNA or a-miRNA, can bind to the 3′-UTR of mRNAs and block the translation of off-target genes via the miRNA pathway.92 Bioinformatic tools are available to avoid such problems81,93 but, in the end, experimental proof of specificity is required.
Photoreceptor Survival Therapy

Neurotrophic Factors

Promoting cell survival is an alternative to correcting or replacing mutant rhodopsin. CNTF (ciliary-derived neurotrophic factor) and GDNF (glial-derived neurotrophic factor) have been tested in animal models of adRP. GDNF is a member of the transforming growth factor-beta family, and its receptors are normally expressed in the retina. In transgenic rats bearing the S334Ter mutation in rhodopsin, subretinal injection of AAV2-GDNF slowed photoreceptor degeneration based on measurement of ONL thickness and on the ERG response. AAV-GDNF also slowed retinal degeneration in 2 other models of retinal degeneration not caused by rhodopsin mutations, which demonstrates the generalizability of targeting prosurvival pathways. Since receptors for GDNF are present on Müller glial cells but not photoreceptors, GDNF probably protects photoreceptors indirectly by acting through Müller cells. Wu et al treated wild-type Sprague-Dawley rats with AAV-GDNF and found no harmful effects or impacts on retinal function over a 1-year time course. Therefore, it appears that AAV-delivered GDNF may be a safe and effective means to prolong photoreceptor survival in adRP.

CNTF, a member of the interleukin (IL)-6 family, has been delivered to the retina using an implantable device containing mammalian cells. This protein has also gained attention in the field of gene therapy. S334ter and P23H RHO transgenic rats were injected in the vitreous with AAV-CNTF, which slowed degeneration of photoreceptors yet suppressed the ERG a-wave and b-wave responses. Diminished ERG responses were also observed with the CNTF secreting implantable devices, suggesting that this neurotrophic factor may be unsuitable for long-term expression by gene delivery due to dampening of photoreceptor function.

The most promising neurotrophic factor tested to date is produced within the retina. Rod-derived cone viability factor (RdCVF), a thioredoxin-like protein lacking oxidoreductase activity, promotes the survival of cone photoreceptor cells. RdCVF operates via the transmembrane protein basigin to promote glucose transport into photoreceptor cells. In P23H Rho transgenic rats, injecting RdCVF increased cone density by nearly 20%. Dalkara et al used an intravitreal injection of AAV7m8, an AAV variant isolated by selection for pan-retinal transduction, to treat the P23H Rho knock-in mice. They reported elevated cone ERG amplitudes in treated eyes compared with those in control-injected eyes at 1 and 4 months, but not at 6 months posttreatment. Intravitreal delivery in these experiments suggest that gene therapy with AAV7m8-RdCVF could be easily translated to the clinic. However, subretinal injection with an AAV vector targeting photoreceptors might prove more effective and long-lasting.
**Suppression of the Unfolded Protein Response (UPR)**

Many class II mutations in *RHO* lead to a misfolded protein that accumulates in the ER. \(^{10,107-109}\) Such misfolded rhodopsin stimulates an ER stress pathway called the UPR, which responds to imbalances between protein synthesis and protein trafficking or degradation. Three transmembrane proteins act as proximal signaling molecules of the UPR: IRE1 (inositol-requiring enzyme 1), ATF6 (activating transcription factor 6), and PERK (protein kinase R-like ER protein kinase).\(^{110}\) Sustained activation of the UPR, and in particular the PERK pathway that reduces protein synthesis, leads to cell death by apoptosis. Stimulation of the UPR by the T17M mutation of rhodopsin also increases the production of inflammatory cytokines such as IL-1β, IL-6, and MCP1 that could promote bystander photoreceptor loss.\(^{111}\) All 3 UPR pathways are kept in an inactive state by binding to the HSP70 family chaperone BiP/Grp78.\(^{112}\) Grp78 binds to each of the transducers of ER stress (IRE1α, ATF6, and PERK) and acts as a sensor of alterations of ER homeostasis, thus increasing expression of Grp78 could reduce the death of photoreceptors in adRP. To this end, Gorbatyuk et al\(^{113}\) used AAV2/5 to deliver the Grp78 gene to the retina of P23H *Rho* transgenic rats via subretinal injection and found improved ERG a-wave and b-wave responses as well as structural integrity of the central retina over a period of 3 months. These changes were associated with decreased apoptosis in the Grp78 treated retinas compared with control. Consequently, targeting the upstream pathways of the UPR or suppressing the downstream signaling pathways\(^{107,114}\) may be of therapeutic value for adRP.

**miRNA Gene Therapy**

miRNAs are ~22 nucleotide RNAs that regulate biological processes by controlling gene expression by binding to mRNAs.\(^{115}\) A number of human diseases have been correlated with dysregulation of miRNA expression, and this holds true for the retina.\(^{116,117}\) To this end, Loscher et al\(^{118}\) reported altered levels of several miRNAs (miR-96, -183, -1, and -133) in the retinas of P347S *RHO* transgenic mice. Furthermore, Conte et al\(^{119}\) identified a mutation in the seed region (nucleotides 2 to 7) of miR-204 that segregates with a form of retinal dystrophy and coloboma in humans. The same group used an AAV2/8 vector expressing the precursor of mouse miR-204 (pre-miR-204) under the control of the cytomegalovirus immediate–early promoter in P247S *RHO* transgenic mice. When neonatal mice were injected, the authors observed a statistically significant increase in the ERG a-wave and b-wave amplitudes, though no apparent difference in ONL thickness. When they injected mice later in the course of retinal degeneration (P30) they observed an increase in the b-wave that lasted until P60. These
investigators reported a similar beneficial result when they used AAV to deliver miR-211, a paralog of miR-204. To analyze the mechanism of protection afforded by AAV-miR-204, they performed RNA sequence analysis and found increased expression of genes involved in visual perception and a downregulation of genes associated with innate immunity and inflammation. They also showed preservation of retinal structure in a recessive inherited retinal degeneration ($Aipl1^{-/-}$). This work suggests that gene delivery using miRNA genes may be beneficial for a variety of inherited retinal disease by stimulating common prosurvival pathways.

### Delivery of Opsins to Surviving Cells

In severe cases of adRP associated with class A $RHO$ mutations, and even in some cases of class B mutations, clinical diagnosis and genetic characterization may come too late to rescue photoreceptor cells. Therefore, gene transfer to these cells is not possible. However, in some forms of adRP, the inner retina is not severely impacted until late in the course of the disease. Several groups have studied the transfer of photoswitch-controlled channels or light-responsive proteins to surviving neuronal cells of the retina. This subject has been reviewed elsewhere and is the subject of a recent collection of papers. Transfer of opsins or channels to cone photoreceptors is not advisable because in severe cases of adRP cone photoreceptors also die, and the cone mosaic is altered. In terms of kinetics and sensitivity to low light, gene transfer of human middle wavelength cone opsin appears to an excellent option. Following intravitreal injection of AAV2 expressing cone opsin in 1- to 2-month-old rd1 mice, Berry and colleagues found that expression of MW-length opsin in 45% of retinal ganglion cells (RGCs) rendered these cells sensitive to flashes of light. The retinas of these mice were as responsive to indoor light as mice treated with AAV2-encoding rhodopsin and 1000-fold more responsive than mice treated with other light-sensitive molecules, including channel rhodopsin or halorhodopsin. Compared with transducing RGCs with rhodopsin, mice expressing MW-opsin exhibited faster kinetics and less reduction in signal after repeated flashes. Furthermore, transduction of RGCs with MW-opsin allowed rd1 mice to distinguish light patterns on an LCD screen and to perform vision-guided behaviors in ambient light. Experiments to restore vision to mice lacking photoreceptor cells is promising and has led to clinical trials (NCT02556736, NCT03326336), but to eliminate signals from endogenous photoreceptors, experiments have been performed primarily on $Pde6B^{rd1}$ mice or on a triple mutant that has no light sensitivity at the time of treatment. Experiments with this methodology have yet to be performed in an animal model of adRP associated with...
rhodopsin mutations. Since most RP patients retain some photoreceptor-based light sensitivity, treating these patients with AAV expressing light-sensing molecules will add difficulty to the interpretation of clinical trials and may produce incoherent, subjective visual responses in patients.

Conclusions

The first-point mutation in RHO leading to adRP was identified in 1990.\textsuperscript{137} At that time, and, indeed, for many years thereafter, advice given to adRP patients and their families involved accommodation for low vision and some estimation of the rate of visual decline. Recent advances, summarized above, enable the discussion of potential gene therapies for rhodopsin-associated adRP and argue for the genetic and phenotypic characterization of patients so that the appropriate treatment is chosen and suitable clinical outcomes measures are employed.

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