



# Inherited diseases of photoreceptors and prospects for gene therapy

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The photoreceptor cells of the retina are subject to a wide range of genetic diseases. This review summarizes current knowledge regarding an important group of retinal diseases caused by mutations in photoreceptor-enriched genes. In addition, progress toward treatment of a variety of these diseases in animal models via adeno-associated virus gene therapy is described. Although no human trials have yet been initiated to treat diseases caused by mutations in photoreceptor-enriched genes, there is a great deal of optimism regarding the prospects of treating these diseases using adeno-associated virus gene therapy.

The photoreceptor cells of the retina are subject to a greater number of Mendelian diseases than any other cell type in the human body [1,2,201]. Human retinal disease is currently known to involve nearly 200 different genetic loci. More than 130 of these loci have been identified, and of those genes whose expression pattern has been characterized, nearly half are enriched in photoreceptors [3,4]. Given the central importance of photoreceptors in the etiology of most forms of inherited blindness, the aim of this review is to survey the variety of heritable retinal diseases that specifically involve genes expressed in photoreceptors and to discuss the progress that has been made toward treatment of these diseases via virus-mediated gene therapy. For broad overviews of retinal gene therapy [5–7], discussions of nonviral strategies for ocular gene therapy [8,9] and reviews focused on specific classes of retinal disease [10–15], the reader is directed to the cited works.

## The structure of the human retina

The retina is an extension of the CNS that lines the back of the eye and mediates the first stages of visual perception [16]. It is divided into three cellular layers and two intervening synaptic layers (Figure 1). The photoreceptor cells, which reside exclusively in a single cellular layer of the retina known as the outer nuclear layer (ONL), mediate the transformation of light into electrical signals via a biochemical process known as phototransduction. This process takes place in an organelle unique to the photoreceptor cell known as the outer segment.

Photoreceptor cells come in two main varieties: rods and cones [17]. Rods mediate dim-light vision and are the predominant cell type of the human retina comprising nearly 95% of the

photoreceptors of the retina outside of the fovea [18]. Cones, in contrast, are responsible for bright-light vision and color vision. Although cones are far outnumbered by rods in the retina as a whole, they are a critically important cell type in humans, since they are the main constituents of the fovea, a small area in the central retina that mediates high-acuity vision. The macula, a somewhat larger area of the central retina that includes the fovea, is the target of a variety of inherited and acquired forms of photoreceptor degeneration, most notably, age-related macular degeneration [11].

Immediately adjacent to the outer segments of the photoreceptors lies a monolayer of pigmented cells known as the retinal pigment epithelium (RPE). These cells serve several important functions, including phagocytosis of the tips of the outer segments, retinoid metabolism and nutritive support of the photoreceptors [19]. After photoreceptors, the cells of the RPE are the second most commonly affected cell type in heritable blindness.

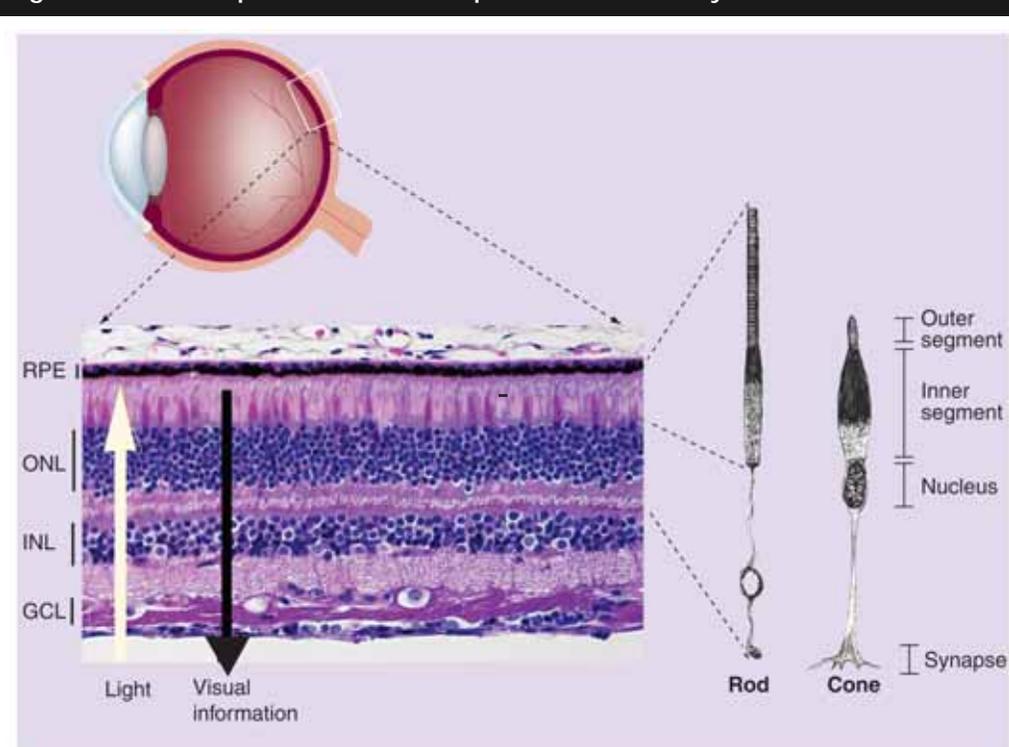
## The remarkable genetic heterogeneity of retinal disease

Inherited retinal diseases that involve photoreceptor-enriched genes can be classified according to a variety of different parameters including the following: mode of inheritance, clinical presentation, disease mechanism, cell type(s) affected and the biochemical function of the gene product involved. These various parameters are highly interdependent, and must therefore be considered together to fully understand the specific cellular and clinical consequences of a given disease-causing mutation. In addition, these parameters will determine the type of gene therapy used to treat specific forms of inherited blindness.

**Keywords:** adeno-associated virus, *cis*-regulatory element, gene therapy, photoreceptors, retina

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Figure 1. Photoreceptors are the nexus point of our visual system.



The human retina (depicted here as a hematoxylin and eosin-stained section) is an extension of the CNS that lines the back of the eye. Photoreceptors reside in the outermost cell layer of the retina known as the ONL. They serve to transduce incoming light into electrical signals that are subsequently processed by the rest of the retina before being transmitted to the brain as visual information. Photoreceptors come in two varieties: rods and cones. Rods are responsible for vision under dim-light conditions (i.e., at night), and cones subserve high-acuity vision under bright-light conditions as well as color vision. The outer segment is a specialized organelle found only in photoreceptors that contains the biochemical machinery responsible for transducing incoming light into an electrical signal. The drawings of human rod and cone photoreceptors depicted here are derived from [17].

GCL: Ganglion cell layer; INL: Inner nuclear layer; ONL: Outer nuclear layer; RPE: Retinal pigment epithelium.

From a clinical perspective, the two most commonly used classification parameters are mode of inheritance and clinical presentation. Retinal diseases can be inherited in an autosomal recessive, autosomal dominant and X-linked fashion. In addition, rare instances of digenic inheritance have been reported in patients with retinal disease [20,21]. Patients showing digenic inheritance carry mutations in two separate genes which, individually, do not cause disease, but together result in photoreceptor degeneration. Such cases frequently involve genes functioning in a common biochemical process. Some forms of photoreceptor degeneration attributable to mutations in mitochondrial DNA are also known [201], but since the genes involved are not preferentially expressed in photoreceptors, they will not be discussed further.

A well-defined clinical phenotype such as is seen in patients with retinitis pigmentosa (RP), can be caused by mutations in a wide range of

different genes [201]. In addition, distinct mutations in a single gene can cause a range of different clinical presentations. One striking example of the latter phenomenon is the gene *ABCA4*. Mutations in this gene can cause Stargardt disease (a frequent cause of macular degeneration in children), fundus flavimaculatus (a Stargardt-like condition with later onset), cone-rod dystrophy or RP [22,23]. The range of conditions caused by *ABCA4* mutations and the frequency with which mutations are found in this gene make it one of the most commonly affected loci resulting in photoreceptor disease [22].

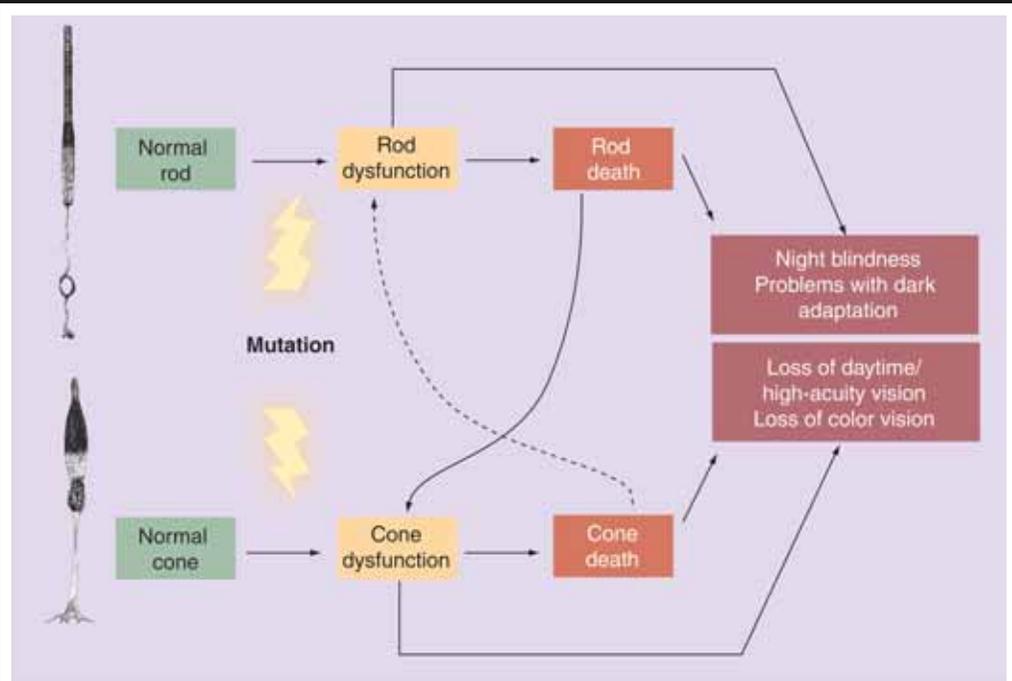
The mode of inheritance in retinal disease is closely tied to the specific mechanism whereby a mutation causes disease. Recessive inheritance is most commonly due to mutations that result in a decrement or complete loss of function of the gene product in question. Since the function of that gene product is required for normal

photoreceptor function or survival, degeneration ensues. Autosomal recessive inheritance can be seen in retinal diseases with many types of clinical presentation [201]. Many of the genes involved in retinal disease subserve functions related to specific aspects of photoreceptor structure/function: the phototransduction cascade (e.g., *RHO*, *GNAT1*, *PDE6A* and *CNGA1*), retinoid metabolism (*RDH12*), the connecting cilium and basal body (*RPGRIP1* and the genes associated with Bardet–Biedl syndrome), outer segment structure (*ROM1* and *RDS*) and synaptic function (*RIMS1*). Loss of function in any of the critical components of these photoreceptor biochemical pathways or structures can predispose the cell to dysfunction and possibly death (Figure 2).

In contrast to recessive inheritance, dominant disease can be caused by mutations that result in loss or gain of function or which generate a gene product that acts in a dominant-

negative fashion. Dominant disease owing to a loss-of-function allele (also known as haploinsufficiency) results when a reduction in the activity of the gene product to half of its normal levels is sufficient to trigger photoreceptor degeneration. This mechanism may be operative in some forms of photoreceptor degeneration, but the evidence is relatively sparse. For example, it has been hypothesized that the *C214S* mutation of peripherin/retinal degeneration slow (*RDS*) represents an instance of photoreceptor disease caused by haploinsufficiency. Humans with this mutation develop a late-onset form of autosomal dominant RP [201]. Naash and colleagues have studied a mouse model carrying the same mutation and concluded that the disease mechanism is likely to be haploinsufficiency [24]. By inference, the human form of the disease may therefore also be due to haploinsufficiency.

Figure 2. Pathways of photoreceptor degeneration in inherited disease.



The extent to which rods, cones or both cell types are affected by a particular mutation depends on a variety of factors, including the expression pattern of the affected gene. Mutations in many rod-specific genes, for example, cause retinitis pigmentosa. In this condition, there is often initial global rod dysfunction, which frequently manifests in adolescence as night blindness and problems with dark adaptation [10]. With time, rods begin to die, usually beginning in the mid-peripheral retina. This rod cell death, in turn, leads to secondary cone dysfunction and death via unknown mechanisms. The net result is a ring scotoma, a circumferential area of decreased rod and cone function in the mid-periphery. Later, rod and cone loss extends further into the far periphery and the patients develop tunnel vision. Ultimately, secondary cone dysfunction and death eventually extend to the central-most portion of the retina, the fovea, resulting in a severe loss of acuity. Mutations in cone-specific genes, in contrast, usually result in isolated cone dysfunction with early loss of central visual function and color vision. In at least one reported case [52], a mutation in a cone-specific gene has been shown to produce secondary rod dysfunction as well (dotted arrow).

Gain-of-function mutations produce a disease phenotype via either misexpression (temporally or spatially) or acquisition of a novel activity, which disrupts normal cellular function [25,26]. Many rhodopsin (*RHO*) alleles are thought to cause disease via a gain-of-function mechanism [25,26]. For example, the *P23H* mutation, which is the single most common mutant *RHO* allele among American patients with RP, has been shown in cell culture to result in retention of the mutant protein in the rough endoplasmic reticulum and subsequent intracellular aggregation [27,28]. The net result is thought to be a backup of the system involved in transporting protein to the outer segment, which ultimately leads to cell death. Although studies carried out in heterologous expression systems are clearly required to dissect the mechanistic basis of the disease, it should be noted that in most cases the proposed mechanisms have not yet been confirmed in diseased human retinas. This fact is attributable, in part, to the paucity of detailed histopathologic studies of retinas from patients with known gene mutations [29].

A second type of gain-of-function mechanism is one in which a mutation results in a gene product that is constitutively activated. Aberrantly activated forms of Rhodopsin have been hypothesized to cause retinal degeneration via constitutive activation of its downstream signaling cascade [25,30]. It is known that exposing an animal to continuous light for extended periods of time can cause photoreceptor degeneration [31]. By analogy, Fain and Lisman have proposed the 'equivalent light hypothesis', which states that some molecular defects that cause constitutive activation of the phototransduction cascade might trigger electrical signals in the photoreceptor equivalent to those produced by continuous light and, thereby, trigger degeneration over time [32,33]. A variety of experiments have been carried out that lend credence to this hypothesis [34,35], but the downstream mechanism whereby constitutive activation might ultimately lead to cell death is not known (but see [33] for a proposed mechanism involving decreased levels of intracellular calcium). Clearly, a range of different types of gain-of-function mutation can result in dominant retinal disease.

Dominant-negative mutations cause disease by producing an aberrant gene product that interferes with the function of the normal protein [36]. Dominant-negative mutations are

often observed in genes whose protein product forms a dimeric complex, thereby 'poisoning' the function of the normal protein. A dominant-negative mechanism of action has been hypothesized to be operative in a number of mutations affecting transcription factors required for photoreceptor gene expression. For instance, mutations in the genes encoding NRL and NR2E3, two rod-specific transcription factors, have been shown to cause autosomal dominant RP [37,38]. NRL is thought to function as either a homo- or heterodimer and NR2E3 as a homodimer. This fact raises the possibility that certain mutations in these proteins can poison the activity of the dimer, as a whole, even though the other member of the dimer is normal. The distinction between gain-of-function and dominant-negative mechanisms of action is an important one as regards gene therapy, since the effect of a dominant-negative allele can, in principle, be overcome by overexpression of a wild-type allele, whereas a gain-of-function allele cannot be so overcome [25].

The clinical features of a patient with inherited retinal degeneration are among the most important criteria used to classify the disease. A wide range of clinical categories of disease exist and reflect such features as funduscopic appearance, electroretinographic findings, cell type(s) affected and the presence/absence of extraretinal manifestations. If such extraretinal manifestations are present, the condition is referred to as syndromic blindness. The two largest categories of syndromic blindness are Usher syndrome and Bardet-Biedl syndrome. The former manifests as a combination of RP and sensorineural hearing loss, and can be caused by mutations in a range of different genes (e.g., *USH2A*, *CDH23*, *PCDH15* and *MYO7A*) [14,39]. Bardet-Biedl syndrome consists of rod-cone dystrophy associated with a spectrum of other abnormalities including postaxial polydactyly, central obesity, mental retardation, hypogonadism and renal dysfunction [40]. All of the genes implicated in this syndrome (*BBS1*, *BBS2*, *BBS3* and so on) appear to function within the basal body of ciliated cells [15,40]. Nonsyndromic forms of blindness, as the name implies, consist of those diseases in which the retina is exclusively affected.

The clinical features of different forms of inherited blindness are frequently related to the spatiotemporal pattern of expression of the affected genes. Photoreceptor genes can have expression patterns restricted to photoreceptors

or can show expression in additional retinal and extraretinal cell types [41]. For example, the genes implicated in Usher syndrome are expressed in photoreceptors (and often RPE), as well as in the hair cells of the inner ear [14,39]. This pattern of expression is consistent with the clinical phenotype. Another striking example is that of a recently reported pedigree with cone–rod dystrophy owing to a mutation in the synaptic protein, RIMS1. Affected members of this pedigree (but not unaffected relatives) show enhanced cognition based on a standardized IQ test [42]. Given the widespread expression of RIMS1 throughout the brain and its role in modulating short- and long-term synaptic plasticity in animal models [43,44], effects on cognition may not come as a surprise. Nevertheless, enhancement of cognition owing to a disease-causing mutation is quite unusual.

Even those genes that are expressed exclusively in photoreceptors can show differences in the time-course of expression during development and the extent of expression in rods, cones or both cell types. For example, in the mouse, photoreceptor genes can show rod-specific expression (e.g., *Rho*, *Gnat1* and *Pde6a*), cone-specific expression (e.g., *Opn1sw*, *Gnat2* and *Cngb3*) or varying degrees of rod/cone coexpression (e.g., *Rdh12*, *Rslh*, *Sag*, *Rgs9*, *Rpgrip1* and *Pcdh15*) [41]. Mice are known to demonstrate some peculiarities of photoreceptor gene expression, such as coexpression of S and M opsins in individual cones [45], and care must therefore be exercised in extrapolating the expression pattern of photoreceptor genes in mice to the expression pattern of the orthologous genes in humans. Nevertheless, cell-type specificity of expression is often a good predictor of disease phenotype. For example, achromatopsia is a severe disorder that typically presents in infancy with a variety of manifestations including total color blindness and poor visual acuity [201]. The primary defect in this disease is a lack of functional cone photoreceptors, which is owing to mutations in one of three genes: *CNGA3*, *CNGB3* and *GNAT2* [46]. Within the retina, all three of these genes appear to be expressed exclusively in cones, a finding that correlates well with the observed phenotype.

Mutations in many rod-specific genes, in contrast, result in RP. Early in this disease, rods are preferentially affected resulting in night blindness and loss of mid-peripheral vision [10]. These clinical findings correlate with the preferential expression of many RP genes in rods. However, with time, patients with RP suffer

progressive loss of cone-dependent vision also (Figure 2). This progression from rod to cone involvement occurs even in those forms of RP that are caused by genes exclusively expressed in rods (e.g., *RHO*). The reasons for the progressive loss of cones in these patients are not entirely clear. Some possible explanations for this ‘bystander’ effect include the release of toxic substances from the dying rods, detrimental effects caused by local collapse of the outer nuclear layer in areas of heavy rod cell loss, or possibly loss of a rod-derived cone viability factor (RDCVF) [47–49]. Sahel and colleagues have isolated a putative RDCVF (*Txn16*) [48]. However, *Nrl*<sup>-/-</sup> retinas, which contain no rods [50,51] and show a 3.5-fold decrease in *Txn16* transcript levels by microarray [4], retain preserved electroretinograms past 6 months of life [50]. These findings suggest that loss of RDCVFs are unlikely to fully account for the cone bystander effect. Recent work has shown that even cone-cell loss owing to mutation in a cone-specific gene, *CNGB3*, can lead to secondary rod dysfunction [52]. This latter finding provides additional support for the notion that bystander effects in photoreceptor cell death may be complex and could relate to the release of toxic substances from dying cells or possibly to the influx and activation of inflammatory cells such as macrophages/microglia in response to cell death in the outer nuclear layer [53,54]. Understanding this bystander effect is important from a clinical point of view, because much of the morbidity associated with rod-based diseases relates to the secondary cone cell loss that eventually supervenes.

Recent progress in treating animal models of blindness via gene therapy Given the bewildering range of photoreceptor genes that have been implicated in hereditary blindness, prospects for treatment may seem daunting. However, there has been remarkable recent progress in gene therapy approaches to treating various preclinical models of inherited blindness [55–58]. Still, significant hurdles exist in translating these results into the clinic. One issue that must be taken into consideration is that retinal degenerations progress at different rates depending upon the particular gene affected, the mutation involved and the patient’s clinical history and genetic background. Compounding this problem is the fact that the time at which treatment can be initiated cannot be controlled as easily in human patients as in animal models.

Ideally, we would treat all patients before the onset of any significant functional compromise, but this is currently not possible. Another important consideration is that gene therapy is only feasible while viable photoreceptors are still present. For successful gene therapy in an individual patient with recessive disease, several additional prerequisites must be met. First, the causative gene must be identified so that a rescue transgene can be constructed. Second, expression of the rescue transgene must be targeted to the correct cell population in the eye. Targeting involves three related factors:

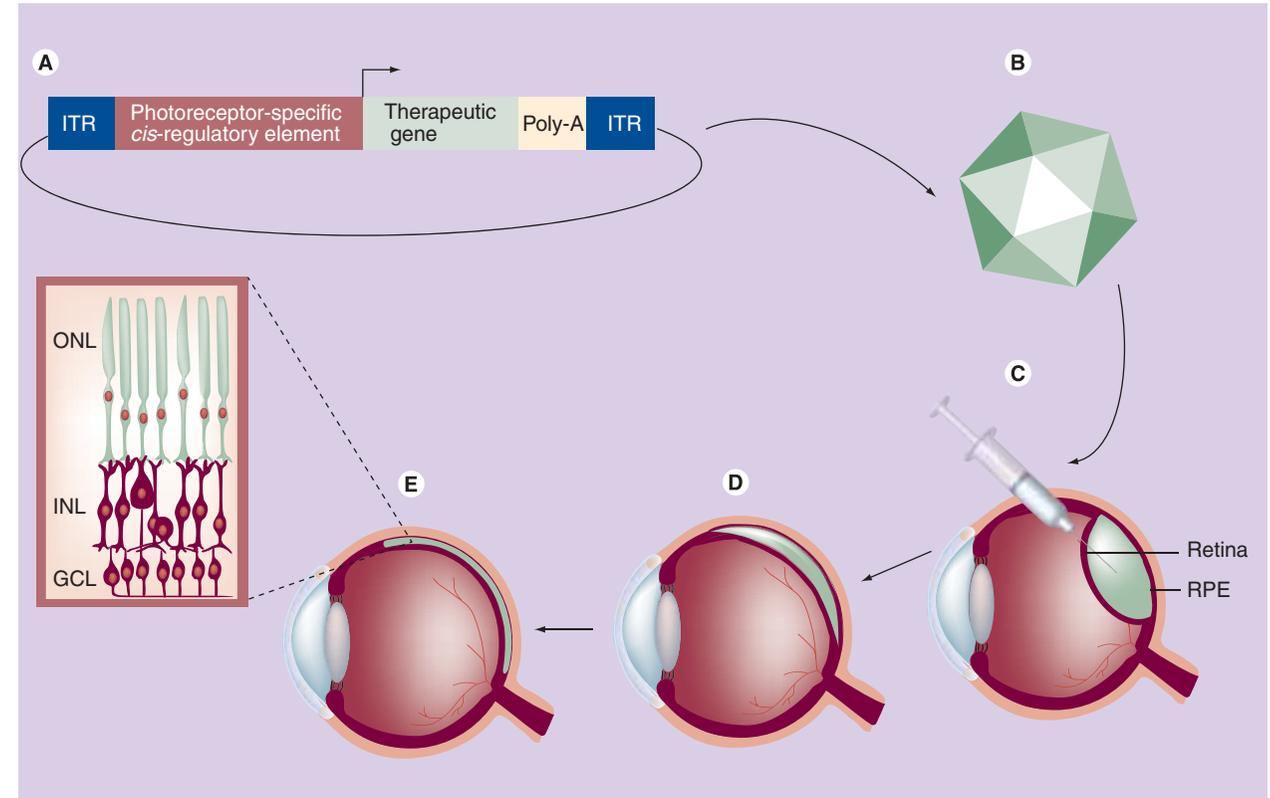
- Anatomic delivery of the gene therapy vector to the target tissue;
- The tropism of the virus for the specific cell population being targeted;
- Inclusion within the therapy vector of an appropriate *cis*-regulatory element (CRE; i.e., promoter/enhancer) to drive expression of the transgene in a spatiotemporally correct pattern and at endogenous levels once it has been delivered to the target cell population (Figure 3).

Recent progress in the mapping and identification of human retinal disease loci has greatly facilitated the characterization of causative mutations in individual patients. In addition, progress is being made in developing a range of viral delivery systems [5,59]: lentiviral, adenoviral and adeno-associated viral vectors have all been tested in preclinical models of blindness [55,60–63]. Although lentiviral vectors have been shown to effectively target RPE [64] and photoreceptors when administered in the early postnatal period in mice [60], they do not efficiently target photoreceptors in the adult mouse retina [65–67]. As with lentiviral vectors, earlier generation adenoviral vectors were shown to successfully transduce photoreceptors and effect rescue of photoreceptor degeneration [61,62], but only when delivered in the early stages of retinal development [68]. However, a recent report has shown that improvements of the adenoviral vector, as well as the use of a photoreceptor-specific promoter to drive transgene expression, can markedly improve transduction of and expression in adult photoreceptors in the mouse [69]. This study opens the door to further use of adenoviral vectors for treating photoreceptor-based diseases. Adenoviruses have a number of advantages in this regard [69], in particular their large packaging capacity (36 kilobases [kb]).

On account of its wide cellular tropism and ability to efficiently transduce adult photoreceptors, recombinant adeno-associated virus (rAAV) is currently the most widely used delivery vector for retinal gene therapy [5]. The virus consists of a 4.7 kb genome flanked by inverted terminal repeats. Although it is possible to replace the entire viral genome with heterologous DNA to create a rescue construct, the packaging capacity is limited to 4.7 kb [59]. Since the coding sequences of some disease genes are this size or larger, using this vector to target such diseases is not feasible. Even when targeting genes whose coding sequence is less than 4.7 kb, relatively little space is left for inclusion of a gene-appropriate CRE to drive transgene expression. Potential solutions to this problem include the use of alternative viral delivery systems with larger cloning capacities, such as adenoviruses [59], and strategies to reduce the size of CREs necessary to drive appropriate patterns and levels of transgene expression. A particularly creative approach to the size limitation problem has been undertaken by Li and colleagues who set out to create a rescue construct to treat mice carrying mutations in *Rpgr*, a model of RP [70]. Since the retina-specific splice variant of the gene *Rpgr-ORF15* is greater than 4.5 kb (and therefore cannot easily be accommodated in AAV-based vectors), the authors demonstrated that a large internal repeat within the coding sequence could be selectively deleted without abrogating the construct's ability to rescue function when introduced on a transgene [70]. This study demonstrated that, in selected cases, it may be possible to accommodate even large genes within AAV-based vectors.

Treatment of recessive photoreceptor degeneration as well as dominant disease caused by haploinsufficiency should, in principle, simply require expression of a normal copy of the mutated gene at endogenous levels and in the appropriate cell types. For this reason, it is not surprising that most examples of successful treatment of retinal degeneration have been in models carrying recessive alleles. One of the first successful rescues was in the retinal degeneration slow mouse, which carries a mutation in *Prph2*, a gene encoding a photoreceptor-specific membrane glycoprotein [71]. Mutations in this gene in humans cause a variety of retinal disease, including dominant RP [201]. In this study, a normal copy of *Prph2* was fused to a 2.2 kb fragment of

Figure 3. Treating photoreceptors with gene therapy.



(A) Structure of a gene therapy vector containing a therapeutic transgene and a photoreceptor-specific *cis*-regulatory element, flanked by AAV-derived ITRs. (B) Packaging of the vector into the AAV capsid and production of recombinant AAV. (C) Delivery of the virus via injection into the subretinal space between the retina and the RPE. This step results in the formation of a small pocket of virus-containing fluid between the retina and the RPE (the size of which is exaggerated in this diagram). (D) The rAAV spreads throughout the subretinal space, and the retina begins to reattach to the RPE. The virus infects retinal cells as well as cells of the RPE. (E) Retinal reattachment is complete and the therapeutic transgene is turned on specifically in photoreceptors under the control of the photoreceptor-specific *cis*-regulatory element.

AAV: Adeno-associated virus; GCL: Ganglion cell layer; INL: Inner nuclear layer; ITR: Inverted terminal repeats; ONL: Outer nuclear layer; RPE: Retinal pigment epithelium.

the bovine *Rhodopsin* (*Rho*) which is known to drive strong expression in rods, and introduced into the mutant retina in an AAV2 virus via subretinal injection at post-natal day 10 (P10). This treatment resulted in temporary improvement in photoreceptor structure and function and evidence of rescued visual function in the superior colliculus, an important visual relay station in the midbrain [71,72].

Recently, two studies demonstrated successful treatment of a murine model of juvenile retinoschisis, an X-linked recessive disease caused by mutations in the *RS1* gene [6,56,73]. In the study by Hauswirth and colleagues, an AAV (serotype 5) vector was used to deliver a normal copy of human *RS1* driven by a 500 bp fragment of the mouse *Rho* promoter to the retinas of P15 mice [73]. This treatment resulted in res-

toration of visual function as assessed by electroretinography, as well as preservation of retinal structure as evidenced by increased thickness of the outer nuclear layer and a reduction in the amount of retinal splitting (a characteristic pathologic feature of this form of degeneration) [73].

In another study, Li and colleagues successfully treated *Rpgrip1*<sup>-/-</sup> mice, a model of Leber's congenital amaurosis, which is a severe photoreceptor degeneration affecting both rods and cones, usually with neonatal onset [201]. The authors were able to achieve significant rescue of rod structure and function by introducing a normal copy of *Rpgrip1* under the control of a 200 bp fragment of the mouse *Rho* promoter into photoreceptors via AAV2-mediated transduction. Despite the rod preservation achieved,

there was no rescue of cone function. This result was expected, since the promoter used to drive expression of the rescue construct was rod-specific. The authors highlight the need for further studies using pan-photoreceptor promoters that can target both rods and cones. In this regard, a recent report of a compact pan-photoreceptor promoter upstream of *Grk1* may prove useful [74].

Recently the first successful treatment of a cone-based disease was achieved [63]. Hauswirth and colleagues treated the *Gnat2*<sup>-/-</sup> mouse, a model of achromatopsia, with an AAV5-based rescue construct containing a 2.1 kb portion of the human red/green cone opsin promoter driving expression of a wild-type copy of *Gnat2*. The treatment resulted in functional rescue as measured by electroretinography. In addition, visual acuity was restored to nearly wild-type levels as measured by optomotor response to a rotating sine-wave grating [63]. Despite these successes, the authors noted that in order to target therapy to blue cones as well as red/green cones in future human therapy, a pan-cone promoter capable of driving expression in all cones would be desirable.

Unlike recessive retinal degenerations that require simple gene replacement, the treatment of dominant disease caused by gain-of-function alleles necessitates either elimination of the abnormal allele or mitigation of its downstream effects. More than 100 different mutations have been identified in the *RHO* gene in patients with RP, most of these mutations being dominant [25]. Given the tremendous costs involved in bringing a novel therapy into the clinical setting, it may not be practical to develop gene therapy vectors, which only target individual mutations. Clearly what is needed is a versatile, mutation-independent therapy that can be applied to patients with a range of different dominant mutations. One approach to this problem is to suppress the abnormal transcript (either via ribozyme treatment or RNA interference [RNAi]) and then replace it with a codon-modified version of the wild-type gene that is immune to degradation [75]. A recent report demonstrated the first successful use of this approach *in vivo* [76]. In this study, the authors showed convincing reduction of endogenous *Rho* transcripts to approximately 10% of wild-type levels in cells transduced with an RNAi construct directed against *Rho*. Next, the authors treated newborn mice carrying a dominant mutant allele of *RHO* (P23H) with an AAV vector containing

this *Rho* RNAi cassette, as well as a rescue construct containing a codon-modified version of wild-type *Rho* driven by a 1.7 kb fragment of the mouse *Rho* promoter. They were able to demonstrate a modest rescue of ONL thickness in the treated retinas, suggesting improved photoreceptor survival. One significant drawback of this study is the rapidity of the degeneration seen in the *RHO* (P23H) mice relative to the kinetics of expression of the AAV-delivered transgene. One study suggested that it can take months to observe full expression of transgenes delivered via AAV [77], a time-course that is not compatible with treating a disease that results in near total photoreceptor loss within weeks. This fact suggests that given a longer time window for expression of the rescue construct (perhaps by using a model with a slower time-course of degeneration), the authors might expect to see even more dramatic rescue. This approach has clear promise for the treatment of dominant retinal disease.

Another recent study demonstrated the feasibility of treating mice with a dominant, gain-of-function allele by introducing another disease gene, albeit one with a counteracting effect [78]. Patients with the Y99C mutation in *GUCAIA* develop a late-onset cone dystrophy [79]. Likewise, mice carrying the Y99C allele show photoreceptor degeneration beginning as early as at 3 weeks of age [80]. The Y99C allele is dominant and appears to represent a gain-of-function allele, which leads to increased production of the photoreceptor second messenger cGMP, thus resulting in degeneration. Another mutant allele in humans, the constitutively active G90D allele of *Rho*, was shown previously to cause congenital stationary night-blindness, which is a mild form of blindness that causes rod dysfunction only [81]. Since rods are not lost in this disease, there is no secondary cone dysfunction or loss, and daytime vision is therefore well preserved. The authors hypothesized that since elevated cGMP caused by the Y99C allele of *GUCAIA* was the cause of the degeneration, introduction of the constitutively active G90D allele of *Rho*, which causes a reduction in cGMP levels, should counterbalance the effect of the Y99C allele and rescue the phenotype of the mice [78]. In fact, the mice carrying both the Y99C and the G90D alleles showed significant rescue of photoreceptor survival and function compared with those mice carrying the Y99C allele alone. Although this study was performed with transgenic mice and not virus-mediated gene therapy, it represents a

fascinating proof-of-principle that it may be possible to treat one form of retinal degeneration by counteracting it with expression of another mutant gene that causes a milder form of blindness. This study also demonstrates the value of exploiting detailed pathophysiologic knowledge of particular forms of retinal degeneration for therapeutic purposes.

One final gene-independent approach to treatment of photoreceptor degeneration is the delivery of neurotrophic factors or anti-apoptotic agents to the degenerating cells. Since apoptosis is thought to be the proximate cause of degeneration in most forms of heritable blindness [82,83], in theory, its prevention should slow the progression of the disease. Similarly, neurotrophic factors could function by replacing endogenous factors lost during the progression of disease or by supplementing natively expressed survival factors in the ONL. A variety of such approaches have been tried in animal models, some with considerable success (reviewed in [6]). One notable case is that of ciliary neurotrophic factor (CNTF), which has been shown to retard retinal degeneration in a range of animal models [84–86], but there are some concerns regarding toxic effects of CNTF at higher doses [87,88]. A recent Phase I clinical trial in humans showed that encapsulated cell intraocular implants expressing CNTF appear to be safe [89]. Further studies to test the efficacy of this treatment in humans are underway.

Overall, there has been significant progress in the development of AAV-mediated gene therapy for a variety of forms of both recessive and dominant photoreceptor degeneration in preclinical models. Although no human clinical trials have yet been undertaken to treat retinal disease caused by mutations in photoreceptor-specific genes, in May 2007, a gene therapy trial in humans for inherited eye disease caused by mutations in the retinal pigment epithelium gene, *RPE65*, was begun [90]. The successful treatment of a canine model with mutations in *RPE65* has raised significant hopes of a positive outcome in human patients [58,91,92]. It is likely that the coming decade will see the initiation of human trials for additional forms of inherited blindness, including those owing to mutations in photoreceptor genes. Enthusiasm for the treatment of previously untreatable forms of inherited blindness is understandably high, but must be tempered by safety concerns. A couple of high-profile cases of patient fatality in the context of gene therapy a number of years ago

led to turmoil in the gene therapy research community and regulatory crackdowns [90,93]. The recent death of a patient receiving AAV-based gene therapy for arthritis has rekindled concern over safety issues [93]. Although it is not clear that this death was directly linked to the gene therapy treatment, caution is still warranted. Furthermore, recent studies have shown that aged mice previously treated systemically with an AAV vector develop hepatocellular carcinoma with increased frequency [94,95]. This effect was shown to be attributable to rare viral integration events into a specific genomic locus [95]. These findings are concerning, but it must be remembered that no such tumorigenic effect has yet been demonstrated in humans and could therefore represent a species-specific event. In addition, intraretinal therapy may minimize systemic dissemination of the virus and thereby circumvent such adverse effects [5]. Clearly, vigilance is warranted in all human gene therapy trials, but there are grounds for optimism regarding the potential of these treatments to cure retinal disease.

### Future perspective

The prospects for gene therapy-based treatment of the nearly 200 different forms of inherited retinal disease are bright. Discovery of the genes mutated in photoreceptor degenerations in humans is proceeding rapidly with new loci being reported almost every month [201]. Detailed knowledge of the genes involved should permit the development of better and more rapid diagnostic tools for quickly pinpointing the precise molecular defect present in individual patients. Furthermore, the continued study of AAV-based gene therapy vectors in a variety of small and large mammalian models will pave the way for direct translation into human therapies. The eye has many advantages for gene therapy: its compartmentalized nature minimizes risk of systemic dissemination of the therapy vector; immune responses to the vector and transgene after intraocular administration are attenuated relative to injection in other sites owing to the immune-privileged status of the retina; and the retina is readily amenable to visual and electrophysiologic monitoring of therapeutic effect [5,6,90]. All of these features make the eye uniquely suited for the application of gene therapy approaches to heritable disease. In the future, we are likely to see the development of novel vectors for gene delivery in the retina [69,96], as well as improvements in

**Executive summary****The remarkable genetic heterogeneity of retinal disease**

- Many forms of inherited blindness are due to mutations in photoreceptor genes, making them important targets for gene therapy.
- In the human retina, 95% of the photoreceptors are rods, but the relatively small cone population is responsible for high-acuity vision and color vision.
- Inherited diseases of photoreceptors can be classified by the mode of inheritance, disease mechanism, clinical features or cell type affected.
- Mutations can affect either cones or rods preferentially, with rod cell death often resulting in secondary cone dysfunction/death.

**Recent progress in treating animal models of blindness via gene therapy**

- Gene therapy for retinal disease requires three basic components: a rescue transgene, a promoter to drive transgene expression and a delivery vector with appropriate cellular tropism.
- Recombinant adeno-associated virus is the most widely used delivery vector for retinal gene therapy.
- There has been remarkable recent progress in the treatment of preclinical models of photoreceptor degeneration using recombinant adeno-associated virus-mediated gene therapy.

**Future perspective**

- In May 2007, the first human gene therapy trial for an inherited form of blindness (caused by mutations in a retinal pigment epithelium-specific gene) was begun.
- Future directions in the field of photoreceptor gene therapy include the development of novel vectors for gene delivery, improvements in the efficacy of existing vectors and the development of more effective promoters for targeting therapeutic transgenes to photoreceptors.
- Given the high costs of clinical trials, there will be a continued focus on developing gene- and mutation-independent approaches to therapy for photoreceptor degenerations.

the efficacy of existing vectors such as AAV [97]. Furthermore, recent studies are paving the way for the development of a wide range of more effective promoters for targeting rescue constructs to photoreceptors [4,74,98–100]. Lastly, given the costs of clinical development, there will be a continuing focus on the development of mutation-independent approaches to therapy that can be applied to a wide range of photoreceptor degenerations.

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### Website

101. link to the RetNet database, which is an excellent resource for current information on all genetic forms of blindness [www.sph.uth.tmc.edu/RetNet/](http://www.sph.uth.tmc.edu/RetNet/)