Transgenic Animal Models for the Study of Inherited Retinal Dystrophies

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Problem of Inherited Retinal Disease

The retina is a complex tissue in the back of the eye containing the rod and cone photoreceptor cells and a network of interneurons that transfer visual information to the brain. The retina is susceptible to a variety of diseases that can lead to compromised vision or blindness. A significant fraction of retinal diseases has been established to have a clear genetic basis. The inherited retinal degenerations are typified by retinitis pigmentosa (RP[1]), an affliction characterized by the gradual breakdown and degeneration of the photoreceptors. This group of debilitating conditions affects approximately 100,000 people of all ages in the United States (NIH 1998). In the elderly, the leading cause of visual loss is age-related macular degeneration (AMD[1]), an eye disease of the macula, a small area in the retina that is responsible for the central vision used in high-resolution activities such as reading, sewing, and driving. A person with AMD gradually loses this clear central vision. It is estimated that AMD causes visual impairment in approximately 1.7 million of the 34 million Americans older than 65 yr (NIH 1997). These inherited diseases are progressive, and no therapies are in clinical use at the time of this writing (NIH 1998). There is a critical need to achieve a better understanding of the disease mechanism in these dystrophies in order to design rational therapies. To this end, a major research effort is under way to identify the cellular, molecular, and systemic factors involved in the pathophysiology of these diseases. Laboratory investigations are using many different approaches, including epidemiology, morphology, cell and molecular biology, and genetics.

Since the late 1980s, researchers have cloned or mapped to a specific chromosome 98 different genes causing inherited retinal diseases. Molecular geneticists have identified the defective genes involved in two types of AMD, and 10 genes have been found to cause RP. Knowing the genes associated with these diseases will enable researchers to determine the relationship of the gene product to the degenerative process. Knowledge of the causative gene permits development of animal models of these dystrophies, using transgenic technology. These models are critical to our understanding of the pathophysiological mechanisms and for the development of methods to modulate the disease process. Therapies found to be most promising in the animal models will be considered for human clinical trials.

Need for Animal Models of Inherited Retinal Disease

Retinal diseases do not carry an increased risk of mortality; consequently, human donor eyes at early stages of the diseases rarely become available. Accordingly, most of our data about human retinal disease have been gleaned from a small number of "informative" patients or donor tissues obtained in an intermediate stage of disease, in which there were surviving photoreceptors to examine (Flannery and others 1989, 1990). Retinal biopsies from affected individuals are extremely rare since it is considered unethical to put a patient's remaining vision at risk from biopsy surgery before there is the potential for treatment (Bird and others 1988). This situation has led scientists worldwide to turn to studying retinal degeneration mutations in laboratory animals. Animal models of human diseases have played a prominent role in vision research, and much has been learned from them about the cellular mechanisms of inherited photoreceptor degenerations and potential therapeutic measures for these diseases. Among the various species with retinal degenerations, mice and rats have been used most extensively. This is primarily because of the following experimental advantages: short gestation time, small size, powerful genetic control in the form of several readily available retinal degeneration mutants, multiple inbred and congenic strains with genetic controls, and different eye pigmentation types. In addition, investigators using rodents can carry out certain embryological procedures such as the production of experimental chimeras and transgenic mice and rats.

The rapidly escalating costs of maintaining animal colonies on individual grant budgets are beyond the means of most vision scientists, and most investigators do not have the time or expertise in mammalian genetics to develop or maintain various inbred, congenic, coisogenic, and transgenic rodent strains. As a result, the National Eye Institute of the

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[1]Abbreviations used in this paper: AAV, adeno-associated virus; adRP, autosomal dominant retinitis pigmentosa; AMD, age-related macular degeneration; cGMP, cyclic guanosine monophosphate; CNTF, ciliary neurotrophic factor; ERG, electroretinogram; GDNF, glial cell line-derived neurotrophic factor; IGF-I, insulin-like growth factor I; PDEb, beta subunit of the cGMP phosphodiesterase; rAAV, recombinant adeno-associated virus; RP, retinitis pigmentosa; RPE, retinal pigment epithelium.

National Institutes of Health and the Foundation Fighting Blindness (Executive Plaza I, Suite 800, 11350 McCormick Road, Hunt Valley, Maryland 21031-1014) have developed and maintained breeding colonies of such mice and rats that are appropriate for studies on various forms of inherited and environmentally induced retinal degenerations and have distributed these animals and eye tissues to investigators who request them.

Naturally Occurring and Transgenic Animal Models

Transgenic techniques have enabled investigators to insert new genes into the retinas of laboratory animals to develop animal models that mimic the human disease. Before the advent of this technology, animal models developed at random through inbreeding or random inheritance of mutations in photoreceptor genes. Examples of these "naturally" occurring retinal degeneration models are the Irish setter (Suber and others 1993), briard dog (Wrigstad and others 1994), Abyssinian cat (Narfström 1983), Royal College of Surgeons (RCS) rat (Edwards and others 1977), and retinal degeneration (*rd*) (Bowes and others 1990) and retinal degeneration, slow (*rds*) (Connell and others 1991) mouse models. The ability to create transgenic animals has made the search for informative patients and naturally occurring animal models less acute because any single gene of interest can now be introduced (Chen and others 1995; Lem and others 1992; Travis and others 1992) or removed (Humprhies and others 1997; Xu and others 1997) from the photoreceptor in a targeted fashion.

Animal studies have not proceeded in isolation; there has been a great deal of interaction between basic researchers and clinical studies involving patients. In fact, animal models of inherited retinal diseases have played a vital role in uncovering the genetic and biochemical defects in some human retinal diseases. For example, the mutations responsible for retinal degeneration in the *rd* and *rds* mouse strains arose spontaneously in the wild many decades ago (Bowes and others 1990; Pittler and others 1991). Very recently, it has been shown that mutations in the human homologues of these murine genes cause some types of RP (NIH 1998). Conversely, gene identifications in patients have provided "candidate genes," which have led scientists to the genetic defects that have spontaneously arisen in animal breeding colonies. As new eye disease-causing genes are identified in the patient population, investigators create new animal models with retinal degeneration. These animals are particularly informative because they carry identical mutations in the same gene as a particular group of patients.

Transgenic mice, rats, and pigs expressing dominant mutations are gradually replacing other animals in ophthalmic research as specific animal models are developed for defined applications. Transgenic models are being used to evaluate compounds and gene therapy protocols before clinical trials in patients. Current needs are to produce results in a shorter time, to use fewer animals, and to provide data more relevant to the prevention of human blindness. Ultimately, the goal of producing these transgenic animal models is development of medical therapies for blinding diseases of the eye. A medical therapy must meet standards of safety, efficacy, delivery, and cost before it can be successfully used in patients.

Production of Transgenic Animals for Retinal Studies

The principles underlying production and breeding of transgenic animals are outlined in a recent issue of *ILAR Journal* (Gordon 1997). Most transgenic animal models of ocular disease have been produced by these general techniques. In this section, I describe some specific technical modifications that have been made to adapt these techniques to the retina.

The design of a transgene must include regulatory elements to enhance and direct tissue expression to a particular target tissue. In the context of vision research, unique regulatory elements, or "promoters," are used to direct cell-specific expression to a single class of cells in the eye. To date, specific promoters have been developed that direct expression exclusively in retinal rod photoreceptors, cone photoreceptors, and lens epithelium. A concerted effort is under way to develop cell-specific promoters targeted to other retinal cell types, specifically, Müller glia cells, retinal pigment epithelium (RPE[1]), and retinal ganglion cells.

Ongoing development of new and improved transgenic animals in vision research is dependent on molecular studies of the regulation of ocular gene expression. These studies build on early work (Lem and others 1991; Zack and others 1991), defining the rhodopsin promoter in an effort to develop photoreceptor-specific promoters with more precise properties. Recently, transgenic mouse analyses of rhodopsin regulatory regions have identified both a rhodopsin proximal promoter region and a rhodopsin enhancer region (Kumar and others 1996; Nie and others 1996). The bovine minimal rhodopsin proximal promoter region is sufficient to direct photoreceptor-specific gene expression, and the rhodopsin enhancer region is important for high-level expression. The definition of rhodopsin promoter fragments that are active in transgenic mice has made possible studies in which exogenous or mutated proteins, such as dominant-negative forms of signaling receptors, are expressed in photoreceptor cells.

Although the transgenic approach for expressing gene products can be powerful, it is limited by poor control of the timing and expression level of the transgene. To address these limitations, several investigators are developing inducible and regulatable photoreceptor-specific promoters by combining the rhodopsin or other retinal cell-specific promoters with the reverse tetracycline transactivator system (see next section).

Inducible Transgenic Animal Models

Bujard, Gossen, and colleagues (1992, 1995) first described regulated high-level gene expression systems. Currently, there are commercially available tetracycline-off and tetracycline-on gene expression systems that allow high-level regulated gene expression in response to varying concentrations of tetracycline or tetracycline derivatives (for example, doxycycline). In the tetracycline-off system, gene expression is turned on in the absence of the antibiotic. In contrast, gene expression is activated in the tetracycline-on system in the presence of the antibiotic.

These systems were originally developed for use with cultured cells, and their use in cell culture is well documented. Inducible promoter elements are now being used in the development of transgenic animals in which expression of the transgene can be controlled by the quantity of tetracycline supplied to the animal in its drinking water or food. This approach will open new avenues for regulated gene expression in vivo (Fishman and others 1994). Potential applications for this technology in transgenic animal systems are very broad.

An application of this technology to retinal disease is the development of transgenic mice carrying an inducible *rds*/peripherin cDNA construct (Chang and others 1998). These transgenic mice have a genetic background with the *rds* mutation, which results in a gradual degeneration of the photoreceptors, caused by a null mutation in the *rds* gene. The inducible promoter-transgene construct allows investigators to control the onset time and dose of the transgenic protein expressed in the retina. These studies temporally and quantitatively assess how late in the degenerative process expression of the wild-type protein can lead to photoreceptor rescue.

Gene Expression Targeted to the Retinal Pigment Epithelium

Photoreceptors depend on cells of the adjacent RPE for metabolic support. Genetic defects in the RPE have been implicated in several ocular diseases, including AMD. There are no naturally occurring or transgenic models that mimic the pathophysiology of AMD. The hallmarks of AMD are cellular accumulations (drusen) in or under the RPE, irregularities in the pigmentation of the RPE, geographic atrophy, serous detachment of the RPE, and neovascularization. Because many of these physiological defects involve the RPE, there is a major effort under way to create an animal model of this disease. Investigators have successfully created mice with transgenes targeted to the RPE. Recently, an RPE-specific promoter (RPE-65) was identified (Marlhens and others 1997; Redmond and others 1998). In the mice expressing this transgene, reporter gene activity is restricted to the RPE, strongly suggesting that the RPE65 promoter is RPE specific and can be used to target genes to the RPE.

Transgenic Mouse Models of Retinitis Pigmentosa

Autosomal dominant mutations in the c-terminus of rhodopsin are found in many RP patients. In one of the first molecular studies of DNA from RP patients, Dryja and others (1990) found a transition from C to T involving the first nucleotide of codon 347—a change from CCG to TCG, which resulted in substitution of one amino acid (serine) for proline in the rhodopsin protein. To explore the pathogenic mechanism of dominant mutations that affect the C-terminus of rhodopsin and cause retinitis pigmentosa, Li and others (1996) generated five lines of transgenic mice carrying an identical proline to serine substitution at codon 347 (P347S) in mouse rhodopsin. The severity of photoreceptor degeneration correlated with the levels of transgene expression in these mice. Visual function was approximately normal at an early stage when there was little histological evidence for photoreceptor degeneration, but it deteriorated as photoreceptors degenerated. This mouse model is important because it is one of the first transgenic models carrying the identical point mutation as observed in a group of retinal degeneration patients.

Unfortunately, this transgenic mouse model has a major drawback for the study of the disease mechanism that is inherent in the transgenic technology itself. In autosomal dominant disease, the patient carries one mutant allele and one normal allele. The addition of a transgene does not inactivate or replace the endogenous gene. The disease mechanism in these transgenic models is different in that they retain two normal rhodopsin alleles in the genome, augmented by the mutant transgene. Therefore, although the rhodopsin transgene has a mutation analogous to that found in RP patients, it is expressed on a normal genetic background. Studies have found that expression of a normal rhodopsin transgene can also lead to retinal degeneration, complicating the interpretation of these transgenic models.

Knockout Animal Models of Retinal Disease

To solve the problem of transgenes augmenting the genome instead of replacing a normal allele, homologous recombination techniques have been developed to inactivate or "knockout" selected genes in the visual system. This technology generates animals with retinal cells that no longer express a normal gene product. Humphries and others (1997) generated mice carrying a targeted disruption of the rhodopsin gene to examine the functional and structural roles of rhodopsin in normal retina and the pathogenesis of retinal disease. Rhodopsin knockout mice failed to make normal photoreceptors and became blind within 3 mo. There was no rod electroretinogram (ERG^1) response in 8-wk-old animals. Heterozygous animals retained most of their photoreceptors, although the inner and outer segments of these cells displayed some structural disorganization, and the outer seg-

ments became shorter in older mice. These animals provide a useful genetic background on which to express other mutant opsin transgenes as well as a model to assess the therapeutic potential of reintroducing functional rhodopsin genes into degenerating retinal tissues.

Combined Transgenic and Knockout Models

Lem and colleagues (Frederick and others 1998) are combining approaches to create rodent models that carry transgenes on a knockout background. Rhodopsin knockout mice are used to study specific rhodopsin mutants on the background of only a single functional rhodopsin allele or in the complete absence of wild-type rhodopsin. Both rhodopsin hemizygous and homozygous knockout mice were developed for these experiments. These rhodopsin knockout mice are crossbred with transgenic animals expressing rhodopsin mutations. These combined transgenic/knockout animals are being used to test the hypothesis that different rhodopsin mutations have different physiological effects on receptor sensitivity. The retinal morphology is examined by light microscopy, and single cell recordings of the light-evoked photoresponse assess physiological function. Effects of the rhodopsin mutation on protein localization in the photoreceptors are assessed by immunohistochemistry.

Transgenic Rescue of Retinal Degeneration in the *rd* Mouse

As mentioned above, mice homozygous for the *rd* mutation display hereditary retinal degeneration, a naturally occurring animal model for human retinitis pigmentosa. Heterozygous *rd/+* mice are phenotypically normal. In affected animals, the retinal rod photoreceptor cells begin degenerating at postnatal day 8, and by 4 wk, no photoreceptors remain. The inherited defect in *rd* mice was recognized more than 70 yr ago and was described as the "rodless retina" mutation (Keeler and others 1924, 1927). Farber and Lolley (Farber and others 1974, 1976a,b) used biochemical measures to establish that *rd* degeneration is preceded by accumulation of cyclic guanosine monophosphate (cGMP[1]) in the retina. Molecular genetic studies determined that the *rd* retinal degeneration is caused by a defect in the gene encoding the beta subunit of the cGMP phosphodiesterase (PDEb[1]) enzyme in the rod photoreceptors .

After the initial identification of the *rd* gene as PDEb, two specific defects were found in the *rd* gene. Sequence analysis uncovered a large piece of viral DNA from a xenotropic murine leukemia virus (Xmv-28) in the promoter region (Bowes and others 1993). Bowes and coworkers suggested that blindness in this mouse strain is a result of infection of a "founder" mouse many decades ago with a virus, which resulted in a random insertion of viral DNA into a gene (PDEb) required for photoreceptor survival. This viral insertion has rendered the PDEb gene incapable of being transcribed into mRNA. Genetic studies by Pittler and others (1991) revealed that *rd* degeneration may have an alternative explanation, a nonsense mutation in the protein-coding region of PDEb. At the time of this writing, it remains unresolved as to which defect in the *rd* gene causes the death of the photoreceptors. Interestingly, both the viral DNA insertion and the point mutation are present in every strain of *rd* mouse tested and are widely distributed through many common inbred strains and recently captured wild mouse strains from different parts of the world. Several investigators have reported inadvertently generating blind laboratory animals by backcrossing normally sighted mice that were heterozygous for *rd* (*rd/+*). The finding of two distinct mutations in many strains suggests that the blinding *rd* defects in mice are ancient. The persistence of this blinding mutation in many mouse strains for decades suggests that vision is not essential for survival of the species.

In 1992, we were able to reverse the retinal degeneration in the *rd* mouse model by transgenic delivery of a normal PDEb gene (Lem and others 1992). The transgene generated normal PDEb protein beginning at postnatal day 5, before the onset of *rd* retinal degeneration. The *rd* mice carrying the transgene expressed normal PDEb mRNA and had PDE enzyme activity. Histological studies revealed that photoreceptor cell death was significantly retarded in these mice. Littermates that did not inherit the transgene were blind by 1 mo after birth. This technique for "rescuing" the *rd* phenotype serves as "proof of principle" for genetic correction of an inherited retinal degeneration. Clearly, injection of a therapeutic gene into the one-cell fertilized egg is unlikely to find clinical application. However, better surgical methods involving direct gene transfer to the eye are in development. Bennett and others (1996) were able to slow retinal degeneration in the *rd* mouse through subretinal injection of an adenovirus that contained the gene for normal PDEb. Subretinal injection of the virus into eyes of *rd* mice was carried out 4 days after birth, before the onset of retinal degeneration. After gene therapy, beta-PDE mRNA and enzyme activity were detected, and histological studies revealed a slowing of the retinal degeneration.

Transgenic Rescue of Retinal Degeneration in the *rds* Mouse Model

In the laboratories of Gabriel Travis and Dean Bok, transgenic mice that model the subclass of human RP caused by mutations in the *rds* gene (Travis and others 1998) are being studied. These mice produce defective *rds*/peripherin, a structural protein of the photoreceptor outer segment that is required for normal photoreceptor morphology and function. These investigators are also using these *rds* transgenic animals to test intraocular therapy by adenoviral delivery of a *rds* minigene similar to the gene construct that produced rescue via the transgenic approach.

Transgenic Pig Model of Retinal Degeneration

Fulton Wong of Duke University and Robert Petters of North Carolina State University have developed a transgenic pig model of retinal disease (Li and others 1998; Petters and others 1997; Tso and others 1997). As in the rodent models, the long-term objective of this research is to find a cure for retinal degenerative diseases such as retinitis pigmentosa. The rodent models indicate that mechanisms other than those immediately caused by the RP-inducing mutations in rhodopsin may play important roles in the human RP phenotype. With an RP-inducing mutation in human rhodopsin, for example, the rhodopsin mutation is expressed only in rod photoreceptors, yet cone photoreceptors die as well.

During the early stages of human RP, some cones die, along with most of the rods; nevertheless, a large population of cones survive but then degenerate slowly, over decades. Saving these cones, which survive long after most of the rods have disappeared, is one of the critical issues for RP therapeutic research.

The pig's eye is anatomically human-like, and unlike rodents, its retina has many cones. The transgenic pig model of RP expresses a rhodopsin mutation that causes retinal degeneration. By 2 mo after birth, most of the rods and some cones have died. A large population of cones degenerate slowly, and residual cones have been identified at 20 mo of age. Any treatment that can prolong the survival of these cones, therefore, will become a potential therapy for human RP.

Transgenic Rat Models for Testing RP Gene Therapy

Currently, animal models are being used to evaluate three modes of therapy for retinal degeneration: transplantation, drug delivery, and gene transfer. These therapies share a common requirement in that the transplanted cell, drug, or vector must be given by injection into the vitreous or subretinal space. The animal models available for in vivo testing of potential therapies include the naturally occurring and transgenic models in the mouse, rat, cat, dog, and pig. Mouse models are the most common, however, as the transgenic technology is most established in this species. Unfortunately, the small eye of the mouse is not ideal for procedures involving intraocular injection. The larger eye of the cat, dog, and pig models is better suited for subretinal injection and transplantation procedures. Unfortunately, these species are difficult and expensive to breed in large numbers.

Our laboratory at the University of California-Berkeley in collaboration with other laboratories developed a new model of retinal degeneration in the rat because this species has specific advantages for the testing of intraocular therapies (Steinberg and others 1997). The rat eye is several-fold larger than the mouse, which simplifies intraocular procedures and electroretinographic evaluation. In addition, rats breed as rapidly as mice, generating large litters in a short gestational time. To create these rats, a mouse opsin transgene was introduced into fertile one-cell wild-type albino Sprague-Dawley rat embryos. Chrysalis DNX Transgenics Sciences (301B College Road East, Princeton, New Jersey) performed the embryo injections.

Retinal degeneration in the transgenic rat model is caused by expression of either a P23H substituted or a c-terminal truncated rhodopsin, a gene defect that is similar to a significant fraction of the RP patient population. Approximately 30% of autosomal dominant retinitis pigmentosa (adRP[I]) families examined to date have mutations in rhodopsin (Inglehearn and others 1992). P23H is the most common rhodopsin mutation in the United States, comprising 24% of adRP rhodopsin mutations. The S334ter mutation is analogous to mutations commonly found in the c-terminal 28 amino acids of the rhodopsin molecule in RP patients.

The rats exhibit a course of retinal disease remarkably similar to that observed in humans bearing the same mutations. Rat photoreceptors have been well studied, and there is extensive knowledge of basic biological mechanisms such as membrane renewal. In addition, earlier experiments in the rat have shown that survival factors administered by intraocular injection can delay retinal degeneration (Steinberg 1994). Survival factor therapy has been successful in the RCS rat degeneration (Faktorovich and others 1990) and with photoreceptor degeneration in the Sprague-Dawley rat resulting from exposure to continuous illumination (Faktorovich and others 1991). In light damage experiments, photoreceptor cell death has been ameliorated with a number of different growth factors or neurotrophic agents, including basic fibroblast growth factor, ciliary neurotrophic factor, and brain-derived neurotrophic factor.

The goals of preclinical safety evaluation for retinal therapies will be to recommend initial safe starting doses in humans, to identify potential target organ(s) of toxicity, to identify appropriate parameters for clinical monitoring, and to identify "at risk" patient populations for exclusion from a clinical trial. A rat homologue will be useful to determine the safety of a therapy. Transgenic rats can be used to establish safety data on repeated administration of drugs, intraocular distribution of the therapy, or potential for autoimmune response. The transgenic rat homologue can also be used to test the safety of multidose administration, induction of inappropriate immune activation, and potential for autoimmune response to therapeutic proteins, as well as histopathology of target organs.

For ocular gene therapy, investigators will need (1) to determine the identity of and access to target tissue and cells, the efficacy of gene transfer and expression, inflammation associated with vector administration, duration of expression, and efficacy of repeat dosing; and (2) to establish clear-cut clinical end points.

Therapies with the Transgenic Rat Model

Viral-mediated Gene Therapy

Our laboratory, in collaboration with the laboratory of William Hauswirth at the University of Florida, has been testing intraocular gene therapy for autosomal dominant retinitis pigmentosa. Successful gene therapy for adRP requires an efficient and cell type-specific gene delivery/expression system, a targeted way to selectively inhibit production of the mutant protein, and valid animal models of adRP in which to test and optimize the viral delivery system.

We are using a recombinant adeno-associated virus (rAAV[1]) in which expression is driven by a portion of the rod opsin promoter, a gene construct analogous to that used to develop the transgenic rats described above. Using rAAV, we have achieved photoreceptor-specific expression of reporter genes in mouse, rabbit, and guinea pig by ocular injection (Flannery and others 1997). Other groups have used similar approaches with other viral vector systems to deliver genes to specific ocular targets (Di Polo and others 1998; Miyoshi and others 1997).

Ribozyme Therapy

We have made synthetic genes for several ribozymes that are catalytic RNA molecules capable of hybridizing to and destroying a specific target RNA (Drenser and others 1998). These ribozymes recognize the nucleotide change causing the P23H mutation or the S334ter mutation in the transgenic rat models. We tested ribozymes with two structural motifs, hammerhead and hairpin, for efficacy in knocking down the level of mutant mRNA. The hammerhead ribozyme is one of the smallest ribozymes known and has attracted much attention because of its potential for sequence-specific inhibition of gene expression. The hammerhead ribozyme cleaves an RNA sequence specifically adjacent to the general triplet sequence NUH, where N is any nucleotide and H can be A, U, or C. The hairpin ribozyme is a similar structure of a slightly smaller size (50 nucleotides) and a different secondary structure that contains four helical elements and two internal loops, which contain most of the catalytically essential groups.

Recombinant adeno-associated virus (AAV[1]) vectors incorporating a rod opsin promoter were used to transfer either hairpin or hammerhead ribozyme genes to photoreceptors (Lewin and others 1998). AAV was administered by subretinal injection in rats at postnatal day 15. In vivo levels of normal and mutant RNAs were measured by allele-specific RT-PCR at postnatal day 60. Eyes were examined by histopathology and morphometric analysis, as well as by ERG at postnatal days 60, 75, and 90. Expression of either a hammerhead or hairpin ribozyme markedly slowed the rate of photoreceptor degeneration for at least 3 mo as determined by counts of the numbers of surviving photoreceptors. The level of mutant opsin RNA relative to wild-type RNA was also reproducibly lower in ribozyme-treated retina. Electroretinography was used to make physiological comparisons of ribozyme-treated versus control eyes and demonstrated functional rescue. The conclusion from these studies is that ribozyme-directed cleavage of mutant mRNAs is a potentially effective therapy for autosomal-dominant retinal degeneration.

ERG for Evaluation of Retinal Therapies

The electroretinogram is an important tool to evaluate photoreceptor function noninvasively in human patients. Recently, the rodent ERG has gained additional importance as a noninvasive tool for evaluating naturally occurring and transgenetically engineered models of retinal dystrophies. The flash ERG is a recording of the electrical response of the entire eye to a flash of light. It is recorded through an electrode placed on the surface of the eye, typically on the cornea. The ERG is produced by the photoreceptors and interneurons of the proximal retina and reflects the state of the entire retina. The response typically consists of a negative-going a-wave, followed by a positive-going b-wave. The leading edge of the a-wave provides a direct measure of photoreceptor activity, and the b-wave provides a reflection of the action of glial and other cells. By changing the stimulus conditions, it is possible to obtain responses from either the rod or the cone systems preferentially. The oscillatory potentials of the ERG comprise a sensitive and objective measure of retinal ischemia.

ERG Measurements of Transgenic Rats

In many experiments, we use the ERG to monitor retinal function and the success of retinal therapies. The typical protocol is as follows: Rats are dark adapted and anesthetized with a ketamine/xylazine mixture. The rats are kept warm on a heating pad at 37°C throughout the recording procedure. ERGs are recorded with an LKC model UTAS-E 3000 electrodiagnostic system (LKC Technologies, Inc., 2 Professional Drive, Suite 222, Gaithersburg, Maryland 20879). ERGs are recorded from both eyes simultaneously using gold foil contact lens electrodes (polymethylmethacrylate) through a layer of 1% methylcellulose. Reference electrodes are placed subdermally between the eyes. Flashes of white light (1 ms in duration) are generated by a stimulator and presented as diffuse, full-field stimulation. The a-wave amplitude in this system is typically between 150 and 300 μV. We use an ERG protocol that defines three basic responses: (1) a rod response (dark-adapted, weak stimulus); (2) a maximal response to mixed rod and cone signals (dark-adapted, strong stimulus); and (3) a record of oscillatory potentials.

Necessity of Animal Models for Development of Human Therapies

Before 1990, retinal research was required to show only that

a treatment improved recovery compared with placebo controls. The necessity for safety was not addressed since progression to clinical trials was not an issue. This situation changed with the publication that retinal cell transplantation, gene transfer, and several cytokines improve photoreceptor survival from injury. A treatment must now be shown to be safe as well as therapeutic before it is eligible for clinical trial. Treatment doses, duration, and timing must be assessed over a range of injury severity. Since treatments may be additive, synergistic, or deleterious when combined with other factors such as survival and antiapoptotic factors, investigators must also evaluate combination therapies. Systematic assessment of dose-response, initiation time, and duration over a range of injury severity and in comparison with vehicle and sham controls is not a trivial undertaking. The best animal models and outcome measures require at least eight to 20 animals per group. Evaluation of a single drug may require thousands of experiments. No single laboratory has the resources to do such studies.

We developed the rat model described above to study and devise therapies to delay or prevent retinal degenerations. The advantages of rat over mouse models have become evident during the development and characterization of the model. Primarily, intraocular delivery of therapeutic agents such as proteins or viral vectors is much more readily accomplished in the larger rat eye, regardless of the route of administration (intravitreal or subretinal).

Clinical Trials in Humans

A number of clinical trials have been considered, have already been attempted, or are in progress using neurotrophins and gene therapies for human diseases. These include work with peripheral neuropathy (nerve growth factor, neurotrophic factor III, and insulin-like growth factor I [(IGF-I[1]), ALS (ciliary neurotrophic factor [CNTF[1]], brain-derived neurotrophic factor, and IGF-I), Alzheimer's disease (glial cell line-derived neurotrophic factor [GDNF[1]]), and Parkinson's disease (GDNF). Clinical trials are being organized or are in progress for cystic fibrosis (cystic fibrosis transmembrane conductance regulator), multiple sclerosis (IGF-I), and retinitis pigmentosa (CNTF). Toxicity studies are being conducted in a careful manner in animals, with testing of both the efficacy and the safety of each factor. Excellent progress is being made to uncover neurotrophic factors and gene therapies that could stop retinal degeneration in humans, and transgenic animals have a major role in the development of these therapies.

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