Characterization of Rhodopsin Mis-sorting and Constitutive Activation in a Transgenic Rat Model of Retinitis Pigmentosa

Eric S. Green,^1 Michael D. Menz,^2 Matthew M. LaVail,^3 and John G. Flannery^2

PURPOSE. To determine the extent to which rhodopsin mis-sorting and constitutive activation of the phototransduction cascade contribute to retinal degeneration in a transgenic rat model of retinitis pigmentosa.

METHODS. Retinas from transgenic rats expressing truncated rhodopsin (Ser334ter) were examined by light and electron microscopic immunocytochemistry at several time points. Retinal degeneration in transgenic rats raised in darkness was evaluated by quantification of outer nuclear layer thickness and by electroretinography.

RESULTS. Mutant rhodopsin was found at inappropriately high levels in the plasma membrane and cytoplasm of Ser334ter rat photoreceptors. When the cell death rate was high this mis-sorting was severe, but mis-sorting attenuated greatly at later stages of degeneration, as the cell death rate decreased. The distributions of two other outer segment proteins (the cGMP-gated channel and peripherin) were examined and found to be sorted normally within the photoreceptors of these rats. Raising Ser334ter transgenic rats in darkness resulted in minimal rescue from retinal degeneration.

CONCLUSIONS. Because dark rearing Ser334ter rats results in little rescue, it is concluded that constitutive activation of the phototransduction cascade does not contribute significantly to photoreceptor cell death in this rat model. The nature of the rhodopsin sorting defect and the correlation between the severity of mis-sorting and rate of cell death indicate that truncated rhodopsin may cause apoptosis by interfering with normal cellular machinery in the post-Golgi transport pathway or in the plasma membrane. (Invest Ophthalmol Vis Sci. 2000;41:1546–1553)

Photoreceptor cells of the mammalian retina perform a wide array of tasks directed toward the goal of fast and reliable signal transduction. In rods, the photopigment molecule rhodopsin is central to many of these tasks, and its complex structure reflects the diversity of roles it must play (reviewed in Ref. 1). The rhodopsin molecule has distinct regions that are specialized for light capture, initiation of the phototransduction cascade, and rapid deactivation after light absorption. Other regions ensure its proper folding and sorting within the photoreceptor. Researchers have identified over 100 mutations in the rhodopsin gene that cause the blinding disease retinitis pigmentosa. Because these mutations are distributed among the many different functional domains of the protein, it is likely that they lead to retinal degeneration through several different pathways.

The C-terminal tail of rhodopsin has two established functions and, therefore, is a potential site of origin for two types of cellular defects. First, this region has been shown to be involved in the sorting of rhodopsin from the photoreceptor inner segment, where protein synthesis and other cellular maintenance occur, to the outer segment, which is the site of phototransduction. Evidence for this is provided by the abnormal protein distributions found in transgenic mice expressing Gln344ter truncated rhodopsin, which lacks the last five amino acids of the C-terminal tail.^2^ Mice expressing rhodopsin with the substitution mutation Pro347Ser also have sorting abnormalities, revealed by the presence of extracellular vesicles near the connecting cilium between the inner and outer segments. Third, the phosphorylation of serine and threonine residues within the last 15 amino acids of the C-terminal tail and the subsequent binding of arrestin to rhodopsin are centrally involved in the deactivation of the protein after light absorption (reviewed in Ref. 4). The presence of rhodopsin that lacks these 15 amino acids in another strain of transgenic mice (Ser334ter mice) leads to responses to a dim light flash that last significantly longer than normal.^5^
Mutations of the C-terminal domain occur in human retinitis pigmentosa patients, and these could interfere with either rhodopsin sorting or the quenching of the light response. Other mutations, like the substitutions Lys296Glu and Gly90Asp, are thought to result in forms of rhodopsin that can be constitutively active, but it is not clear that this defect leads to retinal degeneration.\(^{10-12}\)

The goal of this study was to evaluate the contributions of improper rhodopsin sorting and constitutive activation to rod cell death in transgenic rats with the truncated Ser334ter form of rhodopsin. These rats, and transgenic rats with the substitution mutation Pro23His, used as nontruncation controls throughout this study, undergo severe retinal degeneration during the first several postnatal months. We first performed Western blot analysis to ensure that the introduction of the transgene did not cause exorbitant amounts of total opsin expression, which itself can cause degeneration.\(^{2,13}\) We then characterized the distributions of rhodopsin and other outer segment proteins to determine how abnormal sorting might lead to apoptosis in transgenic rats and monitored the extent of abnormal rhodopsin localization over the course of retinal degeneration to determine whether we could establish a correlation with cell death rates. Finally, we assayed for retinal rescue in Ser334ter rats raised in darkness to determine whether preventing prolonged photoresponses could slow photoreceptor cell death.

**METHODS**

**Animals**

All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. These experiments used TgN(S334ter)4 rats (S334ter-4, line 4 transgenic rats expressing Ser334ter rhodopsin),\(^{14}\) TgN(P23His)3 rats (P23His-3, line 3 transgenic rats expressing Pro23His rhodopsin)\(^{14}\) produced on a Sprague–Dawley background by Chrysalis DNX Transgenic Sciences (Princeton, NJ), and wild-type Sprague–Dawley rats (Simonsen, Gilroy, CA). Cyclic light-reared rats were maintained on a 12-hour light/dark schedule. During the light cycle, in-cage illuminance levels were roughly 20 foot-candles. Dark-reared rats were born in darkness and cared for with the aid of a dim red safelight.

**Western Blot Analysis**

Retinas from postnatal day 15 (P15) rats were dissected and homogenized by sonication in 5 mM Tris-acetate buffer with 65 mM NaCl, 2 mM MgCl\(_2\), and protease inhibitors. Proteins were solubilized by a 4-hour incubation with 2% digitonin at 4°C and deglycosylated by an overnight incubation at room temperature with N-glycosidase F.

Western blot analysis with several serial protein dilutions was performed to locate a concentration within the linear range of detection. Protein in solution with sample dye, with B-ME and sodium dodecyl sulfate for denaturation, was loaded and separated electrophoretically on a 12% polyacrylamide gel. Protein in the gel was then wet-transferred overnight to a polyvinylidene fluoride membrane (Bio-Rad, Hercules, CA). The membrane was blocked, probed with the N-terminal rhodopsin antibody rho4D2, washed, and then probed with horse-radish peroxidase–conjugated secondary antibody (Sigma, St. Louis, MO). Labeling was detected with a Renaissance enhanced chemiluminescence system (NEN Life Science Products, Boston, MA) and hyperfilm-ECL X-ray film (Amersham Life Science, Arlington Heights, IL). Densitometric analysis was performed on NIH image software (available at rsb.info.nih.gov/nih-image). Analysis of relative levels of protein expression was performed on samples with 5 µg of protein per lane. Results from two lanes from each of 4 rats were averaged before relative levels of expression were compared.

**Immunocytochemistry**

In all immunocytochemical experiments, rats were killed soon after the beginning of the 12-hour light cycle. Tissue was processed using the progressive lowering of temperatures method\(^{15}\) for immunofluorescent labeling on Lowicryl plastic sections (Polysciences, Inc., Warrington, PA). First, retinas were removed and fixed at 0°C in a solution of 3.6% formaldehyde and 0.5% glutaraldehyde in phosphate buffered saline (PBS). The tissue was then dehydrated in a graded alcohol series over a period of 5 hours, and the temperature simultaneously lowered to −50°C using a low temperature embedding unit (Bal-tec LTE 020; Forstentum, Liechtenstein). The tissue was embedded at −50°C in Lowicryl K11M resin and polymerized with ultraviolet light. Semi-thin sections were cut and labeled overnight with primary antibodies at a dilution of 1:300, and for 2 hours with fluorescein-conjugated secondary antibodies (Sigma). Prolong Antifade (Molecular Probes, Eugene, OR) was used as a mounting medium to reduce fluorescein photobleaching. For each condition, labeling was performed on sections from at least two rats. Propidium iodide staining at a dilution of 1:20,000 was performed on some plastic sections after antibody labeling.

Immunofluorescence on agarose sections was performed by first fixing dissected retinas in 4% formaldehyde for 30 minutes, washing in PBS, and embedding in 5% agarose in PBS. Sections were then cut at a thickness of 150 µm, and antibody labeling was performed as for plastic sections.

Tissue for immunoelectron microscopy was processed by freezing dissected retinas at high pressure using a Bal-Tec HMP 010 system (Forstentum, Liechtenstein), freeze-substituting for 3 days in aceton, and embedding in LR White resin.\(^{16}\) Ultra-thin sections were cut and labeled overnight in primary antibodies, and for 2 hours with gold-conjugated secondary antibodies (Sigma). Sections were stained with 3% uranyl acetate and photographed at 12,000×. Quantification of subcellular labeling densities was performed by counting gold particles in a minimum of four locations, each with an area of 18 µm\(^2\).

Antibodies rho4D2, rho1D4, pmc1D1, and per3B6 were generous gifts from Robert Molday (Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver). Antibody R215N was a generous gift from Paul Hargrave (Department of Ophthalmology, University of Florida, Gainesville).

**Retinal Morphometry**

The thickness of the outer nuclear layer (ONL) was quantified as previously described\(^{17}\) to obtain degeneration curves for the transgenic rat strains. In brief, rats were killed with an inhalation overdose of carbon dioxide and perfused through the heart with 2.0% formaldehyde and 2.5% glutaraldehyde in PBS. Heads were removed and immersed in fixative for 12 hours. Eyes were then enucleated and bisected along the vertical
meridian through the optic nerve head. Eye halves were osmi
cated, dehydrated, and embedded in Epon–Araldite resin. One-
micrometer-thick cross sections of the retina, spanning from
the ora serrata to the optic nerve head on both the inferior and
superior sides, were cut and stained with toluidine blue. Fifty-
four panretinal measurements of ONL thickness were made
using a camera lucida, digitizing pad, and quantification soft-
ware (Bioquant; R&M Biometrics, Nashville, TN).

Estimates of photoreceptor death rates in transgenic rats
were made by first determining from the degeneration curves
the number of micrometers of ONL thickness lost in the week
centered around a given age and dividing by seven to obtain an
approximation of micrometers lost per day. The number of
photoreceptors each micrometer represents was estimated by
dividing 17 million (the approximate number of photorecep-
tors in a wild-type rat retina) by the thickness of a wild-type
ONL at the given age. The percent of the remaining photore-
cptors that die in a day was calculated by dividing the number
of micrometers lost in 1 day by the thickness on that day.

Electroretinography

Animals were dark-adapted for 12 hours, anesthetized with a
ketamine (87 mg/kg) and xylazine (13 mg/kg) mixture, and
placed on a heating pad. The pupils were dilated with atropine,
and the corneas were locally anesthetized with proparacaine.
Electroretinograms (ERGs) were recorded from both eyes with
cooled chloride silver wire electrodes, contacting the corneas
through a layer of clear gel (Gonak; Akorn, Abita Springs, LA).
Brief (<1 msec) flashes of light from a xenon flashtube (Nova-
tron, Dallas, TX) were filtered (W47B blue filter) and presented
as diffuse full-field illumination with an intensity of −0.15 log
cd/sec · m². A-wave amplitudes were quantified by measure-
ment on averaged ERGs from the baseline amplitude to the
peak of the a-wave. B-wave amplitudes were measured starting
from the peak of the a-wave.

RESULTS

Western Blot Analysis

We performed Western blot analysis to compare the levels of
total opsin expression in wild-type and S334ter-4 transgenic
rats and to compare the relative levels of mutant opsin and
normal opsin in transgenic rats (Fig. 1). Mutant opsin runs as a
smaller fragment (33 kDa) than normal opsin (38 kDa) in the
S334ter-4 lane due to the 15 amino acid deletion. Total opsin
levels in the S334ter-4 rat were 112% of opsin levels in the
wild-type rat. This comparison included quantification of the
dimer band at 76 kDa and the other multimers. Within the lane
representing S334ter-4 rats, Ser334ter opsin levels were 10% of
normal opsin levels. Thus, the Ser334ter opsin in transgenic
rats represented approximately 9% of normal opsin in wild-
type rats.

Characterization of Abnormal Protein
Distributions

We performed immunocytochemistry on 1-micrometer-thick
plastic sections to examine the sorting of rhodopsin in rod
photoreceptors of the S334ter-4 and P23H-3 transgenic rats. In
wild-type rats at P26, high levels of rhodopsin labeling are
restricted to the outer segments (Fig. 2A). In S334ter-4 rats, we
observed labeling throughout the cell, in the outer segments,
inner segments, ONL, and outer plexiform layer (Fig. 2B). This
abnormal labeling was detected with the N-terminal rhodopsin
antibody rho4D2, which recognizes both normal and Ser334ter
rhodopsins. We determined the distribution of normal rhodop-
sin in the S334ter-4 rat retina with the C-terminal rhodopsin
antibody rho1D4, which does not recognize Ser334ter rhodop-
sin because the binding site is deleted. This normal rhodopsin
is only in the outer segments, except in a very few cells that are
labeled in the ONL (Fig. 2C). We conclude from these results
that only mutant rhodopsin is abnormally localized in S334ter-4
retinas.

We probed P23H-3 control rat retinas at P37 (approxi-
mately the same stage of degeneration as an S334ter-4 retina
at P26) with three different rhodopsin antibodies (rho4D2,
rho1D4, and R215N). No abnormal localization of Pro23HIs or
normal rhodopsin was seen (Fig. 2D), except again in rare cells
labeling in the ONL (not shown). Figures 2E and 2F, respec-
tively, show a control section labeled only with secondary
antibody and a toluidine blue–stained section, to indicate the
layers of the retina.

To help determine the nature of the sorting defect of
Ser334ter rhodopsin we performed quantitative immuno-
electron microscopy, comparing the densities of rhodopsin
labeling between wild-type and S334ter-4 rat photorecep-
tors at four different locations within the cell (Fig. 3A). Labeling
densities in wild-type and S334ter-4 rat photoreceptors are not
significantly different in the outer segments, endoplasmic reticulum (ER), and Golgi membranes. Labeling densities are fivefold higher in S334ter-4 rats than in wild-
type rats in areas of the cytosol not associated with any
visible membranes, but which would be expected to include
protein transport vesicles. In the plasma membrane of the
photoreceptor inner segments, labeling is 20-fold higher in
S334ter-4 rats than in wild-type rats. Figure 3B shows the
distribution of rhodopsin found in wild-type rats, and Figure
3C shows an example of the abnormally high plasma mem-
brane labeling seen in the inner segments of S334ter-4 pho-
toreceptors. Similarly, there is sparse labeling in the ONL
and outer plexiform layer of wild-type photoreceptors (Figs.
3D and 3E) but significant labeling in these regions in
S334ter-4 photoreceptors (Figs. 3F and 3G).
To determine whether the improper routing of rhodopsin affects the sorting of other outer segment proteins, we localized the cGMP-gated channel and peripherin in animals with the Ser334ter transgene. Agarose sections of wild-type (Fig. 4A) and S334ter-4 (Fig. 4B) retinas, labeled with the cGMP-gated channel antibody pmc1D1, show that this channel sorts normally in S334ter-4 rat retinas. Retinas sectioned in plastic and labeled with the per3B6 antibody show that peripherin also sorts normally (Figs. 4C and 4D), except in rare cells.

Cell Death Rates and Rhodopsin Mis-sorting

In S334ter-4 transgenic rats, photoreceptor cell death begins at about P13 and proceeds at different rates during different times in the course of degeneration (Fig. 5A). If the improper sorting of rhodopsin is responsible for cell death, then the severity of the abnormality in rhodopsin distributions should correlate with the rate of cell death at a given time point.

At P15, wild-type retinas show the normal adult rhodopsin distribution pattern, with labeling only in the outer segments (Fig. 5B). S334ter-4 retinas show rhodopsin labeling throughout the photoreceptor layer at P15, when cell death is well under way (Fig. 5C). In contrast, the rhodopsin distribution in S334ter-4 rats appears essentially normal at P90, by which time cell death has slowed considerably (Fig. 5D). Note that Figure 2B represents another time point (P26) for S334ter-4 retinas in this sequence.

Contribution of Constitutive Activation to Cell Death

To determine the extent to which constitutive activation of the phototransduction cascade contributes to degeneration in S334ter-4 animals, we compared retinas of rats raised in cyclic light to those of rats raised in darkness. The decreased activation of rhodopsin in darkness should prevent any cell death induced by the activity of the phototransduction cascade, so rescue seen in dark-reared S334ter-4 rats would be an indicator of the degeneration caused by this mechanism. P23H-3 rats were used as nontruncation controls because this rhodopsin mutation would not be expected to exhibit constitutive activation after light absorption.

We quantified the thickness of the ONL as a measure of photoreceptor number at six different ages in wild-type, cyclic light–reared S334ter-4, and dark-reared S334ter-4 rats, and at one age (P60) in cyclic light- and dark-reared P23H-3 rats (Fig. 6). Each data point in the graph represents the average of 162 measurements made from the retinas of three different rats. At later ages, the average ONL thickness of dark-reared S334ter-4 rats was slightly greater than that of cyclic light-reared S334ter-4 rats, but this difference did not rise to the level of statistical significance at any age. Similarly, at P60 the average ONL thickness of dark-reared P23H-3 control retinas was slightly, but not significantly, thicker than that of cyclic light-reared P23H-3 rats. (Most of the decrease of ONL thickness in wild-type rats, as well as some of the decrease in the transgenic rats, is due to the thinning of the retina as the eye grows in size.)

To determine whether functional rescue occurred through dark rearing of transgenic rats, we performed ERGs at the same ages at which morphologic measurements were made. Dark-reared S334ter-4 rats showed a 116% greater a-wave amplitude, and an 87% greater b-wave amplitude than cyclic light-reared S334ter-4 rats. These represent restorations.
of 21% and 32% of the wild-type response in the a- and b-waves, respectively (Fig. 7). P23H-3 rats reared in the dark demonstrated a greater degree of rescue of the ERG response than the S334ter-4 animals (data not shown). Although the increases in a- and b-wave amplitudes were smaller (94% and 48%, respectively), these represented restorations of 70% and 81% of the wild-type response.

**DISCUSSION**

The present results suggest that Ser334ter-truncated rhodopsin is lethal in rat photoreceptors because of its failure to be properly routed to the outer segment, and not because of its improper deactivation after light absorption. Furthermore, our immunocytochemical analysis reveals the subcellular distribution of the mutant rhodopsin and, therefore, sheds light on both the nature of the sorting defect and the mechanisms by which it is transmitted to the outer segment.
which it may lead to apoptosis. High expression of the mRNA of a normal opsin transgene in mice leads to retinal degeneration, but transgene expression at levels comparable to those of the endogenous opsin gene does not. In addition, other lines of S334ter rats show various rates of retinal degeneration that are proportional to the mutant:normal opsin ratio (Flannery et al., unpublished observations, May 1999). Because we find that in S334ter-4 rats the transgene product is present at just 10% of the level of the endogenous rhodopsin, we believe that degeneration in these rats must result from a functional defect in the rhodopsin protein.

The finding that S334ter-4 transgenic rats exhibit a sorting abnormality corroborates several previous reports, which indicate a role for the C-terminal tail in the proper sorting of rhodopsin. Most recently, Deretic et al. showed in a cell-free system that the last five amino acids of the C-terminal tail appear to regulate the packaging of the protein into appropriate vesicles in the trans-Golgi network. The subcellular distribution of mutant rhodopsin that we see—a level in the ER and Golgi comparable to that of normal rhodopsin in wild-type animals, a level 5-fold higher than normal in the cytosol, and a level 20-fold higher than normal in the plasma membrane—fits particularly well with this model. It seems likely that after correct folding and processing in the ER and Golgi, rhodopsin lacking its signal sequence is packaged into vesicles with no explicit destination or with a default destination of the plasma membrane. The higher labeling in the cytosol may reflect the abnormal presence of rhodopsin in these vesicles.

We found no evidence that Pro23His mutant rhodopsin mis-sorts in the photoreceptors of control P23H-3 rats. Results from different animal models that both conflict with ours and agree with ours with respect to the subject of the sorting of rhodopsin containing the Pro23His mutation have been published previously. In this article, the P23H-3 control serves to demonstrate that the introduction of a transgene does not in itself lead to mis-sorted rhodopsin.

Our most convincing evidence that abnormal rhodopsin sorting leads to apoptosis in the S334ter-4 transgenic rats is that the severity of mis-sorting correlates well with the rate of cell death at a given point in the degeneration of the retina (Fig. 5). At P15, mis-sorting is severe and widespread, and we estimate that 620,000 photoreceptors are dying per eye per day at that time. This represents 3.9% of the photoreceptors present at

![Figure 5](image-url) Correlation of the severity of abnormal rhodopsin distributions with cell death rates. (A) The average thickness of the ONL of S334ter-4 rats over the course of retinal degeneration, demonstrating that cell death is faster at younger ages, and slows greatly by P90. (B) A wild-type retina at P15, labeled with the rhodopsin antibody rho4D2. (C, D) S334ter-4 retinas at P15 and P90, labeled with rho4D2. Abnormal labeling is present at P15 but not at P90. Scale bar, 15 μm.

![Figure 6](image-url) ONL thicknesses for wild-type, cyclic light-reared S334ter-4, and dark-reared S334ter-4 rats at six time points, and for cyclic light-reared and dark-reared P23H-3 rats at one time point. Error bars are 1 SD above and below the mean.
antibody and propidium iodide (a chromosomal marker that shows clear signs of being engaged in the process of apoptosis. For example, when sections are probed with both the protein and other membrane-bound proteins of the outer segment do not miss-sort concurrently with Ser334ter rhodopsin. They have abnormal distributions only in occasional cells, which often are dying per eye per day. It is possible that this correlation exists because the rhodopsin mutation causes some other cellular defect that induces apoptosis and exacerbates mis-sorting at times when the cell death rate is greatest. However, the simplest explanation is that more severe mis-sorting in the photoreceptors of young rats, perhaps caused by protein synthesis rates higher than those in adults, leads directly to higher cell death rates. LaVail has shown that outer segment disc synthesis in young mice is up to 1.6 times the adult level, contributing to the process of outer segment elongation during development, and Treisman et al. have shown that opsin synthesis rates are higher in young rats than in adults.

There are at least three possible mechanisms for the induction of apoptosis that are consistent with a sorting defect at or beyond the trans-Golgi network, and that would be expected to produce higher rates of cell death at times of more severe mis-sorting. First, mutant rhodopsin may overwhelm the normal vesicular machinery of the plasma membrane-bound pathway in these cells, interfering with the routing of legitimate cargo. This hypothesis can be contrasted with the proposed mechanism of cell death in some Drosophila rhodopsin mutations, in which improperly folded rhodopsin is retained in large quantities in the ER and Golgi, potentially interfering with protein synthesis and maturation. Second, the physical presence of high levels of mutant opsin in the plasma membrane may interfere with normal cellular processes, such as neurotransmitter release in the synaptic zone. Third, the degradation of large amounts of miss-sorted protein may cause a damaging increase in the metabolic load placed on photoreceptors.

Interestingly, we find in general that normal rhodopsin and other membrane-bound proteins of the outer segment do not miss-sort concurrently with Ser334ter rhodopsin. They have abnormal distributions only in occasional cells, which often show clear signs of being engaged in the process of apoptosis. For example, when sections are probed with both the protein antibody and propidium iodide (a chromosomal marker that stains apoptotic nuclei), cells that show abnormal protein localization also frequently label with the propidium iodide (data not shown). From this we can conclude that apoptosis in the photoreceptors of the transgenic rats is not a result of disruption of the sorting of these other proteins of the outer segment and, instead, that a breakdown in the sorting of many proteins is one of the secondary effects of the cell death process.

Expression of the Ser334ter rhodopsin gene in mice results in photoreceptor flash responses that can be more than 20 times longer than normal and comparable results have been found in the S334ter rats (Shimpei Nishikawa, Roy H. Steinberg, Matthew M. LaVail, unpublished observations, February 1998). We therefore believe that the phototransduction machinery in cyclic light-reared S334ter-4 rats is grossly overactive. We find that dark rearing of S334ter-4 rats results in minimal rescue from retinal degeneration, and none beyond that seen in P23H-3 rats. Dark rearing produced some rescue of the ERG in S334ter-4 rats, but we found equal or greater rescue in the ERG of dark-reared P23H-3 rats. This rescue may therefore reflect an increased sensitivity of any or all of the retina’s signaling pathway components after development in darkness, rather than an effect specific to a given mutation. Indeed, when wild-type albinos are dark-reared, the total rhodopsin level in the eyes is approximately 50% greater than that in cyclic light-reared littermates, and the rod outer segments are approximately 25% longer in the dark-reared rats. Because we find little or no change in the amount of photoreceptor apoptosis, and no change in the ERG beyond that seen in the nontruncation control rats, we conclude that in the S334ter-4 transgenic rat prolonged rhodopsin activation does not contribute significantly to photoreceptor death.

A strong case has been made for the idea that deletion of the gene encoding the cGMP-gated channel leads to retinal degeneration because the lack of this protein produces photoreceptor activity like that which occurs in a constant light environment. However, conclusive evidence that rhodopsin mutations can lead to retinal degeneration by constitutively activating the phototransduction cascade remains elusive. For example, substitution mutations at amino acid position 296 have been shown to be constitutively active in vitro and can lead to photoreceptor degeneration, but ERGs of Lys296Glu transgenic mice did not reveal in vivo evidence of constitutive activation. Furthermore, the night-blindness reported by human patients with a Gly90Asp substitution would be expected if this rhodopsin mutant were constitutively active, but Gly90Asp patients do not undergo severe photoreceptor degeneration. It may be that constitutive activation causes some degeneration in these cases, as pointed out by Lisman and Fain, and that the mild rescue we observed reflects a small contribution of constitutive activation to cell death in S334ter-4 rats. However, our results are more aligned with studies suggesting that rhodopsin mutations induce degeneration primarily through other kinds of functional defects.

**Acknowledgments**

The authors thank Douglas Yasumura, Michelle Mo, Melissa Barnett, Ward Peterson, and Kent McDonald for their valuable advice and technical assistance.

![Figure 7](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932905/)
References