Susceptibility to Retinal Light Damage in Transgenic Rats with Rhodopsin Mutations

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PURPOSE. To determine relative light-induced retinal damage susceptibility in transgenic rats expressing mutations in the N- or C-terminal region of rhodopsin.

METHODS. Heterozygous transgenic rats, including P23H sublines 2 and 3 and S334ter sublines 4 and 9, were reared in dim cyclic light or in darkness before visible light exposure starting at various times of the day or night. Before exposure to light, some rats were given the synthetic antioxidant dimethylthiourea (DMTU). At various times after intense light treatment, rats were killed for determinations of rhodopsin and retinal DNA recovery, DNA fragmentation patterns, and Northern blot analysis of retinal heme oxygenase (HO)-1 and interphotoreceptor retinol binding protein (IRBP). Rod outer segments (ROSs) were isolated for Western blot analysis of rhodopsin using N- and C-terminal–specific monoclonal antibodies.

RESULTS. All rats incurred greater photoreceptor cell damage from exposure to light starting at 1 AM than from exposure at 5 PM. Among cyclic-light–reared rats, P23H line 3 animals were more susceptible to light-induced damage than P23H line 2 animals. S334ter rats exhibited retinal light damage profiles similar to those in normal rats. Dark-rearing potentiated retinal damage by light. However, dark-rearing alone prolonged photoreceptor cell life in P23H rats, but had no such effect in S334ter animals. DMTU pretreatment was effective in preventing or reducing light-induced retinal damage in all transgenic rats. S334ter rat ROSs contained the truncated form of rhodopsin. Intense light exposure resulted in DNA ladders typical of apoptotic cell death and the simultaneous induction of retinal HO-1 mRNA and reduced expression of IRBP.

CONCLUSIONS. Light-induced retinal damage in transgenic rats depends on the time of day of exposure to light, prior light- or dark-rearing environment, and the relative level of transgene expression. Retinal light damage leads to apoptotic visual cell loss and appears to result from oxidative stress. These results suggest that reduced environmental lighting and/or antioxidant treatment may delay retinal degenerations arising from rhodopsin mutations. (Invest Ophthalmol Vis Sci. 2003;44:486–492) DOI:10.1167/iovs.02-0708

At low light levels, rhodopsin bleaching triggers the phototransduction cascade within photoreceptor cell rod outer segments (ROSs). However, prolonged- or intense light-exposure induces retinal damage, also beginning within the ROSs and ultimately resulting in the death and disappearance of visual cells.1 It is well known that the action spectrum for light-induced retinal damage in rats is the same as the rhodopsin absorption spectrum.1–3 Thus, whether white, green, or even blue light4 is used, extensive rhodopsin bleaching leads to visual cell loss. The extent of light-induced retinal damage and photoreceptor loss depends on a variety of extrinsic factors, including the duration of light and its intensity, prior light-rearing conditions, and diet or antioxidant pretreatment (reviewed in Refs. 5–7). Intrinsic factors such as age,8 genetic inheritance,9,10 and circadian rhythms11–12 are also known to affect the outcome of treatment with intense light in experimental animals. The availability of transgenic animal models of human retinal disease has rekindled interest in environmental factors that may impact the rate of retinal degeneration. For example, transgenic mice expressing multiple rhodopsin mutations exhibit different rates of visual cell loss from light- or dark-rearing13 and increased susceptibility to intense-light–induced damage.14 Similarly, animals with a single amino acid substitution at position 23 in rhodopsin (P23H), a common form of autosomal dominant retinitis pigmentosa (RP),15 exhibit enhanced rates of light-dependent retinal degeneration.16,17 However, retinal degenerations in transgenic mice18 or rats19 expressing rhodopsin with a truncated C-terminal region may depend on the accumulation of mutant opsin within the photoreceptor cell body and appear to be unaffected by dark-rearing.19

Photoreceptor cell degenerations arising from the expression of mutated opsin(s) or from treatment with intense light occur by an apoptotic process involving endonuclease-mediated DNA fragmentation.20–22 Whereas the rate of visual cell death in transgenic animals appears to be related to relative transgene expression, exposure to intense light typically results in the synchronous involvement of numerous photoreceptors during a relatively short period. Caspase activation occurs as an early apoptotic step in both inherited and light-induced retinal degenerations, but the caspase cascades may be different in different animal models.23–26

Herein, we describe the relative rates of light-induced retinal degeneration in transgenic rats expressing a P23H mutation near the amino terminal of rhodopsin and others having a truncation of the C-terminal region (S334ter). For each transgenic animal model we compared two sublines, each exhibiting different rates of degeneration under normal light-rearing conditions. Both transgenic animal models exhibit circadian-dependent retinal damage by light that is influenced by prior light- or dark-rearing conditions, but P23H rats incurred more damage than S334ter animals. Antioxidant treatment prevented or reduced intense light-mediated retinal damage in both types
of transgenic animals. Our findings suggest that, as previously reported in some patients with RP, avoiding bright-light environments may slow the progression of visual cell loss but will not prevent the ultimate loss of vision.

**MATERIALS AND METHODS**

**Animals and Rearing Conditions**

Homozygous albino breeding pairs of P23H rats, lines 2 and 3, and S334ter rats, lines 4 and 9, were generously provided by Matthew M. LaVail (University of California, San Francisco, CA). These animals were maintained and bred in barrier cages and fed a fatty-acid-rich diet (Teklad #7904; Harlan-Teklad, Inc., Indianapolis, IN). Heterozygous litters were obtained from crossing homozygous transgenic male offspring with female Sprague-Dawley rats, obtained from Harlan-Teklad, Inc. These animals were fed standard rat chow (#8640; Harlan-Teklad, Inc.). On weaning, heterozygous rats were segregated by sex and moved to a room with dim cyclic light, consisting of 12 hours white light per day (20–30 lux); lights on 8 AM off at 8 PM, or a dark room environment. They, along with weaning male Sprague-Dawley rats, were maintained in their respective rearing environments for 6 weeks. At approximately P60, experimental animals were dark adapted for 16 hours and then treated with intense visible light. After the treatment, the animals were either killed immediately or placed in a dark environment for as long as 14 days before death. All rats were killed in a CO₂-saturated chamber. The use of rats in this study conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Intense Light Treatment**

Dark-adapted rats were treated with intense light in circular green Plexiglas chambers (490–580-nm light), beginning at 1 AM, 9 AM, or 5 PM. Two rats per chamber were exposed to light (1200–1400 lux) for as long as 8 hours. To assess relative susceptibility to light damage the duration of exposure was adjusted as needed to produce 50% visual cell loss in comparison with unexposed control animals from each group of rats. Some animals received two intraperitoneal injections of dimethylthiourea (DMTU), an antioxidant obtained from Sigma, Inc. These animals were fed standard rat chow (#8640; Harlan-Teklad, Inc.). On weaning, heterozygous rats were segregated by sex and moved to a room with dim cyclic light, consisting of 12 hours white light per day (20–30 lux); lights on 8 AM off at 8 PM, or a dark room environment. They, along with weaning male Sprague-Dawley rats, were maintained in their respective rearing environments for 6 weeks.

**Rhodopsin and Retinal DNA Levels**

End point measurements of whole-eye rhodopsin levels and retinal DNA were performed 2 weeks after treatment with intense light, as described. Briefly, rhodopsin was extracted from one eye with extraction reagent (1.5% Emulphogene BC-720 detergent; Sigma Inc.) and retinal DNA was extracted from the fellow eye. Photoreceptor cell DNA was then calculated from retinal DNA by subtracting the DNA content of the inner retinal layers of 7-month-old RCS rats (75 μg/retina) from the retinal DNA content. Because the inner retinal layers are unaffected by light damage, the difference provides a good estimate of the remaining photoreceptor cell DNA content in the rat retina (187 μg in control retina). To estimate the loss of visual cells, rhodopsin and DNA levels were then compared with their respective control levels measured in unexposed rats for each of the groups studied. When possible, equal numbers of male and female heterozygous rats were used for each experiment. In unexposed transgenic control rats, there were no statistically significant differences in rhodopsin content or in retinal DNA levels, based on the sex of the animals.

**Electrophoretic Analysis of DNA and mRNA**

At periods of from 12 hours to 4 days after treatment with intense light, some rats were killed for neutral gel DNA electrophoresis, to detect apoptotic DNA fragmentation, and Northern blot analysis of mRNA for heme oxygenase (HO)-1 and interphotoreceptor retinol binding protein (IRBP). Procedures for the extraction, electrophoresis and detection of retinal DNA and mRNAs have been described. For these measurements DNA and total RNA were obtained from the fellow eyes of four animals at each time point.

**Western Analysis of Rhodopsin**

ROS were isolated from rat retinas by discontinuous sucrose density gradient ultracentrifugation. Proteins from band I ROSs, the purest fraction, and band II, a less pure fraction, were electrophoresed on 10% polyacrylamide gels containing 0.1% SDS. The proteins were transferred to nitrocellulose membranes and then probed with rhodopsin monoclonal antibodies. The N-terminal (mAb B6-30) and C-terminal (mAb K16-155) antibodies were a generous gift from Paul A. Hargrave (University of Florida, Gainesville, FL).

**Statistical Evaluation**

The data were analyzed with Student’s two-sample, two-tailed t test. P < 0.05 was considered to represent a statistically significant difference.

**Results**

The effects of dim cyclic light- and dark-rearing environments on photoreceptor cell survival were examined by measuring rhodopsin and visual cell DNA levels in 60-day-old animals. Table 1 shows that transgenic rats maintained in dim cyclic light had significantly lower levels of rhodopsin than was found in normal Sprague-Dawley rats. The S334ter rats had 83% (line 4) and 45% (line 4) of the rhodopsin content in normal animals. P23H rat rhodopsin levels were 76% (line 2) and 53% (line 3) of normal. The relative levels of photoreceptor cell DNA in S334ter rats were higher: 100% for line-9 rats and 45% for the line-4 animals. In P23H rats DNA levels were 77% of normal for

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**Table 1. Rhodopsin and Photoreceptor Cell DNA in Normal and Transgenic Rats**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Cyclic Light-Reared</th>
<th>Dark-Reared</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhodopsin DNA</td>
<td>Rhodopsin DNA</td>
</tr>
<tr>
<td>Normal</td>
<td>1.82 ± 0.14</td>
<td>2.25 ± 0.18</td>
</tr>
<tr>
<td>S-334ter line 9</td>
<td>1.51 ± 0.13*</td>
<td>1.94 ± 0.18</td>
</tr>
<tr>
<td>S-334ter line 4</td>
<td>0.58 ± 0.05*</td>
<td>0.84 ± 0.07†</td>
</tr>
<tr>
<td>P23H line 2</td>
<td>1.42 ± 0.06†</td>
<td>1.75 ± 0.12†</td>
</tr>
<tr>
<td>P23H line 3</td>
<td>0.97 ± 0.07†</td>
<td>1.27 ± 0.13†</td>
</tr>
</tbody>
</table>

Results are the mean ± SD for the number of determinations shown. Rhodopsin nmol/eye; photoreceptor cell DNA μg/retina. Rats 60 days of age. All rhodopsin data in dark-reared rats were significantly greater than in their respective cyclic light-reared counterparts.

* By Student’s t test, results significantly lower than in normal rats.
† DNA level significantly higher than in cyclic light reared P23H rats.
line-2 rats and 75% for the line-3 animals. The lower rhodopsin levels in P23H line-3 rats relative to DNA, agrees with measurements of shorter ROS length in these animals (Coulibaly S, ARVO Abstract #3372, 2001). With the exception of DNA levels for S334ter line-9 animals, all results in the heterozygous transgenic rats were significantly lower than in normal rats ($P < 0.001$).

Dark-rearing led to the expected increase in rhodopsin levels in normal rats and in transgenic animals. In normal and P23H rats, these increases were from 20% to 24% of the levels measured in cyclic-light-reared animals. However, dark-reared S334ter line-9 rats had the same rhodopsin level as normal animals, a 33% increase compared with their cyclic-light-reared counterparts. Line-4 rats also exhibited a similar (30%) increase in rhodopsin. With the exception of S334ter line-9 rats, rhodopsin levels in other dark-reared transgenic rats were still significantly lower than in normal animals ($P < 0.001$). Compared with cyclic-light-reared animals, photoreceptor cell DNA levels in S334ter rats were unaffected by dark-rearing. However, dark-rearing of P23H rats prolonged photoreceptor cell life, as shown by the significantly higher DNA levels than in cyclic-light-reared animals ($P < 0.025$). Our DNA data for cyclic-light-reared transgenic rats are in good agreement with the number of rows of photoreceptor nuclei in the ONL (LaVail MM, unpublished data, 1999) and with published results, showing that dark-rearing has no effect on photoreceptor cell survival in S334ter line-4 rats.19

**Light-Induced Retinal Damage in S334ter Rats**

To compare susceptibility to damage by light we studied S334ter line-9 rats, which have a slow rate of spontaneous visual cell loss, and line-4 rats, which have a more rapid rate of retinal degeneration. Transgenic rats and normal Sprague-Dawley animals were treated with intense light, beginning at various times during the day and night. The data contained in Figures 1A and 1B indicate that cyclic-light-reared line-9 rats and normal animals had similar rhodopsin and retinal DNA levels. Both the normal and line-9 rats exhibited significant losses of rhodopsin and DNA when exposed to light at 1 AM ($P < 0.001$), with little effect at 9 AM and 5 PM. Although rhodopsin and DNA levels in S334ter line-4 rats were much lower than in line-9 animals, they were similarly affected by 8-hour light exposures. The loss of rhodopsin was less than in line-9 rats at 1 AM, but greater at 9 AM and 5 PM, whereas DNA levels in line-9 and line-4 rats were nearly the same at 1 and 9 AM.

Figures 1C and 1D indicate that rats previously reared in darkness exhibited more damage at 1 AM than at 5 PM. In contrast to those reared in cyclic light, dark-reared line-9 animals incurred greater losses of rhodopsin and DNA than line-4 rats or normal animals. In the line-9 rats, the decreases in these visual cell components were significant at all time points ($P < 0.001$), whereas only the 1 AM levels were significantly lower in line-4 rats ($P < 0.001$). In normal dark-reared animals visual cell loss was approximately 50% of the unexposed control animals at both 1 and 9 AM with approximately 10% loss at 5 PM. Actual percentages of light-induced changes in rhodopsin and DNA have been summarized in Table 2.

To determine whether antioxidant treatment would prevent light-induced photoreceptor cell loss, some rats were given DMTU 24 hours before and just before exposure to intense light at 1 AM. The data in Figure 1 indicate that DMTU pretreatment completely prevented light-induced retinal damage. In each case, rhodopsin and DNA recoveries in DMTU-treated rats were no different from their respective unexposed control.

**Rod Outer Segment Opsin in S334ter Rats**

We used Western analysis and antibodies directed against the N- or C-terminal regions of opsin to detect the truncated form
of rhodopsin in S334ter rat ROSs. Using the N-terminal antibody, both the normal and lower-molecular-weight forms of opsin can be detected (Fig. 2). The truncated opsin was found to be present in purified ROSs (arrows band I) and in a less pure fraction (band II) isolated from either line-9 or line-4 rats (Fig. 2, top). As expected, normal-molecular-weight opsin was present in ROS fractions from Sprague-Dawley rats. Using the C-terminal probe (Fig. 2, bottom) only the normal-molecular-weight opsin was detected in all samples. Similar results were found in ROSs from dark-adapted animals and in those isolated from rats exposed to light for 30 minutes. Thus, brief light exposure had no effect on the relative migration of opsin in normal or transgenic animals, and there was no shift in the distribution of opsin between the two ROS fractions.28

Light-Induced Retinal Damage in P23H Rats

We also studied light damage susceptibility in P23H rats with a slow rate of retinal degeneration (line 2) and a more rapid rate of visual cell loss (line 3). After the same 8-hour (cyclic reared) and 3-hour (dark reared) exposure to intense light used for S334ter rats, the loss of visual cell components was extensive.17 Therefore, we used 4-hour exposures to light for cyclic-light–reared rats and 1-hour exposures for dark-reared animals. Figure 3 shows that P23H line-2 rats incurred less light damage than line-3 animals. At all time points, rhodopsin levels were significantly higher in line-2 rats than in the more sensitive line-3 animals \((P < 0.001)\). In line-2 rats from the dim cyclic light condition, exposure to intense light at 1 AM resulted in 20% to 30% losses of rhodopsin and DNA, whereas exposure at 9 AM or 5 PM resulted in losses of 10% or less (Figs. 3A, 3B). Although unexposed line-3 rats had lower levels of rhodopsin and DNA than line-2 animals, they were more affected by intense light. Rhodopsin levels were reduced by 40% to 70%, whereas DNA levels decreased by 30% to 50% at the various time points. The same relative susceptibility to light damage was found in dark-reared rats exposed to intense light for only 1 hour. Line-2 animals exhibited a circadian profile, with 30% to 40% rhodopsin and DNA loss at 1 AM, 15% to 30% at 9 AM, and only approximately a 5% loss of visual cell components at 5 PM (Figs. 3C, 3D). In line-3 rats rhodopsin losses were 60% to 75%, whereas retinal DNA levels were reduced by 40% to 50% at all time points. These data have been summarized in Table 2.

Table 2. Changes in Rhodopsin and Photoreceptor Cell DNA in Light-Exposed Rats

<table>
<thead>
<tr>
<th>Time of Exposure</th>
<th>Cyclic Light-Reared</th>
<th>Dark-Reared</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 AM</td>
<td>9 AM</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+3 h light</td>
<td>48/42</td>
<td>4/11</td>
</tr>
<tr>
<td>+8 h light</td>
<td>44/32</td>
<td>8/10</td>
</tr>
<tr>
<td>S334ter line 9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+3 h light</td>
<td>25/55</td>
<td>25/20</td>
</tr>
<tr>
<td>P23H line 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+1 h light</td>
<td>22/32</td>
<td>5/12</td>
</tr>
<tr>
<td>P23H line 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+1 h light</td>
<td>72/51</td>
<td>48/30</td>
</tr>
<tr>
<td>+4 h light</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Results are presented as the average % loss, (+) gain, in rhodopsin/photoreceptor cell DNA compared with the respective unexposed rat control data shown in Figures 1 and 3.

Figure 2. Western blot analysis of rhodopsin isolated from ROSs of S334ter rats. Cyclic-light–reared normal (CN), line-9, and line-4 rats were exposed to light for 30 minutes or were not exposed and ROSs isolated by sucrose density ultracentrifugation. ROS fractions (bands I, II) were electrophoresed, transferred to membranes, and probed with an N-terminal mAb, stripped and reprobed with a C-terminal-specific mAb. Arrows: lower-molecular-weight opsin. For both bands, each lane was loaded with 5 μg protein.

Figure 3. Northern Blot Analysis and DNA Fragmentation in Transgenic Rats

To further characterize susceptibility to light damage in transgenic rats, we compared the most sensitive P23H line-3 rats and the least sensitive S334ter line-9 animals, both reared in dim cyclic light. Figure 4 contains the mRNA expression profiles of retinal HO-1, an inducible marker of oxidative stress and IRBP, a constitutive marker of normal photoreceptor cell transcription.12 DNA was extracted from the fellow eyes to detect
the presence of DNA ladders, an indicator of apoptotic cell death. The data show that HO-1 was induced in both types of transgenic rats after 4 (P23H) or 8 (S334ter) hours of treatment with intense light. Maximum expression of HO-1 was found 12 hours after treatment, with reduced expression for as long as 4 days later. In both types of rats, exposure to light at 1 AM resulted in greater expression of HO-1 than found at 9 AM or 5 PM. The expression of IRBP mRNA, which was constitutively expressed at all times in unexposed rats, was decreased at the same times that retinal expression of HO-1 was greatest. DNA ladders were present immediately after exposure to light in both P23H and S334-ter rats. The appearance of ladders was also more prominent at 1 AM than at the other times. Comparison of DNA gel patterns at the 1 AM time point seemed to show that DNA fragmentation occurs in multiple phases after exposure to light. These prominent DNA ladder profiles cor-

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932919/)

**Figure 3.** Rhodopsin and retinal DNA in P23H rats exposed to intense light at different times. Cyclic-light-reared (A, B) and dark-reared (C, D) rats were treated with intense light for 4 hours and 1 hour, respectively. Open symbols: line-2 and -3 rats; (+) rats given DMTU and exposed to light at 1 AM; filled symbols: unexposed control rats. Results are the mean ± SD of results in 8 to 10 animals.

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932919/)

**Figure 4.** Changes in retinal DNA and selected mRNAs after treatment with intense light. Cyclic-light-reared P23H line-3 rats were exposed to light for 4 hours; S334-ter line-9 rats were given 8 hours of light exposure starting at 1 AM, 9 AM, or 5 PM. At various times after light treatment, rats were killed and retinas excised for DNA and mRNA extraction. DNA was isolated from four retinas from four different rats and electrophoresed on neutral polyacrylamide gels, 2 µg per lane. Total RNA preparations from the fellow eyes were loaded at 10 µg/lane, electrophoresed, and blots probed for HO-1 and IRBP mRNA. 18S rRNA was used for identification of loading differences.
related with increased expression of HO-1 and reduced IRBP message levels, suggesting that visual cell death occurs in different populations of cells at different times over the post-exposure time course.

**DISCUSSION**

This study shows that transgenic rats having mutations in the photopigment rhodopsin are more susceptible to light-induced retinal degeneration than normal animals. By most criteria, transgenic rats reared in either dim cyclic light or in darkness lost a greater fraction of their rhodopsin and retinal DNA than normal animals. Susceptibility to light-induced damage was also found to depend on the time of day or night that treatment with intense light started. In each case exposure to light starting at 1 AM resulted in greater photoreceptor cell loss than that starting at 5 PM. Thus, both S334ter and P23H rats exhibited circadian-cycle–dependent light-induced retinal damage similar to that found in normal animals. Because neonatal rat pinealocytes exhibit photoreceptor-cell–like morphology and a low level of rhodopsin is present in the adult rat pineal gland, we measured pineal melatonin levels in transgenic rats. Radioimmunoassay revealed that both types of transgenic rats expressed pineal melatonin levels similar to those of normal cyclic-light– and dark-reared Sprague-Dawley rats at various times of the day or night (Darrow RM, unpublished results, 2002). Accordingly, S334ter and P23H rats do not appear to have altered circadian rhythms that could influence retinal gene expression and their enhanced susceptibility to light damage.

For transgenic rats having a slower rate of spontaneous visual cell loss, the extent of retinal light damage was generally less than in others with a more rapid rate of degeneration. The single exception was S334ter line-9 rats reared in darkness, in which light-induced damage far exceeded that found in comparable cyclic light reared rats or in line-4 animals. The reasons for this difference are not understood at present and necessitate additional work. However, if rhodopsin, which accumulates in the cell body of S334ter rats is bleached in a fashion similar to that in ROSs, it may help to explain their enhanced susceptibility to light-induced retinal damage. Among the transgenic rats studied, P23H line-3 animals were the most sensitive to light-induced damage, a finding in general agreement with studies using other transgenic animal models. Heckenlively et al. reported that patients with the P23H rhodopsin mutation were more likely to lose vision when working in bright-light environments than in dim-light environments. In our study, prior light-rearing environment did not alter the relative susceptibility to light-induced damage of P23H rats, but both sublines exhibited prolonged visual cell life when reared in darkness (Table 1). No such protective effect was found in the S334ter rats reared in darkness. Antioxidant pretreatment of transgenic rats was also effective in preventing or reducing the intense-light–mediated loss of rhodopsin and photoreceptor cell DNA. This effect was remarkable in dark-reared S334ter rats, but protection in P23H line-3 rats was incomplete. Because P23H line-3 rats are exquisitely sensitive to intense light, their susceptibility to retinal light damage may overwhelm any protective effect of antioxidant treatment. It remains to be determined whether regular and repeated antioxidant treatment will prolong visual cell life in transgenic rats maintained only in a dim-cyclic-light environment.

Light-induced retinal damage is triggered by rhodopsin bleaching, and, in this respect, transgenic rats appear to be no different from normal animals. Both the normal-molecular-weight rhodopsin and the lower-molecular-weight truncated form were present in ROSs isolated from S334ter rats (Fig. 2). Previous work has shown that the mutated P23H opsin is also present in ROSs from line-3 animals, and we found no differences in the rates of rhodopsin bleaching or regeneration in transgenic rats compared with normal animals (Organisciak D, ARVO Abstract #1195, 1999). The mechanism of light-induced retinal damage in both types of transgenic rats appears to involve an oxidative stress, as shown by the induction of retinal HO-1 (Fig. 4) and the generally protective effect of treatment with DMTU. In normal rat exposure to intense light induces the expression of HO-1, a 3.2-kDA stress protein, treatment with DMTU suppresses its expression. In mice, light-induced photoreceptor cell death also involves caspase-1 activation and the early gene response transcription factor, c-fos. In the current study, both P23H line-3 and S334ter line-9 rats exhibited DNA fragmentation patterns indicative of apoptosis, and both exhibited more extensive DNA laddering at 1 AM than at 9 AM or 5 PM. However, as shown by the recurrent patterns of DNA ladders after light exposure at 1 AM, visual cell loss may be related to the degree of light-induced damage in different hemispheres of the eye.

Defective rhodopsin localization has been reported in the transgenic animal models with rhodopsin truncation mutations, Q-344ter or S334ter, and disc morphogenesis and rod length appear to be altered in mice with multiple missense mutations near the amino terminal of rhodopsin. Our P23H mutant rats also have shortened ROSs compared with normal animals (Coulibaly S, ARVO Abstract #3572, 2001). Thus, although exposure to intense light appears to initiate the same oxidative process in both types of transgenic rats, the rates and extent of visual cell death may be affected by rhodopsin mis-sorting or disc abnormalities. Further studies of the regional and morphologic differences among transgenic rats arising from light-induced retinal damage should provide additional insights into the relationship between inheritance and retinal degeneration.

**References**


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