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DNA Repair in Photoreceptor Survival

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Abstract

Light triggers a sequence of events that damage photoreceptor cells within the superior central portion of the retina, resulting in apoptotic cell death. This damage is mediated by energy absorbed by rhodopsin and the intermediates of the rhodopsin-bleaching process. Furthermore, inhibition of the visual cycle and the re-isomerization of all-*trans* retinol preserve photoreceptors. We have recently shown light-induced DNA fragmentation to occur only within photoreceptors, and, in time-courses following light treatment, these cells exhibit two peaks of damage, approx 24 h apart. This was also observed by quantification of nucleosome-length DNA fragments and their multimers (DNA ladders) as well as by highly repetitive short interspersed nuclear element (SINE) analysis. This bimodal pattern of photoreceptor DNA fragmentation suggests two populations of cells, and each of these were affected by light at a different rate or time. However, the rat retina is composed of 500 nm-sensitive rods, and approx 2% cones, suggesting that a two-cell-type hypothesis is incorrect. Thus, there is a possibility that light-induced DNA fragmentation is triggered and that some photoreceptors are able to initiate a repair mechanism, resulting in a temporary decrease in DNA damage followed by another wave of fragmentation that ultimately leads to cell death. Subsequently, we observed that the repair enzyme DNA polymerase β was upregulated following light treatment, again suggesting the presence of a repair mechanism. Our results suggest that a DNA-repair mechanism exists within photoreceptors, and indicate that manipulation of this process may provide additional protection and/or recovery from events that trigger DNA fragmentation and apoptotic cell death in photoreceptors.

Index Entries: Light damage; photoreceptors; DNA fragmentation; DNA repair; DNA polymerase β.

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Introduction

Neurons survive potentially damaging events (e.g., ischemia) by unregulating cell-protective signaling. Three days after ischemia-induced neuronal injury in gerbils, one-third of the CA3 hippocampal neurons become TdT-Mediated dUTP nick-end labeling (TUNEL)-positive, indicating extensive DNA fragmentation. However, after an additional 4 d, all TUNEL labeling is gone, and there is no evidence of cell loss in the CA3 region, indicating that the fragmented DNA must have been repaired *(1).* Other studies have further demonstrated ischemia-induced early DNA single-strand nicks in brain, followed by delayed fragmentation of DNA *(2).* In general, these studies suggest that a DNA-repair mechanism operates in neurons during oxidative stress, in which DNA nicks or breakage occur. Little is known about similar mechanisms within the retina, but, because photoreceptors have extremely high metabolic activity, absorb large numbers of photons, contain relatively large amounts of highly unsaturated fatty acids in their phospholipids, and generally have a strong potential for the generation of free radicals, it is likely that highly efficient DNA-repair mechanisms are present. In fact, in the case of the retina, excessive light triggers damage, and some photoreceptors recover *(3–8).* In this article, we summarize recent studies that examine the events that are triggered by excessive light in photoreceptors and demonstrate that specific DNA polymerases are induced.

The Use of Light-Damage Models

Most of the light-damage methods use cool, white fluorescent light placed above *(9–11)* or surrounding *(12,13)* the animals. However, some models make use of green *(14,15)* or blue fluorescent light *(16,17).* Xenon sources and fiberoptics have also been used when specific wavelengths were required *(3,18).* There are also continuous *(19)* and intermittent *(9)* light models. Intensities also vary greatly among models, as do the duration of exposure and the time of day *(20).*

We chose an array of circular, cool white fluorescent lamps because the emission spectrum spans the green-rod rhodopsin-absorption curve. Comparisons of fluorescence emission and rod-absorption curves reveal that these photoreceptors can absorb 34% of the available white light. This stimulator produces 20 kLx of light, and triggers photoreceptor injury with 50% cell loss within 36–48 h *(21).* The damaged area consistently involves only the superior central region of the retina (Figs. 1, 2), as shown in rodents by other investigators *(7,10,22,23).* Finally, the short stimulus period—5 h—of our model greatly reduces animal stress.

It has been suggested that exposure to sunlight contributes to the development of age-related macular degeneration, and that bright light accelerates its progression *(24–26).* Retinitis pigmentosa (RP) may also be influenced by light. In animal models for RP, in which there are mutations similar to those expressed in human RP patients, there is an accelerated progression and increased severity of retinal degeneration when excessive light is present *(27,28).* In RP patients who suffer from retinal degeneration, its progression may be slowed by uniocular occlusion *(29).* Thus, light-damage models may be useful for the study of photoreceptor degeneration. After exposure to bright light, photoreceptors die by apoptosis *(14),* the mode of cell death recognized as the final common pathway in many human retinal diseases *(30).* Although photoreceptors die over an extended period of time in long-term degenerative diseases of the retina, light-induced retinal-degeneration models synchronize this process, resulting in significant cell dropout during a very brief interval. The changes that occur during this induced photoreceptor degeneration are, therefore easier to detect. Thus, the light-damage model represents an experimental system with which to study mechanisms that may be involved in the physiopathology of retinal diseases or degenerative conditions in the nervous system.

Light-Induced Retinal Degeneration

The damaging effect of visible light on photoreceptor cells was described by Noell and col-

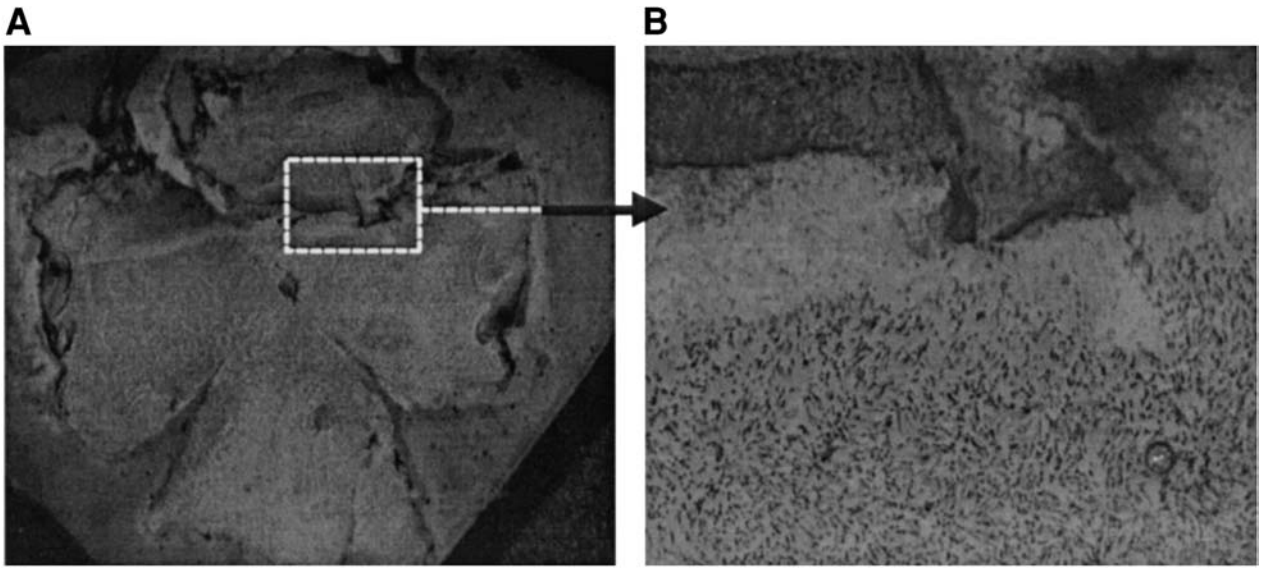

Fig. 1. Whole-mount retina after photoreceptor dropout. Retina labeled with peanut agglutinin after 5 h of light treatment plus 10 d. Damage appears in the superior light-"sensitive" region. 100× and 400× photographs (left and right, respectively) show an absence of photoreceptors in the damaged area.

leagues in 1966 *(22).* Since then, numerous studies have further characterized this response. Excessive light triggers apoptosis in photoreceptors *(14),* and morphologic changes appear that include disc vesiculation, mitochondrial swelling, and densification of cytoplasm *(6,8).* Finally, there is nuclear membrane fragmentation and pyknosis, signifying inevitable DNA loss and cell death.

Exposure to bright light triggers rapid degeneration of the rat retina. This process is initiated upon photon absorption by rhodopsin *(16),* and the action spectrum of light damage matches the green-sensitive rod rhodopsin-absorbance curve *(18).* Therefore, the extent of rhodopsin bleaching and its subsequent regeneration determines the degree of injury *(31).* Moreover, when the visual cycle and rhodopsin re-isomerization are inhibited by the anesthetic halothane, rhodopsin-mediated absorbance of the critical number of photons is impeded, and light-induced photoreceptor death is prevented *(32).* Inhibition of the visual cycle by 13-*cis*-retinoic acid also offers protection from light damage *(33).* However, blue light enables *in situ* photoreversal of rhodopsin bleaching, and increases the photon-catching capacity of the retina, thereby making photoreceptors even more susceptible to light damage *(16).* Finally, rhodopsin knockout mice (Rho−/−) are completely protected against light-induced photoreceptor apoptosis *(34).*

Oxidative stress is involved in the initiation of damage after excessive light exposure. The interaction of light with visual pigments results in the production of reactive oxygen species (ROS) *(19,22).* In the rat, photoreceptor cell death from exposure to bright light can be prevented by treatment with antioxidants such as dimethylthiourea *(35),* ascorbic acid *(36),* and beta-carotene *(37).* Moreover, the metal-independent superoxide dismutase mimic (TEMPOL) reduces light-induced retinal degeneration *(38).* Therefore, large amounts of ROS are produced in the outer and inner segments of photoreceptors when they are exposed to light.

The generation of ROS in the outer segment requires rhodopsin activation. However, free radicals produced in the ellipsoid are a result of mitochondrial metabolism responding to the requirement for adenosine 5′ triphosphate (ATP) generated by the increase of inward Na^+

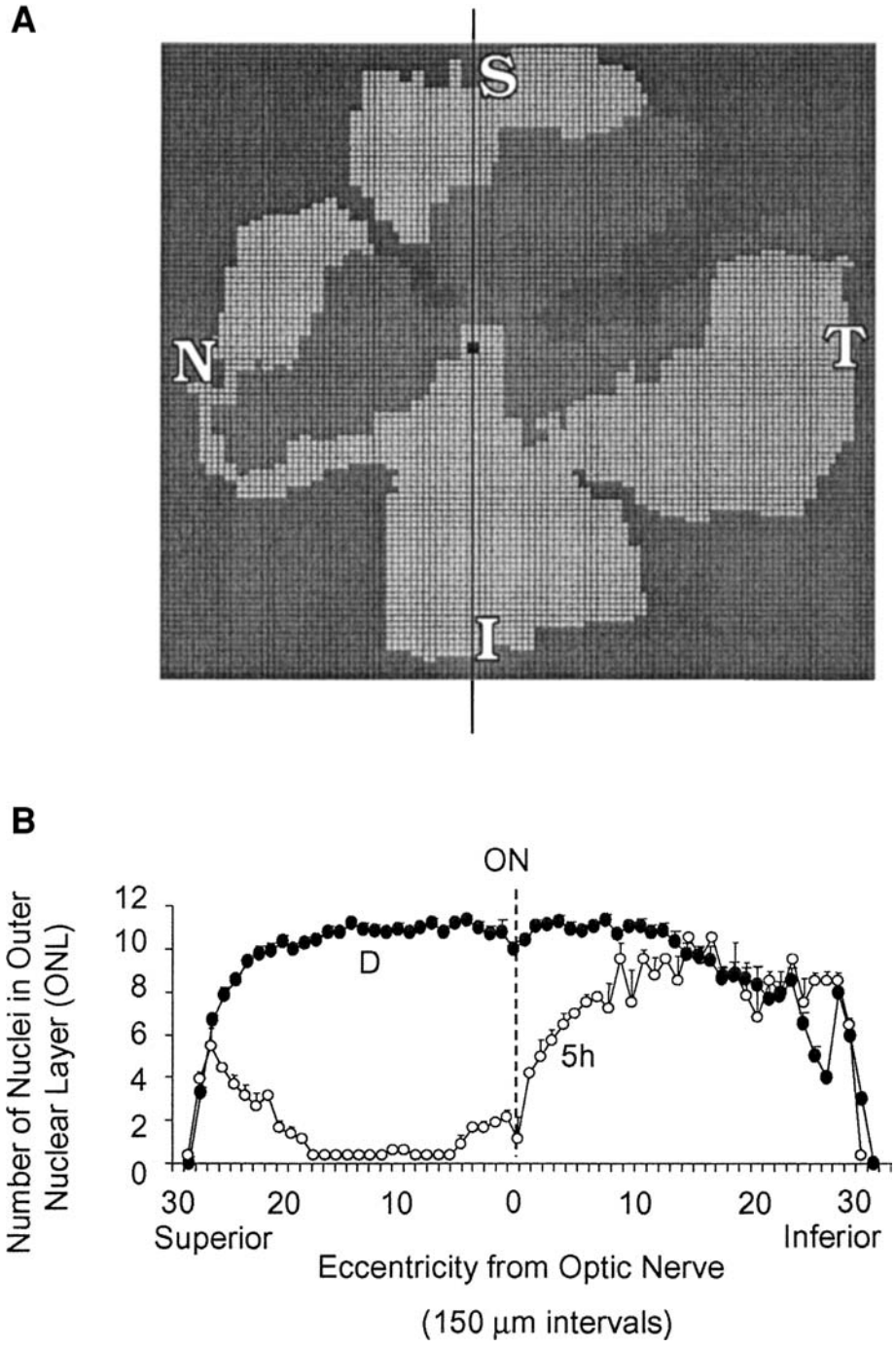

Fig. 2. Light-induced photoreceptor dropout regions in rat retina at 10 d following 5 h of light treatment. **(A)** Peanut agglutinin-labeled photoreceptors in whole mounts delineate the region of photoreceptor loss within the superior retina. Microscope fields at a magnification of ×200 were scored for presence or absence of labeled cells. Plots were then assembled to map the region of light damage in whole retinas. The plot shown here, oriented with the superior retina at the top and the nasal margin to the left, shows the entire photoreceptor dropout area for a single retina. Sections, cut from the superior edge of the retina through the optic nerve to the inferior margin (along the vertical line on this plot), allow the thickness of the outer nuclear layer (ONL) to be measured along the retina. **(B)** Retinal ONL thickness (number of nuclei) plots along meridianal sections of dark-control (D) and light-treated (5h) rat retinas. Control rat ONLs are 12 nuclei thick. After photoreceptor loss, a region of damage appears within the central superior retina, centered approx 1.2 mm above the optic nerve (reprinted from *21* with permission).

current upon light exposure *(17,39,40).* Moreover, excessive light may cause membrane lipid peroxidation *(41,42).* Finally, because of the geometric arrangement of densely packed mitochondria in the ellipsoid, interactions between mitochondria are reinforced, so that oxidative stress generated locally may induce the entire mitochondrial network to produce and release more free radicals *(17).*

It is generally accepted that the accumulation of oxidative damage contributes to the aging process, and this concept has been extensively discussed (*see* reviews by Neely and Montine *[43]* and Liang and Godley *[44]*). Therefore, light-induced free radical formation may play an important role in the pathogenesis of age-related macular degeneration *(26,45).* Several factors make the retina especially susceptible to oxidative stress. First, it contains chromophores and photosensitizers such as rhodopsin, lipofuscin, melanin, cytochrome c oxidase, and blood-borne pigments that, when activated, may generate ROS *(46).* Second, the outer segments of photoreceptors contain high concentrations of polyunsaturated fatty acids, specifically, docosahexaenoic acid (22:6) *(47,48).* Retinal 22:6, a highly unsaturated fatty acid, is affected by intense light *(49),* and may undergo oxidation when exposed to oxygen or its derivatives. However, it has also been suggested that 22:6 release may be protective *(50,51).* The accumulation of lipid peroxidation products in photoreceptors contributes to their degeneration *(19).* Third, photoreceptors and the retinal pigment epithelium are exposed to light and high oxygen tension, providing ideal conditions for the generation of ROS. Finally, photoreceptors are electrically coupled through gap junctions that may provide a pathway for free radical propagation, which may compromise nearby photoreceptors and cause additional cell loss. Ripps *(52)* refers to this indirect cell loss as the "bystander effect." Thus, photoreceptors exposed to bright light may produce potentially damaging amounts of ROS within both the outer and inner segments.

When cells are under stress, mitochondria play a crucial role in the determination of cell fate. Mitochondria are responsible for oxidative phosphorylation, which generates ATP by way of the respiratory chain. However, approx 2% of the oxygen that is consumed during this process is released as ROS *(53).* Free radicals are generated during oxidative phosphorylation, and they can also, in turn, affect ATP production. For example, nitric oxide binds to the iron of heme-containing complexes of the respiratory chain and inactivates them, potentially leading to mitochondrial dysfunction *(54).* Moreover, mitochondrial DNA is directly damaged by ROS *(53),* which could result in the alteration of functional genes and a further decline in oxidative phosphorylation. The decrease in ATP availability may lead to permeabilization of mitochondrial inner and outer membranes with the release of cytochrome C and subsequent activation of caspases. A caspase-independent pathway may also be activated with the release of apoptotic-inducing factor *(55,56).* The mechanisms involved in photoreceptor apoptosis are not well-understood; however, the downregulation of NF-κB *(57)* and the upregulation of c-Fos-containing AP-1 activity are essential steps in light-induced photoreceptor death *(58).* Ultimately, DNA fragmentation is induced by the activation of endonucleases. Apparently, the alteration of mitochondrial physiology can induce a cellular stress response that includes the release of pro-apoptotic factors and the regulation of gene expression that executes the apoptotic program.

Photoreceptor DNA Damage: An Apoptotic Event

Light exposure triggers DNA damage. Before nuclear pyknosis is evident, DNA single- and double-strand breaks are detected by gel electrophoresis *(59).* This type of DNA fragmentation, in the form of internucleosome-strand breaks, is one of the hallmarks of apoptosis. Blue light produces TUNEL label of photoreceptor nuclei 8–16 h after light exposure, as well as DNA internucleosome cleavage into 180–200 basepairs, or their multimers, by gel electrophoresis (DNA ladders) at corresponding

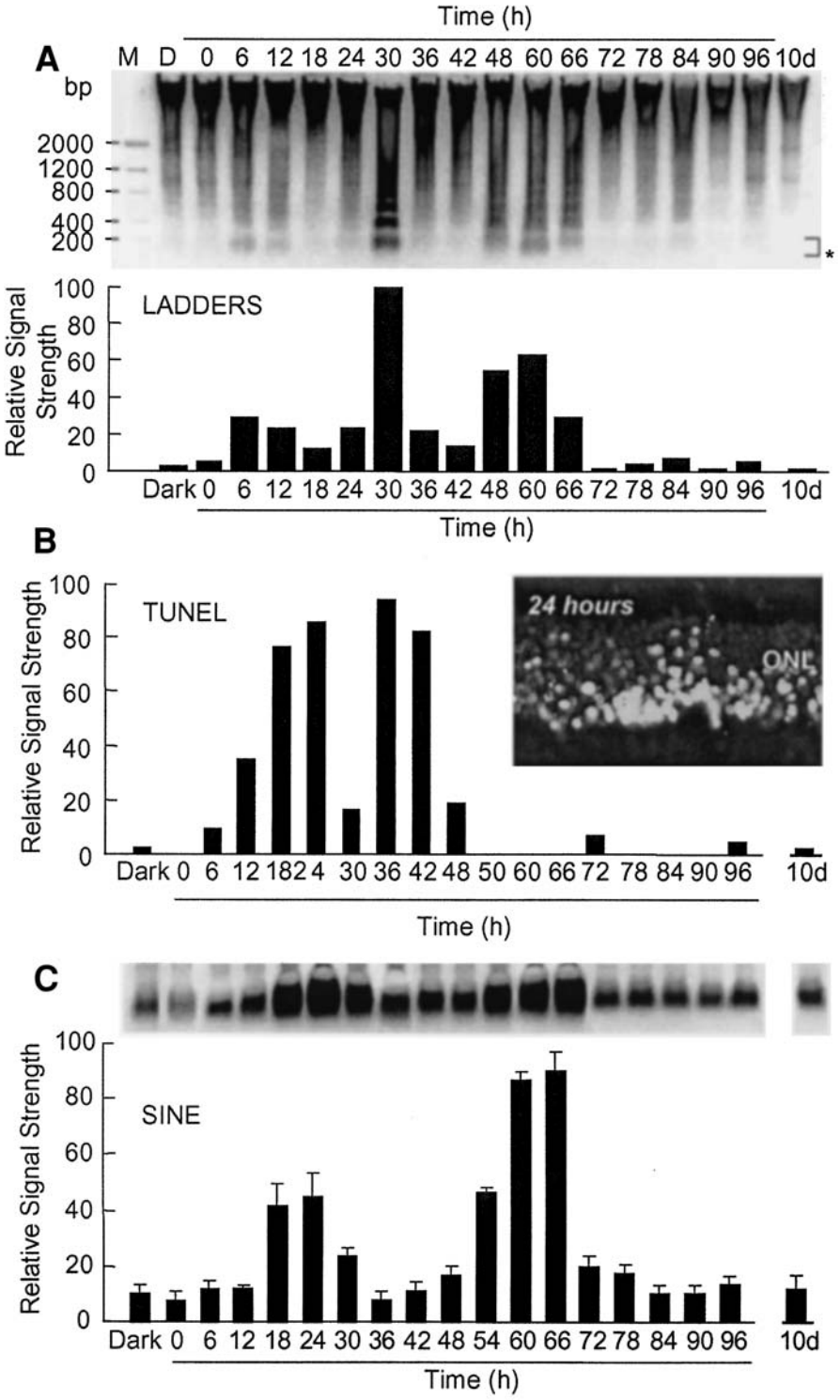

Fig. 3. Analysis of photoreceptor nuclear DNA fragmentation. **(A)** DNA laddering measured at 6-h intervals after 5 h of light treatment. DNA gel ladders are shown at the top. Density measurements of the 180- to 200-bp bands (*) are shown below. **(B)** TUNEL labeling of photoreceptor-cell nuclei at 6-h intervals after 5 h of light treatment. Total labeled photoreceptor-cell nuclei for each time-point are shown. TUNEL-positive nuclei appear up to 60 h following light treatment. The photograph corresponds to 24 h post-light-treatment retina with TUNEL-positive nuclei. **(C)** Highly repetitive SINE PCR of total retinal DNA after 5 h of light treatment. Rat SINE-specific primers were used to detect low-mol-weight and mononucleosome-size DNA fragments. Density measurements were taken for each time point of three complete time-courses and averaged. ([A], inset of [B], and [C] reprinted from *[21]* with permission.)

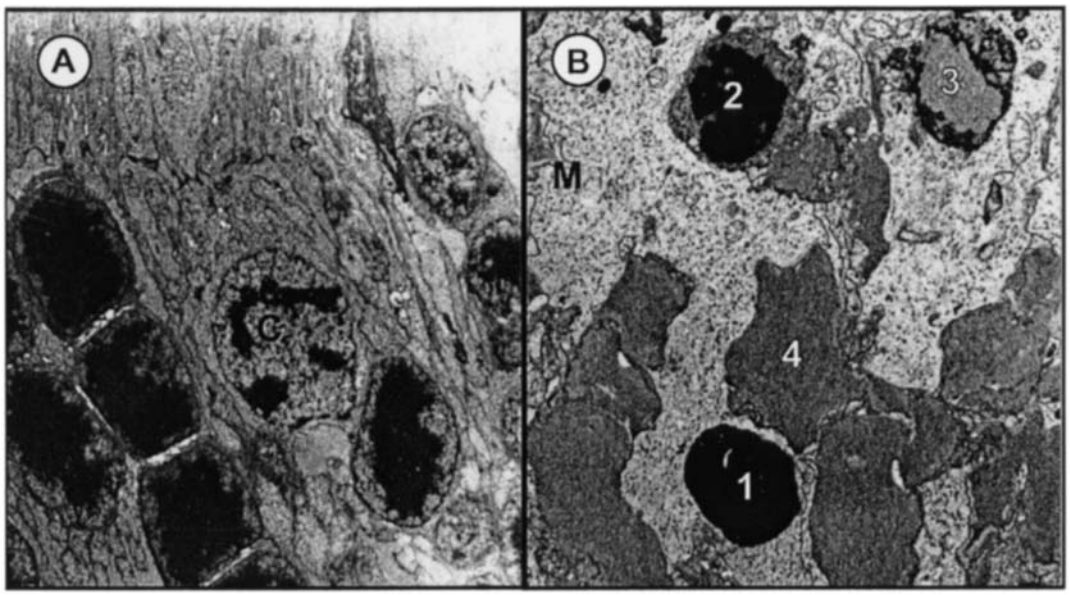

Fig. 4. Electron micrographs of the ONL within the superior central sensitive region of the rat retina. These micrographs demonstrate changes in photoreceptor nuclei at different times following 5 h of light treatment. Micrographs are generally oriented with inner segments up, and are shown at the same size (original magnification 3000×). **(A)** Dark-control retina showing cone (c) and rod (dark) nuclei. Rod nuclei are smaller and denser than cone nuclei, and are surrounded by a thin border of lighter chromatin. These nuclei are organized into columns of about 12 nuclei. **(B)** By 36 h post-light treatment, rod nuclei present several appearances, depending on the progression of apoptosis. Some, just beginning this process, appear as dense spherical packages **(1)**, and others show an irregular profile as the lighter chromatin region thickens and invades the denser area **(2)**. Eventually, the central chromatin becomes lighter with remnants of the dense nuclear material forming a surrounding ring **(3)**. Finally, only lighter material remains in very irregular nuclei that appear to be compacted together **(4)**. Plastic sections stained with toluidine blue will not reveal this last stage. During this process, the Müller cells (M) expand as photoreceptors disintegrate.

time-points *(60).* It has also been suggested that the generation of DNA single-strand breaks, as well as nucleosome-size DNA fragments, implies two phases of light-induced damage, with ROS attacks on DNA preceding enzymatic degradation *(61).*

Recent studies in our lab have demonstrated photoreceptor DNA fragmentation in a biphasic pattern *(21).* Two peaks of photoreceptor nuclei, positive for TUNEL (Fig. 3A), are evident 24–36 h apart. The first peak occurs at 18–24 h and the second at 36–42 h after light exposure. We have also observed two peaks of DNA fragmentation with short interspersed nuclear elements (SINE) analysis (Fig. 3B) and by DNA laddering (Fig. 3C). The appearance of DNA fragmentation coincides with the beginning of progressive condensation in photoreceptor nuclei as shown by electron microscopy, and successive nuclear changes, which are typical of the apoptotic process, follow (Fig. 4).

DNA Repair in Photoreceptors

Four processes have been described by which cells can repair damaged DNA. Most repair systems are based on excision of the DNA lesions. Nucleotide-excision repair (NER) involves about 30 different proteins in human cells that excise damaged oligonucleotide segments approx 30 nucleotides in length *(62).* NER removes major ultraviolet (UV)-induced photoproducts from DNA *(63).* Base-excision

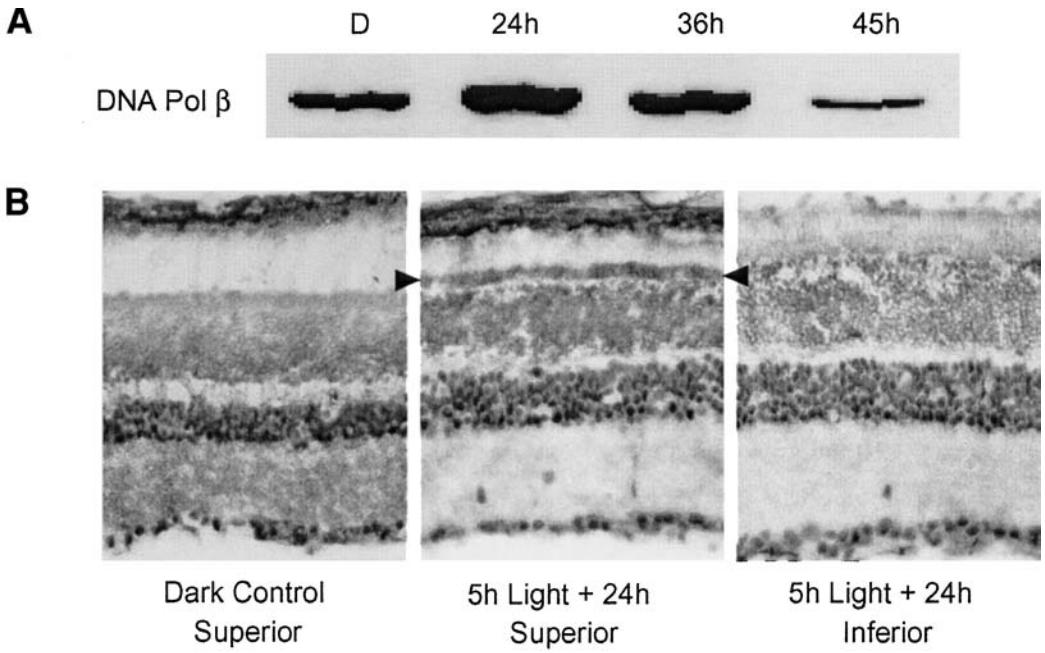

Fig. 5. **(A)** Western-blot analysis showing an increase in DNA polymerase β at 24 h after light treatment. **(B)** Immunohistochemical localization of DNA polymerase β. Image from superior region of the rat retina 1.2 mm from the optic nerve in dark-control retina (left panel) compared with the same region in 24 h post-light-treatment retina (center panel) and corresponding inferior region of the same retina (right panel). Arrowheads indicate a dark immunopositive label corresponding to photoreceptor inner segments. Reprinted from *(21)* with permission.

repair (BER), however, repairs shorter fragments, usually one nucleotide long (although they can be up to six nucleotides in length), using a different set of repair enzymes that act on single-base residues damaged by hydrolysis, ROS *(64),* or simple alkylating agents *(63).* In mismatch repair (MMR), mistakes that occur during DNA replication are recognized and mended *(65).* Some organisms make use of photolyases and specific ultraviolet (UV)-endonucleases to repair thymidine dimers caused by UV damage to DNA *(66).* Finally, double-strand breaks are repaired by a process known as recombination, which involves exchange of equivalent regions between homologous chromosomes *(62).*

DNA repair is a complex process that requires multiple steps and enzymes. However, one major required step is the resynthesis of the excised portion of damaged DNA that restores the original nucleotide sequence. This is performed by a DNA polymerase. Six DNA polymerases have been well-characterized in mammalian cells: α, β, γ, δ, ε, and ζ. DNA polymerase γ is specific to mitochondrial DNA repair, and the others mend nuclear DNA.

Although DNA polymerase β is responsible for 99% of the polymerase activity in neurons *(67),* under certain circumstances, when longer fragments must be repaired, both β and δ, or ε, will be engaged. This suggests that the main repair pathway used by neurons is BER *(68).*

Western-blot analysis has demonstrated upregulation of DNA polymerase β approx 24 h after light exposure (Fig. 5; *21*). This event coincides with the first peak of DNA fragmentation, suggesting that the process leading to breakage, or the actual act of DNA fragmentation, triggers signaling events that lead to the induction of repair enzyme(s). The subsequent decrease in TUNEL label after the initial wave of damage may be the result of polymerase β

activity and repaired DNA. In fact, several studies with many cell types have demonstrated the presence of mechanisms that can repair damage to mitochondrial and nuclear DNA when induced (*see 69*). Neurons possess these mechanisms, and the repair molecules are present. There is now strong evidence that photoreceptors regularly police the condition of their DNA and successfully respond to nicks and fragmentation.

Photoreceptors may be able to recover from damage caused by bright light if the critical number of photons needed to induce apoptosis is not achieved. Moriya and colleagues *(6)* showed that structural changes in photoreceptor cells were reversible when the exposure time to light did not exceed 12 h, although intensities brighter than 80 lx were not tested. Other studies, using 1000 lx from above for 2 h, found evidence of photoreceptor recovery in the inferior nasal retina within 6 d of light exposure, as opposed to the inferior temporal retina that showed extensive and irreversible damage *(8).* This study documented outer segment disc disruption, but did not determine whether nuclear DNA was damaged, so it has not been determined whether fragmented DNA was repaired.

In our studies, using 18–20 klx, we found that the time period between the two waves of TUNEL labeling was too brief to ascribe the large decline of TUNEL label to photoreceptor dropout, suggesting that some DNA damage must have been repaired. Thus, there is evidence for an active DNA-repair mechanism that is able to rescue at least a small number of photoreceptors after light exposure *(21).*

Conclusion

Photoreceptors undergo various levels of oxidative stress during their lifetimes. The products of oxidative stress may lead to DNA damage. Photoreceptors induce repair events in response to DNA fragmentation, and the biochemical events leading to it, in order to preserve cell homeostasis. However, when photoreceptors are exposed to relatively high levels of oxidative stress, such as excessive light, a series of events occurs that leads to changes in mitochondria and alteration of the mitochondrial membrane potential, with a subsequent release of pro-apoptotic factors that are involved in caspase activation. Oxidation-sensitive transcription factors are involved with pro-inflammatory gene expression, which contributes to increased levels of free radicals. Eventually, endonucleases are activated and DNA is fragmented. The events leading to photoreceptor DNA fragmentation trigger the upregulation of DNA polymerase β in an attempt to repair the damaged DNA. This response, although initially adequate, is ultimately insufficient, and is therefore unable to prevent the photoreceptor from undergoing apoptosis. Although bright light triggers this response in most photoreceptors, repair is successful in many cells, halting the path to cell death. We hypothesize that if the repair enzymes and/or their regulating mechanisms are affected, either in quantity or quality, photoreceptors may no longer be able to sustain the fine equilibrium that exists between damage and repair. The upset of this balance may be an important factor that contributes to the development of retinal degenerative diseases such as age-related macular degeneration, in which retinal pigment epithelial cells are lost first, followed by photoreceptors. In the future, the development of pharmacologic strategies to control and upregulate the expression and activity of repair enzymes may make it possible to arrest the progression of photoreceptor degenerations.

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References

1. Gordon W.C., Colangelo V., Bazan N.G., and Klatzo I. (1999) Aspects of the maturation phenom-

enon observed by the TUNEL method. In: *Maturation Phenomenon in Cerebral Ischemia III* (Ito U., Fieschi C., Orzi F., Kuroiwa T., Klatz I., eds.), Springer-Verlag, Berlin, pp. 15–23.
2. Tobita M., Nagano I., Nakamura S., Itoyama Y., and Kogure K. (1995) DNA single-strand breaks in postischemic gerbil brain detected by in situ nick translation procedure. *Neurosci. Lett.* **200,** 129–132.
3. Gorn R.A. and Kuwabara T. (1967) Retinal damage by visible light: a physiologic study. *Arch. Ophthalmol.* **77,** 115–118.
4. Wyse J.P.H. (1980) Renewal of rod outer segments following light-induced damage of the retina. *Can. J. Ophthalmol.* **15,** 15–19.
5. Henton W.W. and Sykes S.M. (1984) Recovery of absolute threshold with UVA-induced retinal damage. *Physiol. Behav.* **32,** 949–954.
6. Moriya M., Baker B.N., and Williams T.P. (1986) Progression and reversibility of early light-indued alterations in rat retinal rods. *Cell Tissue Res.* **246,** 607–621.
7. Rapp L.M. and Smith S.C. (1992) Morphologic comparisons between rhodopsin-mediated and short-wavelength classes of retinal light damage. *Investig. Ophthalmol. Vis. Sci.* **33,** 3367–3377.
8. Szczesny P.J., Munz K., and Remé C.E. (1995) Light damage in the rat retina: Patterns of acute lesions and recovery. In: *Cell and Tissue Protection in Ophthalmology* (Pleyer U., Schmidt K., and Thiel H.-J., eds.), Hippokrates Verlag, Suttgart, Germany, pp. 163–175.
9. Penn J.S. and Williams T.P. (1986) Photostasis: Regulation of daily photon-catch by rat retina in response to various cyclic illuminances. *Exp. Eye Res.* **43,** 915–928.
10. La Vail M.M., Gorrin G.M., Repaci M.A., and Yasumura D. (1987) Light-induced retinal degeneration in albino mice and rats: Strains and species differences. In: *Degenerative Retinal Disorders: Clinical and Laboratory Investigations* (Hollyfield J.G., Anderson R.E., and La Vail M.M., eds.) Alan R. Liss, New York, pp. 439–454.
11. Bush R.A., Remé C.E., and Malnoe A. (1991) Light damage in the rat retina: the effect of dietary deprivation of n-3 fatty acids on acute structural alterations. *Exp. Eye Res.* **53,** 741–752.
12. Shahinfar S., Edward D.P., and Tso M.O. (1991) A pathologic study of photoreceptor cell death in retinal photic injury. *Curr. Eye Res.* **10,** 47–59.
13. Darrow R.A., Darrow R.M., and Organisciak D.T. (1997) Biochemical characterization of cell-specific enzymes in light-exposed rat retinas: oxidative loss of all-*trans* retinol dehydrogenase activity. *Curr. Eye Res.* **16,** 144–151.
14. Abler A.S., Chang C.-J., Fu J., Tso M.O.M., and Lam T.T. (1996) Photic injury triggers apoptosis of photoreceptor cells. *Res. Commun. Mol. Pathol. Pharmacol.* **92,** 177–189.
15. Organisciak D.T., Darrow R.M., Barsalou L., Kutty R.K., and Wiggert B. (2000) Circadian-dependent retinal light damage in rats. *Investig. Ophthalmol. Vis. Sci.* **41,** 3694–3701.
16. Grimm C., Wenzel A., Williams T.P., Rol P.O., Hafezi F., and Remé C.E. (2001) Rhosopsin- mediated blue-light damage to the rat retina: effect of photoreversal of bleaching. *Investig. Ophthalmol. Vis. Sci.* **42,** 497–505.
17. Yang J.H., Basinger S.F., Gross R.L., and Wu S.M. (2003) Blue light-induced generation of reactive oxygen species in photoreceptor ellipsoids requires mitochondrial electron transport. *Investig. Ophthalmol. Vis. Sci.* **44,** 1312–1319.
18. Williams T.P. and Howell W.L. (1983) Action spectrum of retinal light-damage in albino rats. *Investig. Ophthalmol. Vis. Sci.* **24,** 285–287.
19. Wiegand R.D., Giusto N.M., Rapp L.M., and Anderson RE. (1983) Evidence of rod outer segment lipid peroxidation following constant illumination of the rat retina. *Investig. Ophthalmol. Vis. Sci.* **24,** 1433–1435.
20. Organisciak D.T, Darrow R.M., Barsalou L., and Kutty K. (2003) Susceptibility to retinal light damage in transgenic rats with rhodopsin rutations. *Investig. Ophthalmol. Vis. Sci.* **44,** 486–492.
21. Gordon W.C., Casey D.M., Lukiw W.J., and Bazan N.G. (2002) DNA Damage and Repair in Light-Induced Photoreceptor Degeneration. *Investig. Ophthalmol. Vis. Sci.* **43,** 3511–3521.
22. Noell W.K., Walker V.S., Kang B.S., and Berman S. (1966) Retinal damage by light in rats. *Investig. Ophthalmol.* **5,** 450–473.
23. Howell W.L., Rapp L.M., and Williams T.P. (1982) Distribution of melanosomes across the retinal pigment epithelium of a hooded rat: implications for light damage. *Investig. Ophthalmol Vis. Sci.* **22,** 139–144.
24. Mainster M.A. (1987) Light and macular degeneration: a biophysical and clinical perspective. *Eye* **1,** 304–310.
25. Young R.W. (1988) Solar radiation and age-related macular degeneration. *Surv Ophthalmol.* **32,** 252–269.
26. Winkler B.S., Boulton M.E., Gottsch J.D., and Stenberg P. (1999) Oxidative damage and age-related macular degeneration. *Mol. Vis.* **5,** 32.
27. Nash M.C., Peachey N.S., Li Z.-Y., Gryczan C.C., Gota Y., Blanks J., et al. (1996) Light-induced acceleration of photoreceptor degeneration in transgenic mice expressing mutant rhodopsin. *Investig. Ophthalmol. Vis. Sci.* **37,** 775–782.
28. La Vail M.M., Gorrin G.M., Yasumura D., and Matthes M.T. (1999) Increased susceptibility to con-

stant light in *nr* and *pcd* mice with inherited retinal degenerations. *Investig. Ophthalmol. Vis. Sci.* **40,** 1020–1024.
29. Berson E.L. (1971) Light deprivation for early retinitis pigmentosa: a hypothesis. *Arch. Ophthalmol.* **85,** 521–529.
30. Remé C.E., Grimm C., and Hefezi F. (1998) Apoptotic cell death in retinal degenerations. *Prog. Retina Eye Res.* **17,** 443–464.
31. Organisciak D.T., Xie A., Wang H.M., Jiang Y.L., Darrow R.M., and Donoso L.A. (1991) Adaptive changes in visual cell transduction protein levels: effect of light. *Exp. Eye Res.* **53,** 773–779.
32. Keller C., Grimm C., Wenzel A., Hafezi F., and Remé C.E. (2001) Protective effect of halothane anesthesia on retinal light damage: Inhibition of metabolic rhodopsin regeneration. *Investig. Ophthalmol. Vis. Sci.* **42,** 476–480.
33. Sieving P.A., Chaudhry P., Kondo M., Provenzano M., Wu D., Carlson T.J., et al. (2001) Inhibition of the visual cycle in vivo by 13-*cis* retinoic acid protects from light damage and provides a mechanism for night blindness in isoretinoin therapy. *Proc. Natl. Acad. Sci. USA* **98,** 1835–1840.
34. Grimm C., Wenzel A., Hafezi F., Yu S., Redmond T.M., and Remé C.E. (2000) Protection of Rpe65-deficient mice identifies rhodopsin as a mediator of light-induced retinal degeneration. *Nat. Genet.* **25,** 63–66.
35. Organisciak D.T., Darrow R.A., Barsalou L., Darrow R.M., and Lininger L.A. (1999) Light-induced damage in the retina: differential effects of dimethylthiourea on photoreceptor survival, apoptosis and DNA oxidation. *Photochem. Photobiol.* **70,** 261–268.
36. Organisciak D.T., Bicknell I.R., and Darrow R.M. (1992) The effects of L-and D-ascorbic acid administration on retinal tissue levels and light damage in rats. *Curr. Eye Res.* **11,** 231–241.
37. Rapp L.M., Fisher P.L., and Suh D.W. Evaluation of retinal susceptibility to light damage in pigmented rats supplemented with beta-carotene. *Curr. Eye Res.* **15,** 219–223.
38. Wang M., Lam T.T., Fu J., and Tso M.O. (1995) TEMPOL, a superoxide dismutase mimic, ameliorates light-induce retinal degeneration. *Res. Commun. Mol. Pathol. Pharmacol.* **89,** 291–305.
39. Ames A. III, Li Y.Y., Heher E.C., and Kimble C.R. (1992) Energy metabolism of rabbit retina as related to function: high cost of Na+ transport. *J. Neurosci.* **12,** 840–853.
40. Demontis G.C., Longoni B., and Marchiafava P.L. (2002) Molecular steps involved in light-induced oxidative damage to retinal rods. *Investig. Ophthalmol. Vis. Sci.* **43,** 2421–2427.
41. Handelman G.J. and Dratz E.A. (1986) The role of antioxidants in the retina and retinal pigment epithelium and the nature of prooxidants-induced damage. *Adv. Free Radic. Biol. Med.* **2,** 1–89.
42. Kayatz P., Heimann K., and Schraermeyer U. (1999) Ultrastructural localization of light-induced lipid peroxides in the rat retina. *Investig. Ophthalmol. Vis. Sci.* **40,** 2314–2321.
43. Neely M.D. and Montine T.J. (2002) CSF lipoproteins and Alzheimer's disease. *J. Nutr. Health Aging* **6,** 383–391.
44. Liang F.Q. and Godley B.F. (2003) Oxidative stress-induced mitochondrial DNA damage in human retinal epithelial cells: a possible mechanism for RPE aging and age-related macular degeneration. *Exp. Eye Res.* **76,** 397–403.
45. Beatty S., Koh H.H., Phil M., and Henson D. (2000) The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv. Ophthalmol.* **45,** 155–134.
46. Rapp L.M. (1995) Retinal phototoxicity. In *Handbook of Neurotoxicology* (Chang L.W. and Dyer R.S., eds.) Marcel Dekker, New York, pp. 963–1003.
47. Rodriguez de Turco E.B., Gordon W.C., Peyman G.A., and Bazan N.G. (1990) Preferential uptake and metabolism of docosahexaenoic acid in membrane phospholipids from rod and cone photoreceptor cells of human and monkey retinas. *J. Neurosci. Res.* **27,** 522–532.
48. Rodriguez de Turco E.B., Jackson F.R., Parkins N., and Gordon WC. (2000) Strong association of unesterified [3^H] docosahexaenoic acid and [3^H-docosahezaenoyl] phosphatidate to rhodopsin during *in vivo* labeling of frog retinal rod outer segments. *Neurochem. Res.* **25,** 695–703.
49. Organisciak D.T., Wang H.-M., Xie A., Reeves D.S., and Donoso L.A. (1989) Intense light-mediated changes in rat rod outer segment lipids and proteins. *Prog. Clin. Biol. Res.* **314,** 493–512.
50. Reinboth J.J., Clausan M., and Remé C.E. (1996) Light elicits release of docosahexanoic acid from membrane phospholipids in the rat retina in vitro. *Exp. Eye Res.* **63,** 277–184.
51. Kim H.-Y., Akbar M., Lau A., and Edsall L. (2000) Inhibition of neuronal apoptosis by docosahexanoic acid (22:6n-3). *J. Biol. Chem.* **275,** 35,215–35,225.
52. Ripps H. (2002) Cell death in retinitis pigmentosa: gap junctions and the 'bystander' effect. *Exp. Eye Res.* **74,** 327–336.
53. Yakes F.M. and Van Houten B. (1997) Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Cell Biol.* **94,** 514–519.
54. Mattson M.P. and Liu D. (2002) Energetics and oxidative stress in synaptic plasticity and neurodegenerative disorders. *Neuromol. Med.* **2,** 215–231.

55. Kroemer G. and Reed J.C. (2000) Mitochondrial control of cell death. *Nat. Med.* **6,** 513–519.
56. Kaufmann S.H. and Hengartner M.O. (2002) Programmed cell death: alive and well in the new millennium. *Trends Cell Biol.* **11,** 526–534.
57. Krishnamoorthy R.R., Crawford M.J., Chaturverdi M.M., Jain S.K., Aggarwal B.B., Al-Ubaidi M.R., et al. (1999) Photo-oxidative stress down-modulates the activity of nuclear factor-KB via involvement of caspase-1, leading to apoptosis of photoreceptor cells. *J. Biol. Chem.* **274,** 3734–3743.
58. Wenzel A., Grimm C., Marti A., Kueng-Hitz N., Hafezi F., Niemeyer G., and Reme C.E. c-Fos controls the "private pathway" of light-induced apoptosis of retinal photoreceptors. (2000) *J. Neurosci.* **20,** 81–88.
59. Messing S., Leffak M., Darrow R.M., Fleishman D., Organisciak D.T. (1995) Visible light induced damage in rat retinal genes. *Investig. Ophthalmol. Vis. Sci.* **Suppl 36,** S520.
60. Wu J., Seregard S., Spangberg B., Oskarsson M., and Chen E. (1999) Blue light induced apoptosis in rat retina. *Eye* **13,** 577–583.
61. Specht S., Leffak M., Darrow R.M., and Organisciak D.T. (1999) Damage to rat retinal DNA induced *in vivo* by visible light. *Photochem. Photobiol.* **69,** 91–98.
62. Friedberg E.C., Walker G.C., and Siede W. (1995) *DNA Repair and Mutagenesis.* American Society of Microbiology Press, Washington, DC.
63. Wood R.D. (1996) DNA repair in eukaryotes. *Annu. Rev. Biochem.* **65,** 135–167.
64. Lindahl T. and Wood R.D. (1999) Quality control by DNA repair. *Science.* **286,** 1897–1905.
65. Friedberg E.C. (2003) DNA damage and repair. *Nature* **421,** 436–440.
66. Sancar G.B. (2000) Enzymatic photoreactivation: 50 years and counting. *Mutat. Res.* **451,** 25–37.
67. Waser J., Hubscher U., Kuenzle C.C., and Spadari S. (1979) DNA polymerase β from brain neurons is a repair enzyme. *Eur. J. Biochem.* **97,** 361–368.
68. Wood R.D. and Shivji M.K.K. (1997) Which DNA polymerases are used for DNA-repair in eukaryotes? *Carcinogenesis* **18,** 605–610.
69. Jinno S., Kida K., and Taguchi T. (1995) Induction of DNA polymerase β and γ in the lungs of age-related oxygen tolerant rats. *Mech. Aging Devel.* **85,** 95–107.