Photoreceptor Autophagy: Effects of Light History on Number and Opsin Content of Degradative Vacuoles

Charlotte E. Remé,[1] *Uwe Wolfrum,*[2] *Cornelia Imsand,*[1] *Farhad Hafezi,*[1] *and Theodore P. Williams*[3]

Purpose. To investigate whether regulation of rhodopsin levels as a response to changed lighting environment is performed by autophagic degradation of opsin in rod inner segments (RISs).

Methods. Groups of albino rats were kept in 3 lux or 200 lux. At 10 weeks of age, one group was transferred from 3 lux to 200 lux, another group was switched from 200 lux to 3 lux, and two groups remained in their native lighting (baselines). Rats were killed at days 1, 2, and 3 after switching. Another group was switched from 3 lux to 200 lux, and rats were killed at short intervals after the switch. Numbers of autophagic vacuoles (AVs) in RISs were counted, and immunogold labeling was performed for opsin and ubiquitin in electron microscopic sections.

Results. The number of AVs increased significantly after switching from 3 lux to 200 lux at days 1 and 2 and declined at day 3, whereas the reverse intensity change did not cause any increase. Early time points after change from 3 lux to 200 lux showed a significant increase of AVs 2 and 3 hours after switching. Distinct opsin label was observed in AVs of rats switched to 200 lux. Ubiquitin label was present in all investigated specimens and was also seen in AVs especially in 200-lux immigrants.

Conclusions. Earlier studies had shown that an adjustment to new lighting environment is performed by changes in rhodopsin levels in ROSs. Autophagic degradation of opsin or rhodopsin may subserve, at least in part, the adaptation to abruptly increased habitat illuminance by removing surplus visual pigment. (*Invest Ophthalmol Vis Sci.* 1999;40:2398–2404)

The light-sensitive rod outer segments (ROSs) are continually renewed by the shedding of their tips, degradation of the shed tips in the retinal pigment epithelium (RPE), and membrane synthesis at the ROS base.[1] In rod inner segments (RISs), bulk degradation, termed autophagy, is observed.[2] Autophagic vacuoles (AVs) that are ubiquitous in many cell types contain membrane-bound cytoplasmic components such as mitochondria, which are degraded by the lysosomal system in contrast to other cellular degradative routes.

Major parts of renewal follow a circadian rhythm[3] and thus represent a highly regulated process. Inner segment autophagic degradation also shows rhythmicity, which persists in constant light but is rapidly abolished in constant darkness. In addition, both disc shedding and autophagy can be evoked by light pulses, further suggesting regulative functions of these processes.[4]

It has been proposed that those degradative processes may serve to adjust the light sensitivity of rods as a means to regulate the visual input stage for circadian rhythm regulation.[5] Indeed, the adjustment to new lighting environment is in part performed by changes in disc-shedding patterns and is associated with the adaptation of rhodopsin levels in outer segment disks.[6,7]

In view of recent thinking that protein degradation constitutes an important mechanism of cellular regulation, including adaptation and control of timing of cellular programs,[8] it is conceivable that opsin or rhodopsin or both are degraded by autophagy in photoreceptors under conditions in which adaptation is required. In the present study, we investigated whether an abruptly reversed lighting environment—a condition that would require adaptation—results in a modification of the number of AVs and whether these AVs contain rhodopsin. The existence of a ubiquitin-dependent pathway regulating phototransduction protein levels in mammalian ROSs has been shown previously.[9] Therefore, we also tested for ubiquitin labeling in rods under our experimental conditions.

Materials and Methods

Animals

All experiments described herein conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Albino rats (ZUR/SIV; Institute of Animal Breeding, University of Zurich, Switzerland) were obtained as weanlings and were maintained in the animal quarters of the University Hospital, Zurich, for 9 to 10 weeks.

From the [1]University Eye Clinic, Zurich, Switzerland; the [2]Institute for Zoology, University of Mainz, Germany; and the [3]Department of Biological Science, Florida State University, Tallahassee.

CER and UW contributed equally to this work.

Supported by an Alexander von Humboldt Stiftung Senior Scientist Award (TPW), Swiss National Science Foundation Grant 31-40791.94; the Suva Research Foundation, Luzern; the Ernst and Bertha Grimmke Foundation, Düsseldorf, Germany (CER); and Deutsche Forschungsgemeinschaft Grant Wo 548/3-1, FAUN-Stiftung, Nürnberg, Germany (UW).

Submitted for publication December 23, 1998; revised April 30, 1999; accepted May 27, 1999.

Proprietary interest category: N.

Corresponding author: Charlotte E. Remé, Department of Ophthalmology, University Hospital, 8091 Zurich, Switzerland.
E-mail: chreme@opht.unizh.ch

Investigative Ophthalmology & Visual Science, September 1999, Vol. 40, No. 10

Experimental Setup

Five groups were studied: one maintained at 3 lux (3-lux natives) and one at 200 lux (200-lux natives) for the entire time, 1 group that was switched to 3 lux from 200 lux (3-lux immigrants) for 3 days and two groups that were switched to 200 lux from 3 lux (200-lux immigrants) for 3 hours or for 3 days before death. All rats were maintained on a 12-hour light–12-hour dark cycle with lights on at 6 AM and food and water provided ad libitum. Rats were moved into the prospective new light regimen at the beginning of the preceding dark period, and light onset in the new environment was at 6 AM.

The following parameters were studied in duplicate experiments: the number of AVs in RISs, immunostaining of opsin in AVs, and immunostaining of ubiquitin.

Native and immigrant rats were killed in dim red light between 10 and 11 AM, at a time when autophagic activity was expected to be high. One of the two 200-lux immigrant groups was killed at 15, 30, 45, 60, 120, and 180 minutes after the change in light regimen at day 1. Eyes were rapidly enucleated and placed in fixative for electron microscopy or immunogold staining.

For quantification of AVs the retinas from five groups of rats were analyzed (n = 2 rats per experimental parameter): 3- and 200-lux natives; 3- and 200-lux immigrants at days 1, 2, and 3 after switching; and 200-lux immigrants killed on the first day of switching at 0 (baseline) 15, 30, 45, 180, and 240 minutes after lights on.

Quantification of AVs by Electron Microscopy

After rats were killed and the eyes enucleated, the anterior half of each eye was removed and the posterior half fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer and processed for electron microscopic observation, as described.[4] Particular care was taken to prepare identical retinal areas (the central upper and the central lower retinal regions) for the quantitative analysis. Autophagic vacuoles are defined as membrane-bound, roundish bodies with more or less degraded cytoplasmic contents.[2] For each retina, only one electron microscopic section mounted on a carbon-coated, single-hole grid was quantified, to ensure that no double counting of vacuoles occurred. In each section, 100 well-oriented RISs were counted. The area of counting comprised the ellipsoid and myoid region up to the outer limiting membrane. The perinuclear area and the synaptic body were excluded from counting.

Immunogold Labeling

Monoclonal antibodies against bovine rod opsin were kindly provided by Paul A. Hargrave (University of Florida, Gainesville). The clones used in the present study were B6-30a1, K16-155, and R2-15 and were described by Adamus et al.[10] The anti-opsin monoclonal antibodies were applied separately, diluted (1:100 to 1:800) in blocking solution (0.5% fish gelatin; Sigma, Deisenhofen, Germany) or 0.1% ovalbumin (Sigma) in phosphate-buffered saline (PBS), or the diluted antibodies were used in a cocktail of all three clones (1:1:1). The monoclonal antibody against ubiquitin was purchased from Boehringer, Ingelheim, Germany, and used in a dilution of 1:1000 in blocking solution. This antibody recognizes both free and conjugated ubiquitin.

After the animals were killed, retinas were removed through a slit in the cornea and fixed in 0.1% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 3 hours at room temperature. Fixed tissue was dehydrated to 98% ethanol, embedded (LR White; Science Services, Munich, Germany), and polymerized at 4°C under UV light for 48 to 60 hours.

Ultrathin sections (60–70 nm) were collected on formvar-coated nickel grids. Sections were first etched with saturated sodium periodate (Sigma) at room temperature for 3 minutes. The grids were preincubated with 0.1% Tween 20 in PBS and blocked with 50 mM NH_4Cl in PBS and in blocking solution. Sections were incubated with primary antibodies (anti-opsin or anti-ubiquitin) diluted in blocking solution at 4°C for 60 hours and washed once in PBS and twice in a mixture of 0.1% ovalbumin, 0.5% cold-water fish gelatin, 0.01% Tween 20, and 0.5 M NaCl in 10 mM phosphate buffer (pH 7.3). The sections were incubated for 2 hours with goat anti-mouse IgG conjugated to nanogold (Nanoprobes, Stony Brook, NY), diluted in 0.1% ovalbumin, 0.5% fish gelatin, 0.01% Tween 20, and 0.5 M NaCl in 10 mM phosphate buffer (pH 7.3). Washed sections were postfixed in 2% glutaraldehyde for 10 minutes and air dried. The nanogold labeling was silver enhanced as described by Danscher.[11] The grids were then washed in distilled water and stained with 2% ethanolic uranyl acetate for 10 minutes before observation by electron microscope (model 912Ω; Zeiss, Oberkochen, Germany).

The following controls were applied: Primary or secondary antibodies were omitted, and secondary antibodies against antibodies differing from original primary antibodies and antibodies against different molecules such as anti-tubulins and anti-centrins were used. All those showed staining patterns different from those for ubiquitin.

Statistical Methods

Evaluation of differences in AV counts between natives and immigrants during the first 3 days of switching the light regimens was performed by one-way analysis of variance and Dunnett's multiple comparison tests. Evaluation of early time points in 200-lux immigrants was performed by fitting the data points to a quadratic polynomial $A + Bx + Cx^2$.

RESULTS

Quantification of AVs and Opsin Labeling

Figure 1A represents a schematic drawing of a rod cell showing the main localizations of AVs in RISs. Disc shedding and membrane renewal of ROSs are also indicated. Figure 1B depicts a schematic drawing of the formation and degradation of AVs. Figure 2A shows the effect on AV count of switching the habitat intensity from 3 lux to 200 lux. The 3-lux native animals had an average of 20 AVs/100 rods. On day 1 in 200-lux lighting, the number dramatically increased ($P < 0.01$). The AV count peaked on day 2 ($P < 0.01$) and decreased to almost baseline on day 3 ($P > 0.05$). When 200-lux natives were switched to the 3-lux environment, the number of AVs did not decrease significantly, remaining near baseline levels of approximately 20 AVs (Fig. 2B).

We resolved the early time course of AV appearance in the retinas of 200-lux immigrant animals (Fig. 3). After a short induction period, the count rose from a baseline of approximately 20 AVs and approached that seen in Figure 2A in the new, more intense lighting. This increase was not linear but

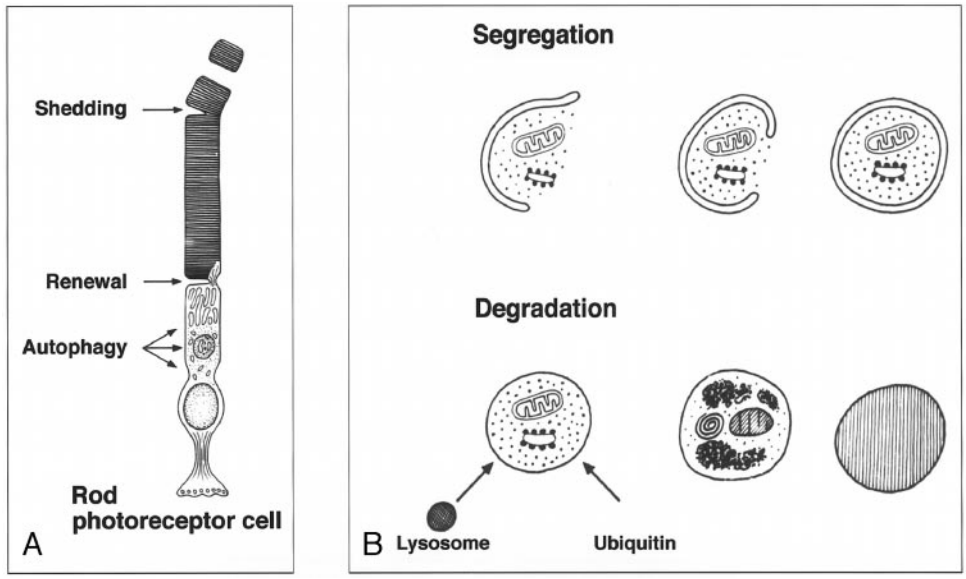

FIGURE 1. Schematic drawings depicting the localization and the formation of AVs. (**A**) Schematic drawing of a photoreceptor cell indicating the main localization of AVs in the ellipsoid and myoid regions and to a lesser degree in the perinuclear area. Vacuoles located in well-oriented RISs in the ellipsoid and myoid regions up to the outer limiting membrane were included in the counts. In this drawing, membrane renewal at the ROS base and disc shedding from the ROS tip are also indicated. (**B**) Schematic drawing showing the formation (segregation) and degradation stages of an AV. After inclusion in one or more membranes and subsequent fusion with lysosomes, the contents of a vacuole are degraded. Alternatively, ubiquitination may occur under some conditions.

quadratic and was statistically significant at 2 and 3 hours after the light switch ($P < 0.01$).

Figure 4A shows an electron micrograph of newly formed and partially degraded AVs. Newly formed AVs contained recognizable cytoplasmic components such as cytoplasm, mitochondria, ribosomes, or parts of the Golgi apparatus. Old AVs displayed degraded, and therefore unrecognizable, condensed contents. Occasionally, AVs may display electron-lucent areas indicating a removal of some of their contents. Figure 4B depicts label in a 200-lux native. Apart from AV labeling, the anti-opsin labeling was seen at sites of rod cells where opsin is expected to be located. These are disc membranes of ROSs and membranes of the Golgi apparatus and transport vesicles in RISs (the latter not shown). In contrast, cone photoreceptor cells remained totally unlabeled.

Rods of 200-lux immigrants reacted to the new, higher illuminance. Figures 4C and 4D show examples of AVs immunogold stained with anti-opsin antibody. Figure 5A shows AVs in the synaptic body, and Figure 5B shows opsin staining in an AV within the synaptic body, at the perinuclear region and in the cytoplasm. Figure 5C displays opsin staining in the plasma membrane of an RIS, and Figure 5D exhibits disrupted ROS tips indicating early signs of moderate light damage.

Ubiquitin

Ubiquitin label was observed in the cytoplasm of RISs and in ROSs (Fig. 6A, 6B, 6C) of all specimens including those from baseline animals. Cone outer and inner segments showed no staining in all investigated retinas (Fig. 6B). Ubiquitin label was also seen in AVs of RISs, especially in 200-lux immigrants (Fig. 6D).

DISCUSSION

In our experiments the number of AVs changed with sudden switches from lower to higher habitat light intensity. Whereas 200-lux immigrants showed a significant increase in AVs during the first 3 days, the 3-lux immigrants had numbers near and below baseline. The AVs were found to contain opsin. Furthermore, they contained ubiquitin, suggesting that their contents, including opsin, were destined for degradation by ubiquitin-regulated autophagy.[12] In this and previous studies,[2] membranes and vesicles of the Golgi apparatus were observed within AVs. Because newly synthesized opsin passes through the Golgi apparatus for glycosylation and is sequestered in transport vesicles[13] it can be included in those AVs that contain transport vesicles and/or Golgi membranes.

Based on this and previous studies we conclude that the abrupt switching of habitat intensities requires rod adaptation to the new lighting regimens.[14,15] The 200-lux immigrants adapted by reducing the optical density of rod cells, perhaps to avoid damage caused by absorption of "too many" photons. The 3-lux immigrants increased the optical density of their rods to achieve a "set number" of photons per day.[14] This means that retinal rhodopsin levels must be upregulated for the 3-lux immigrants and downregulated for the 200-lux immigrants. Furthermore, this adaptation must be rapid to maintain retinal homeostasis and prevent damage.

Apart from rhodopsin levels, other photoreceptor components such as phospholipid composition, antioxidant levels, and phototransduction proteins have also been found to change as a function of an altered habitat light intensity.[16–20] Such observations further emphasize the role of light as a modulator of photoreceptor function[21] and the significance of processes regulating the optical density of ROSs.

That opsin is found in the AVs suggests that autophagic degradation of opsin is one possible way to regulate the rhodopsin content of ROSs. In particular, Penn and Williams,[14] using retinal sections and Schremser and Williams[7] using single rods, both showed that rhodopsin concentration in ROSs is inversely proportional to habitat light intensity. Thus, degradation of opsin in RIS may be one mechanism for controlling the rhodopsin content in ROSs. If so, the switch to higher light intensity, accompanied by a dramatic increase in AVs, is consistent with an initial downregulation of the opsin targeted to the ROSs.

Schremser and Williams[6] did not observe an increase in disc shedding when rats were switched from 3 lux to 200 lux.

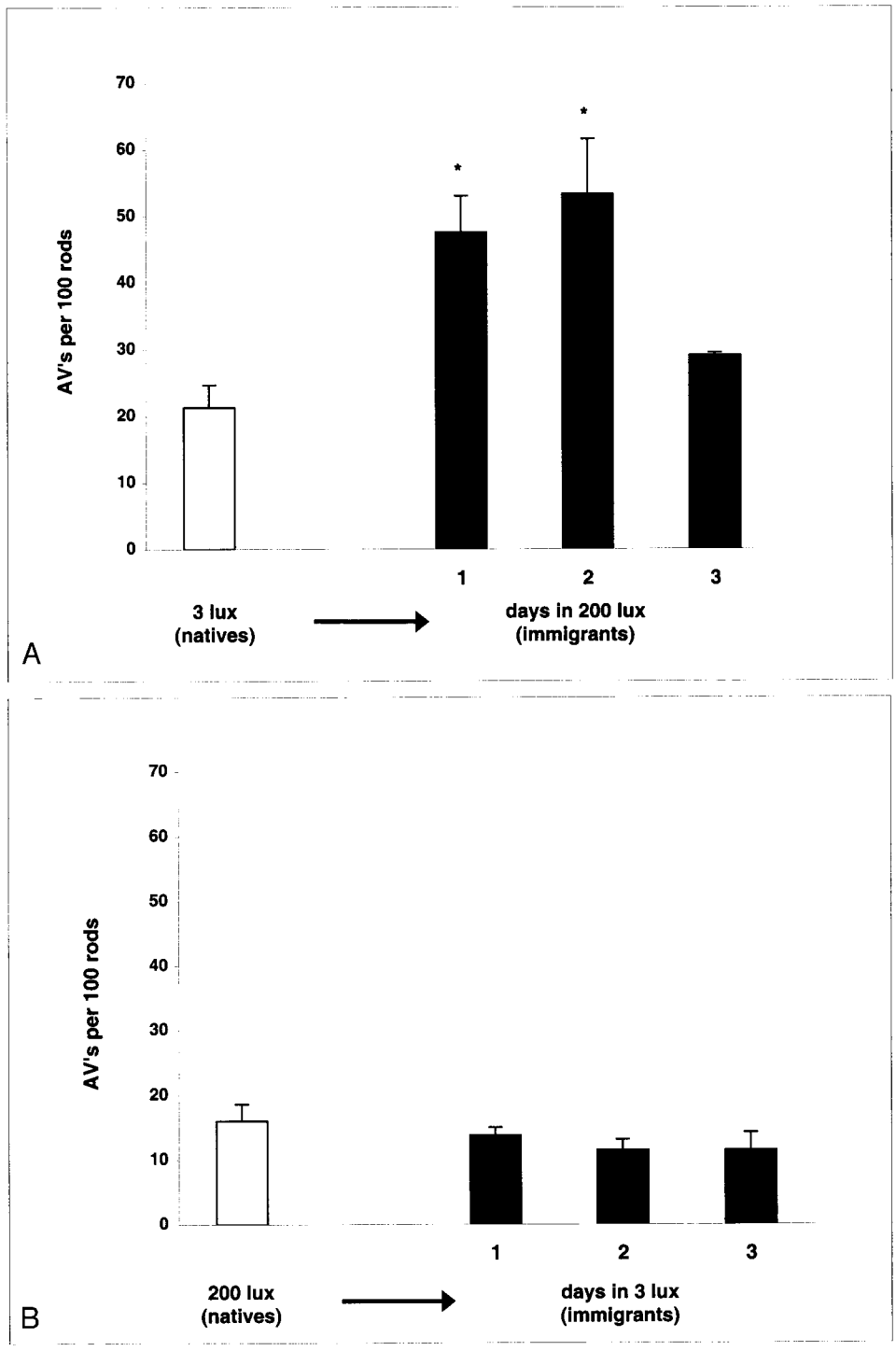

FIGURE 2. Counts of AVs in natives and immigrants. (**A**) Intensity switch from 3 lux to 200 lux. Baseline levels of AVs in 3-lux natives show approximately 20 vacuoles/100 rods. After animals were switched to 200 lux (immigrants), the number of AVs increased up to 50 to 60 during days 1 and 2 and approached baseline levels at day 3 (days 1 and 2, $P < 0.01$; day 3, $P < 0.05$). (**B**) Intensity switch from 200 lux to 3 lux. Baseline levels of AVs in 200-lux natives show approximately 20 vacuoles/100 rods. After animals were switched into 3 lux (immigrants), these values declined to approximately 15 AVs/100 rods, which was not significantly different from baseline levels. $N = 2$ rats per parameter and time point.

Earlier studies demonstrated that bursts of disc shedding as well as autophagy could be evoked several times within 24 hours by light pulses followed by a dark period.[22] Such an increase of phagosomes would rapidly reduce ROS length and rhodopsin levels. To date, it is unclear to what extent AV, disc shedding and/or the proteasome system contribute to the downregulation of rhodopsin levels. Whereas disc shedding removes the complete visual pigment from ROSs, AV and proteasome degradation would modify the amount of apoprotein to be inserted into the disc membrane after translation.

Schremser and Williams[7] used a microspectrophotometer to study single rat rods and were able to show that the concentration in ROS tips was predictably different from that in the base if an animal was switched from 3 lux to 200 lux. Single ROSs were found with gradients of rhodopsin concentration in them that matched those of 200-lux immigrants at the base and those of 3-lux immigrants at the tip. Their findings demonstrated one of the end-stages of photostasis, and the current results provide the next level of explanation for those early observations.

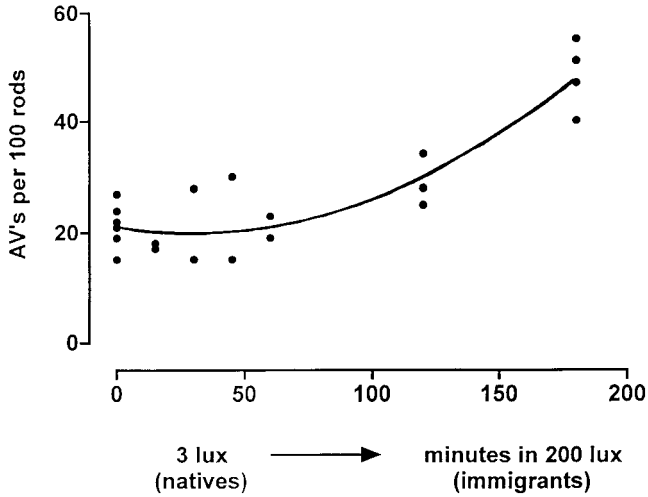

FIGURE 3. Counts of AVs at early time points after switching from 3 lux to 200 lux at day 1. There was a gradual increase in the number of AVs from 15 minutes to 2 hours after changing the light environment, which became statistically significant at 2 and 3 hours after the light switch ($P < 0.01$). Data points were fitted by a quadratic polynomial that demonstrated a quadratic rather than linear increase of AVs.

In retrospect, the rapid dampening of the rhythm amplitude of AV in constant darkness found by Remé et al.[4] can now be interpreted in terms of this proposed regulation: When switched into low photon fluxes, rod cells upregulate the rhodopsin content of their ROSs.[6,7] In such an environment the demand on newly synthesized opsin would increase, and there would be little or no need to degrade opsins in the RIS by means of the AVs.

It can be inferred from our data that the prevention of damage is required for 200-lux immigrants. Their retinas reveal dilations and vesiculations of ROS tips as indicators of threshold light damage and opsin in the synaptic region and RIS plasma membrane as a possible sign of cellular injury. Similar mislocations of opsin were also found in photoreceptors of animal models of retinitis pigmentosa.[23] In light of those and other observations, increased AV-removal of surplus opsin may also be present in light damage pathology when ROSs are injured or even absent. In our study, light damage was confined to the distal parts of ROSs and thus was considered as moderate threshold damage that is reversible within the regular ROS renewal cycle.[24] This condition would still permit disc membrane synthesis at the base of ROSs but would require adaptation to avoid further damage. Therefore, conditions in our study may represent intermediate stages between pure adaptation and pure light damage. Furthermore, several studies did not detect any significant cell loss when animals were switched from 3 lux to 200 lux[6,7,14]

Ubiquitin label in AVs, in the cytoplasm and in ROSs was observed in all our specimens including baseline retinas. The AV label was distinct in 200-lux immigrants. There are two major ways of intracellular protein degradation, cytosolic degradation by the proteasome system, and lysosomal degradation by membrane endocytosis or by autophagy. Ubiquitin conjugation is known to target proteins to degradation by proteasomes. Ubiquitin appears to be present in all eukaryotic cells and degrades proteins including those with important regulatory functions such as transcription factors, the tumor suppressor p53 and cyclins. Numerous different conditions are known to use the ubiquitin pathway, those comprise cell differentiation and cell cycle, apoptosis, DNA repair, rapid stress responses, and signal transduction.[25–27] Recent studies show that ubiquitination may target proteins also for lysosomal degradation, perhaps by a different type of ubiquitination than that used for proteasomes.[12,28]

Obin et al.[9] suggested a role for ubiquitination of transducin and rhodopsin in the regulation of levels of phototransduction proteins. Notably, ubiquitination of transducin is modulated by light. They also suggested that removal of phototransduction proteins through ubiquitination is a protective measure against light damage.[29] Naash et al.[30] found that exposure to light higher than habitat intensity induces the ubiquitination of molecules in the inner retina. It was thus confirmed in their study that ubiquitin may act as a stress protein that helps to protect cells against damage. A similar condition may exist in our study for the 200-lux immigrants. The ubiquitination of opsin for proteasome and/or autophagic degradation may serve to regulate the level of rhodopsin and thus protect the retina against absorbing "too many" photons. Supporting the observations by Obin et al.,[9] there was ubiquitin label in ROSs that may represent transducin destined for removal. This removal would reduce the capacity of ROSs to transduce the initial light signal.

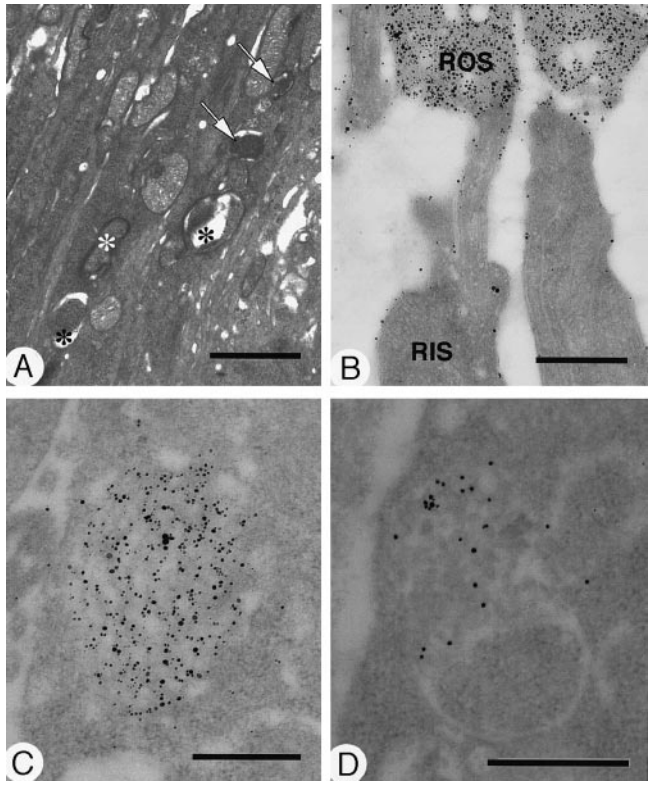

FIGURE 4. Electron micrographs depicting AVs and immunogold labeling of opsin. (**A**) The myoid region of RISs shows newly formed AVs (*asterisks*) with recognizable contents and electron-dense vacuoles with degraded contents (*arrow*). (**B**) Specimen from a 200-lux native showing dense opsin label in ROSs and weak label in RISs. (**C, D**) Silver-enhanced immunogold labeling of opsin in AVs in RIS from 200-lux immigrants. The staining shows a distinct accumulation of anti-opsin label within AVs. Bar, (**A**) 2 μm; (**B**) 450 nm; (**C, D**) 500 nm.

The regulatory mechanism suggested by our results may have its counterpart in an invertebrate eye. Huber et al.[31] studied the synthesis, maturation, and targeting of opsin to rhabdomeric membranes in the eye of the blow fly, *Calliphora*. They suggested that opsin without chromophore was degraded in photoreceptor cell bodies. Furthermore, AVs containing both rhodopsin and ubiquitin have been identified in the photoreceptor cells of opsin mutants of the fruit fly *Drosophila* before photoreceptor degeneration.[32]

In conclusion, our data show that autophagic degradation of opsin may contribute to the downregulation of rhodopsin levels in animals abruptly experiencing an increase of habitat light intensity. This adaptational regulation would enable the retina to reach the photostasis number of photons. Moreover, the downregulation of light absorption would help to prevent light-induced damage to photoreceptors. Further studies are needed to clarify in a quantitative manner the extent to which AV and other proteolytic pathways contribute to the regulation of opsin levels.

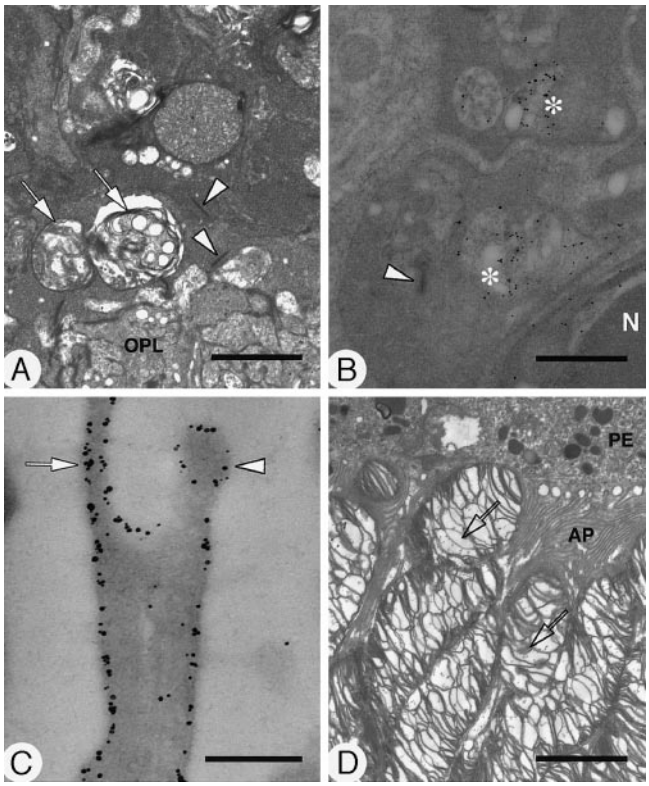

Figure 5. Electron micrographs depicting AVs and opsin label in various regions of rods as well as the characteristic disruptions of light-damaged ROS tips in 200-lux immigrants. (**A**) Electron micrograph showing AVs within the synaptic body (*arrows*). Synaptic ribbons (*arrowheads*) and synaptic vesicles are apparent. (**B**) Silver-enhanced immunogold labeling of opsin of AVs (*asterisks*) in the synaptic body. The perinuclear area and cytoplasmic domains are also labeled. Synaptic ribbons are visible (*arrowhead*). (**C**) Silver-enhanced immunogold labeling of opsin at the plasma membrane of a RIS. A calycal process (*arrow*) and parts of the connecting cilium (*arrowhead*) appear. (**D**) ROSs showing disc disruptions and dilations (*arrow*) which are typical for early stages of threshold light damage. N, nucleus; OPL, outer plexiform layer; PE, pigment epithelium; AP, apical processes. Bar, (**A**) 2 μm; (**B**, **C**) 500 nm; (**D**) 3.2 μm.

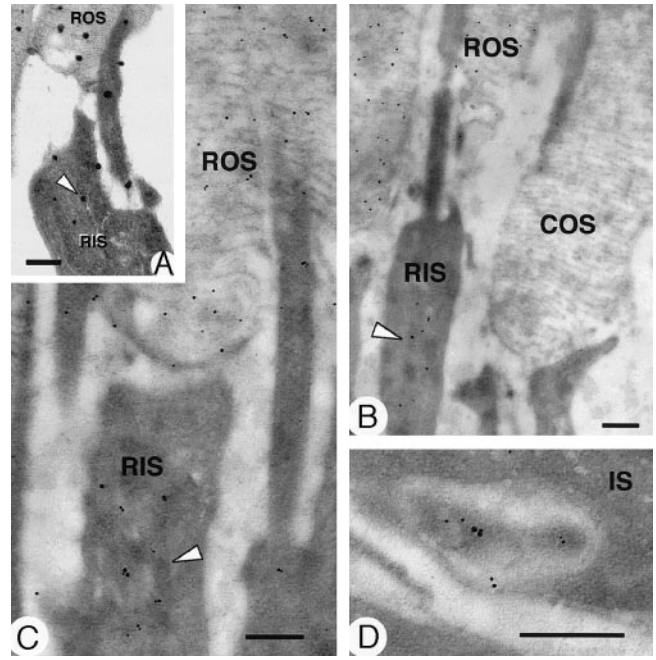

Figure 6. Electron micrographs depicting silver-enhanced immunogold labeling of ubiquitin in photoreceptors of a 200-lux native rat and of those switched from 3 lux to 200 lux for 1 hour and then killed. (**A**) Ubiquitin label located in mitochondrial membranes of a RIS (*arrowhead*) and at the base of a ROS from a 200-lux native. (**B**) Longitudinal section through a cone outer segment (COS) and a rod from a 200-lux immigrant. Ubiquitin label is restricted to the ROSs and RIS (*arrowhead*). (**C**) Longitudinal section through a rod from a 200-lux immigrant. Ubiquitin label is present in the ROSs and in cytoplasmic domains of the RISs (*arrowhead*). (**D**) Ubiquitin label within an AV of an inner segment (IS) from a 200-lux immigrant. Bar, (**A**) 350 mm; (**B**) 350 nm; (**C**) 250 nm; (**D**) 100 nm.

Acknowledgments

The authors thank Rainer Müller, University of Karlsruhe, for skillful technical assistance and Fabio Valeri, University Hospital Zürich, for valuable advice and performance of the statistical tests.

References

1. Young RW. Visual cells and the concept of renewal. *Invest Ophthalmol.* 1976;15:700–725.
2. Remé C. Autophagy in rods and cones of the vertebrate retina. *Dev Ophthalmol.* 1981;4:101–148.
3. LaVail MM. Rod outer segment disc shedding in the rat retina: relationship to cyclic lighting. *Science.* 1976;194:1071–1073.
4. Remé CE, Aeberhard, B, Schoch, M. Circadian rhythms of autophagy and light responses of autophagy and disc shedding in the rat retina. *J Comp Physiol A.* 1985;155:669–678.
5. Reme CE, Wirz–ustice A, Terman M. The visual input stage of the mammalian circadian pacemaking system, I: is there a clock in the mammalian eye? *J Biol Rhythms.* 1991;6:5–29.
6. Schremser JL, Williams TP. Rod outer segment renewal as a mechanism for adaptation to a new intensity environment, I: rhodopsin levels and ROS length. *Exp Eye Res.* 1995;61:17–24.
7. Schremser JL, Williams TP. Rod Outer Segment (ROS) renewal as a mechanism for adaptation to a new intensity environment. II. Rhodopsin synthesis and packing density. *Exp Eye Res.* 1995;61: 25–32.
8. Hilt W, Wolf DH. Proteasomes: destruction as a programme. *Trends Biochem Sci.* 1996;21:96–102.

9. Obin MS, Jahngen–Hodge J, Nowell T, Taylor A. Ubiquitinylation and ubiquitin-dependent proteolysis in vertebrate photoreceptors (rod outer segments). *J Biol Chem.* 1996;271:14473–14484.
10. Adamus G, Zam ZS, Arendt A, et al. Anti-rhodopsin monoclonal antibodies of defined specificity: characterization and application. *Vision Res.* 1991;31:17–31.
11. Danscher G. Histochemical demonstration of heavy metals: a revised version of the sulphide silver method suitable for both light and electron microscopy. *Histochemisry* 1981;71:1–16.
12. Hicke L, Riezmann H. Ubiquitination of a yeast plasma membrane receptor signals its ligand-stimulated endocytosis. *Cell.* 1996;84: 277–287.
13. Papermaster DS, Converse CA, Siu J. Membrane biosynthesis in the frog retina: opsin transport in the photoreceptor cell. *Biochemistry*. 1975;14:2438–2442.
14. Penn JS, Williams TP. Photostasis: regulation of daily photon catch by rat retinas in response to various cyclic illuminances. *Exp. Eye Res.* 1986;44:915–928.
15. Williams TP. Light history and photostasis: What is a "normal" rat retina? In: Williams TP, Thistle AB, eds. *Photostasis and related phenomena.* New York: Plenum Press; 1998;17–32.
16. Penn JS, Anderson RE. Effect of light history on rod outer-segment membrane composition in the rat. *Exp Eye Res.* 1987;44:767–778.
17. Penn JS, Anderson RE. Effects of light history on the rat retin. In: Osborne N, Chader G, eds. *Progress in Retinal Research*. New York: Pergamom Press; 1991:75–97.
18. Penn JS, Tolman BL, Thum LA, Koutz CA. Effect of light history on the rat retina: timecourse of morphological adaptation and readaptation. *Neurochem Res.* 1992;17:91–99.
19. Organisciak DT, Xie A, Wang H-M, et al. Adaptive changes in visual cell transduction protein levels: effect of light. *Exp Eye Res.* 1991; 53:773–779.
20. Farber DB, Danciger JS, Organisciak DT. Levels of mRNA encoding proteins of the cGMP cascade as a function of light environment. *Exp Eye Res.* 1991;52:781–786.
21. Remé CE, Bush R, Hafezi F, Wenzel A, Grimm C. In: Williams TP, Thistle AB, eds. *Photostasis and beyond, where adaptation ends*. New York: Plenum Press; 1998:199–206.
22. Remé CE, Wirz–Justice A, Rhyner A, Hofmann S. Circadian rhythm in the light response of rat retinal disk-shedding and autophagy. *Brain Res.* 1986;369:356–360.
23. Roof DJ, Adamian M, Hayes A. Rhodopsin accumulation at abnormal sites in retinas of mice with a human P23H rhodopsin transgene. *Invest Ophthalmol Vis Sci.* 1994;35:4049–4062.
24. Szczesny PJ, Munz K, Remé CE. Light damage in the rat retina: patterns of acute lesions and recovery. In: Pleyer U, Schmidt K , Thiel HJ, eds. *Cell and Tissue Protection in Ophthalmology*. Stuttgart: Hippokrates Verlag; 1995:163–175.
25. Varshavsky A. The ubiquitin system. *Trends Biochem Sci.* 1997; 22:383–387.
26. Liakopoulos D, Doenges G, Matuschewski K, Jentsch S. A novel protein modification pathway related to the ubiquitin system. *EMBO J.* 1998;17:2208–2214.
27. Jentsch S, Schlenker S. Selective protein degradation: a journey's end within the proteasome. *Cell.* 1995;82:881–884.
28. Roth AF, Davis NG. Ubiquitination of the yeast a-factor receptor. *J Cell Biol.* 1996;134:661–674.
29. Obin M, Nowell T, Taylor A. The photoreceptor G-protein transducin (Gt) is a substrate for ubiquitin-dependent proteolysis. *Biochem Biophys Res Commun.* 1994;200:1169–1176.
30. Naash MI, Al-Ubaidi MR, Anderson RE. Light exposure induces ubiquitin conjugation and degradation activities in the rat retina. *Invest Ophthalmol Vis Sci.* 1997;38:2344–2354.
31. Huber A, Wolfrum U, Paulsen R. Opsin maturation and targeting to rhabdomeral photoreceptor membranes requires the retinal chromophore. *Eur J Cell Biol.* 1994;63:219–229.
32. Bentrop J, Schwab K, Pak WL, Paulsen R. Site-directed mutagenesis of highly conserved amino acids in the first cytoplasmic loop of Drosophila Rh1 opsin blocks rhodopsin synthesis in the nascent state. *EMBO J.* 1997;16:1600–1609.