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Light Damage Susceptibility and RPE65 in Rats

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A sequence variation in the pigment epithelial protein RPE65 has been shown to correlate with RPE65 protein levels, rhodopsin regeneration kinetics and light damage susceptibility in different mouse strains. Here, we tested whether such a correlation can also be found in rats. We examined four rat strains for RPE65 protein levels and the *Rpe65* gene sequence. In two strains, we additionally determined *Rpe65* mRNA levels, rhodopsin regeneration and light damage susceptibility (LDS).

RPE65 protein levels were higher in Lewis and Brown Norway rats compared to Wistar and Long Evans. The albino strains Wistar and Lewis were investigated further. Lewis had higher *Rpe65* mRNA levels than Wistar. Sequence analysis of the coding region of the *Rpe65* cDNA revealed no relevant sequence variations in the two strains. Content and regeneration of rhodopsin were comparable in both strains. However, Wistar rats were more susceptible to light damage than Lewis. We conclude that lower RPE65 protein levels in Wistar may have been caused by decreased gene expression and not by a sequence variation as suggested for mice. In rats, RPE65 may not be a limiting factor for rhodopsin regeneration. Since LDS in rats did not directly correlate with RPE65 protein levels and rhodopsin regeneration, other yet unidentified (genetic) factors may account for the susceptibility differences observed in rats.

Key words: retina; photoreceptor; rhodopsin; light damage; rat; rhodopsin regeneration; RPE65; gene expression.

1. Introduction

Apoptosis is the main mechanism of cell death in retinal diseases such as glaucoma, age related macular degeneration (AMD) and retinitis pigmentosa (RP). Although many gene mutations have been identified as the underlying cause of the diseases, little is known about signaling pathways and molecular mechanisms leading to apoptotic cell death in the diseased retina. Epidemiological and experimental evidence suggests that exogenous stimuli like light exposure are important cofactors for the development and severity of retinal degenerations as they occur during the course of AMD and RP (Taylor et al., 1990; Cruickshanks et al., 1993, 2001; Simons, 1993; Cideciyan et al., 1998). In addition, the progression of retinal degeneration is enhanced by light in an increasing number of animal models for human retinal diseases (Sanyal and Hawkins, 1986; Wang et al., 1997; Chen et al., 1999a,b; LaVail et al., 1999; Organisciak et al., 1999). The capacity of visible light to induce photoreceptor apoptosis in wild-type vertebrates (Reme et al., 1998) underscores the importance of excessive photon absorptions for retinal degenerations. However, different species are differently susceptible to light-induced retinal degeneration. Rats generally need less intensive light than mice for comparable damage (Reme et al., 1998). Furthermore, strains within a particular species show considerable differences in their sensitivity to light damage (LaVail et al., 1987a,b; Borges et al., 1990; Wenzel et al., 2001).

The susceptibility to light damage is at least partially determined by genetic factors (LaVail et al., 1987a; Danciger et al., 2000; Wenzel et al., 2001). Recent studies have shown that the substitution of leucine by methionine at position 450 of the retinal pigment epithelial protein RPE65 cosegregates with a reduced susceptibility to long term (2 weeks) light exposure in mice (Danciger et al., 2000). Similarly, the *Rpe65* methionine variant was also found in mice with reduced age-dependent loss of photoreceptors suggesting that this sequence variation might also influence effects of life-long light exposure. The substitution does not influence *Rpe65* gene expression but correlates with low levels of RPE65 protein, probably caused by a post-translational process (Wenzel et al., 2001). Low levels of RPE65 and the methionine substitution correlate with slow rhodopsin regeneration rates after bleaching in mice. This in turn may be the underlying cause for the increased resistance against light damage because photon absorption by rhodopsin is essential for light damage to occur. In the absence of the visual pigment, acute light exposure does not activate the intracellular death cascade and light damage is prevented (Grimm et al., 2000c). Multiple photon absorptions seem to be

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required for the induction of light damage since a single bleach of rhodopsin is not sufficient to induce photoreceptor apoptosis in wild-type animals. Inhibition of rhodopsin regeneration after bleaching by a pharmacological approach protects against light damage in mice (Keller et al., 2001; Sieving et al., 2001). Similar mechanisms may account for variability of LDS also in rats: Dietary reduction of levels of docosahexaenoic acid (DHA; 22:3*n*–6), a fatty acid which is essential for photoreceptor function, significantly impaired rhodopsin regeneration, photon catch and LDS (Bush et al., 1991, 1994; Reme et al., 1994).

Here, we show that strain differences with respect to RPE65 protein levels also exist in rats. In contrast to mice, however, levels of RPE65 protein may be determined on the gene transcription level. No relevant sequence variations were found in the coding regions for the rat RPE65 protein. Furthermore, RPE65 may not be rate limiting for rhodopsin regeneration in rat and RPE65 protein levels did not correlate with light damage susceptibility. Strain differences for LDS may therefore depend on other, yet unidentified mechanisms.

2. Materials and Methods

Animals

All procedures concerning animals were in accordance with the regulations of the Veterinary Authority of Zurich and with the statement of The Association for Research in Vision and Ophthalmology for the use of animals in research. Male rats (4–6 week old) of the albino strains Wistar (outbred) and Lewis (inbred) and of the pigmented strains Brown Norway (inbred) and Long Evans (inbred) were purchased and reared for additional 10–15 days in a light/dark cycle (12 hr:12 hr) with 60–100 lux within the cages. Experiments were performed at the age of 6–8 weeks.

Western Blotting

Eyecups (including the retina) from dark-adapted rats were homogenized in 100 mM Tris–HCl, pH 7·4 using a sonifier. Protein concentrations were determined by the Bradford assay using BSA as standard. Western blotting was performed according to standard procedures. For Immunodetection, polyclonal rabbit antisera directed against RPE65 (Redmond and Hamel, 2000) and interphotoreceptor retinoid binding protein (IRBP) (Smith et al., 1997) and polyclonal goat antisera directed against β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A. sc-1616) were applied. After application of HRP-conjugated secondary antibodies (Santa Cruz Biotechnology, sc-2004 and sc-2031) immunoreactivity was visualized using the Renaissance-Western blot detection kit (Perkin Elmer Life Sciences, Emeryville, U.S.A.).

RT–PCR and Rpe65 Sequence Analysis

Total RNA was prepared from eyecups using the RNeasy kit (Qiagen, Hilden, Germany). Remaining genomic DNA was digested with deoxyribonuclease RQ (Promega, Madison, WI, U.S.A.), and 1 μg of total RNA was reverse-transcribed with oligo(dT) and M-MLV reverse transcriptase (Promega, Madison, WI, U.S.A.). cDNAs corresponding to 10 ng of total RNA were amplified during 23 cycles in a thermocycler with ^{32}P-endlabelled primers specific for β-actin (Grimm et al., 2000b). Amplification products were quantified on a PhosphorImager (Fuji, Japan) for standardization. Standardized cDNAs corresponding to about 10 ng of total RNA were amplified by PCR for 30 cycles using the following ^{32}P-endlabelled primer pair for *Rpe65* cDNA: up: 5-ATGATCGAGAAGAGGATTGTC-3′, down, 5′-CTGCTTTCAGTGGAGGGATC-3′ (Redmond et al., 1998). Although primers were originally designed for amplification of mouse cDNA and contained a mismatch to the rat sequence at position 5 of the 'up' primer, they efficiently amplified rat *Rpe65* cDNA. Products were separated by native polyacrylamide gel electrophoresis (6 %), stained with ethidium bromide, and quantified on a PhosphorImager.

To determine the sequence of *Rpe65* coding region, cDNA was prepared as above and five overlapping fragments were amplified by PCR using the following primer pairs: F1, 5′-AATTCTCTCTAGTCTTCACTG-3′ and R1, 5′-CACCATTGACGGAAACATAGTTGC-3′; F2, 5′-CAAAGATTAACCCAGAGACCTTGG-3′ and R2, 5′-TGTCAGCAACATGAAGCCAAA-3′; F3, 5′-TTGGCTTGACTCCCAACTATATCG-3′ and R3, 5′-CATAAGTGTAAGGTTTCCCCCCAC-3′; F4, 5′-GTCAGGAGATACGTTCTTCC-3′ and R4, 5′-CTCACCACCACACTCAGAACT-3′; F5, 5′-CGGATTCTTACCCATCTGAACCC-3′ and R5, 5′-ACACACACACACACACACATACAC-3′. Fragments covered the cDNA from position −28 (with respect to first nucleotide of start codon) to +181 (with respect to last nucleotide of stop codon) (Manes et al., 1998). Products were purified using QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced in both directions (Microsynth GmbH, Balgach, Switzerland).

Rhodopsin Content and Regeneration

The total rhodopsin content was determined after 16 hr of dark adaptation as described (Wenzel et al., 2001). To assess rhodopsin regeneration, levels of the visual pigment were determined at various times (0, 30, 60 and 90 min) in darkness after an almost complete bleach of rhodopsin. The latter was achieved by exposing dark-adapted rats to 3000 lux of white light for 10 min.

Induction and Assessment of Light Damage

Rats were dark adapted for 16 hr before exposure to 3000 lux of diffuse white fluorescent light (no u.v.) for various times as indicated in Fig. 4. Animals were returned to darkness for 24 hr before retinas were analyzed morphologically and by the TdT-mediated dUTP nick-end labelling assay (TUNEL). Tissue preparation was as described (Grimm et al., 2001). The superior and the inferior halves of the retinas were trimmed and embedded in Epon 812. Sections (0·5 μm) were prepared from the lower retina (most affected in our light-damage model) and stained with methylene blue.

For the TUNEL assay, eyes were fixed in 2% paraformaldehyde for 2 hr at 4°C followed by dehydration and paraffin embedding. The assay was performed with modifications using the 'in situ cell death detection kit' (Roche Diagnostics, Rotkreuz, Switzerland) on 5 μm paraffin sections. DNA strand breaks were labelled with fluorescein and visualized with a FITC filter as described (Hafezi et al., 1998).

3. Results

Different Levels of RPE65 Protein in Rat Strains

RPE65 protein levels were detected in homogenates from eyecups by Western blotting. Age matched animals from four rat strains (Wistar, Lewis, Long Evans and Brown Norway) were screened initially. Judged by visual impression, Lewis and Brown Norway rats showed higher levels of RPE65 protein than animals of the Wistar and Long Evans strains (Fig. 1(A)). β-actin was used to demonstrate equal loading. Wistar and Lewis rats are albinotic whereas Long Evans and Brown Norway have pigmented eyes. To have equal conditions in terms of pigmentation, we concentrated on the two albinotic strains for further analysis. The differential expression of RPE65 protein in these strains was verified in additional blot experiments (Fig. 1(B)). In contrast to RPE65, levels of IRBP were not significantly different in the two strains and β-actin served as control for equal loading.

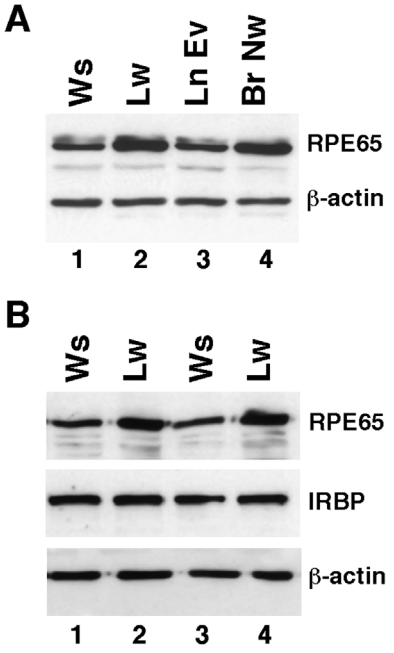

FIG. 1. RPE65 protein levels in eyecups of different rat strains. Total proteins were isolated from eyecup (including the retina) preparations, separated by SDS–PAGE and specific proteins were detected by Western blotting. (A) Comparison of RPE65 protein levels in four rat strains. (B) Comparison of RPE65 and IRBP protein levels in eyecups of two additional rats of each albino strain. Each lane in (A) and (B) corresponds to proteins of individual animals. Ws: Wistar; Lw: Lewis; Ln Ev: Long Evans; Br Nw: Brown Norway.

Rpe65 Gene Expression in Wistar and Lewis Rats

In mice, different levels of RPE65 protein may be due to post-translational mechanisms possibly influenced by a sequence variation at position 450 of the protein. Levels of *Rpe65* mRNAs are similar in various mouse strains despite different RPE65 protein amounts (Wenzel et al., 2001). In contrast, Wistar and Lewis rats expressed *Rpe65* mRNA at statistically different levels when standardized to β-actin gene expression (Fig. 2). Lewis rats had higher *Rpe65* mRNA levels, which may explain the increased RPE65 signal intensities observed in the Western blots shown in Fig. 1. Sequence analysis of the complete *Rpe65* coding region of the two rat strains revealed no relevant (see discussion) sequence difference to the published sequence (Manes et al., 1998). However,

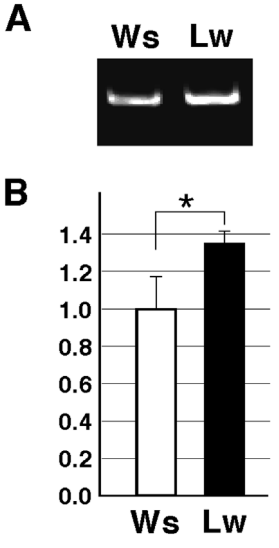

FIG. 2. *Rpe65* mRNA levels in eyecups of different rat strains. Total RNA was prepared from eyecups of Wistar (Ws) and Lewis (Lw) rats, reverse transcribed and *Rpe65* semiquantitatively amplified by PCR. (A) *Rpe65* amplification products after RT–PCR. (B) Relative *Rpe65* mRNA levels in Wistar and Lewis rats. Expression in Wistar was set as 1·0 arbitrary unit. RT–PCR amplifications from three different rats were done in triplicates. Shown are mean values ± S.D. $*P < 0{\cdot}008$, unpaired *t*-test.

two out of four tested Lewis rats had a G to A transition at position 382 of the coding sequence leading to a substitution of valine by isoleucine at position 128 of the protein (data not shown).

Rhodopsin Metabolism and Light Damage Susceptibility in Wistar and Lewis Rats

In mice, RPE65 protein levels correlate with rhodopsin regeneration kinetics and LDS. We therefore tested rhodopsin in dark adapted rats and determined regeneration of rhodopsin after bleaching. The difference in the levels of dark adapted rhodopsin was not significant with a P value larger than 0·1 in an unpaired t-test (Fig. 3(A)) and both strains regenerated rhodopsin with comparable kinetics (Fig. 3(B)).

To test light damage susceptibility, rats were dark adapted for 16 hr overnight and exposed to 3000 lux of white light for different durations (Fig. 4). Unexposed control retinas of both strains showed normal morphology (Fig. 4(A) and (E)) and no TUNEL positive cells in the outer nuclear layer (Fig. 4(I) and (N)). Whereas 10 min of exposure induced apoptotic cell death only in very few scattered nuclei (Fig. 4(B), (F), (K) and (O)) in both rat strains, a 20 min exposure was sufficient to induce massive apoptosis in Wistar but not in Lewis rats (Fig. 4(A), (G), (L) and (P)). Apoptosis manifested itself by the appearance of pycnotic photoreceptor nuclei and TUNEL positive cells in the ONL as well as by the disorganization of ROS and RIS and the beginning of RPE disintegration. An exposure duration of 30 min was needed to induce photoreceptor apoptosis in Lewis rats to a similar degree as in Wistar rats after a 20 min exposure (Fig. 4(H), (Q) and (C), (L)). A 30 min exposure further increased damage in Wistar (Fig. 4(D) and (M)) as did a 40 min exposure in Lewis rats (data not shown).

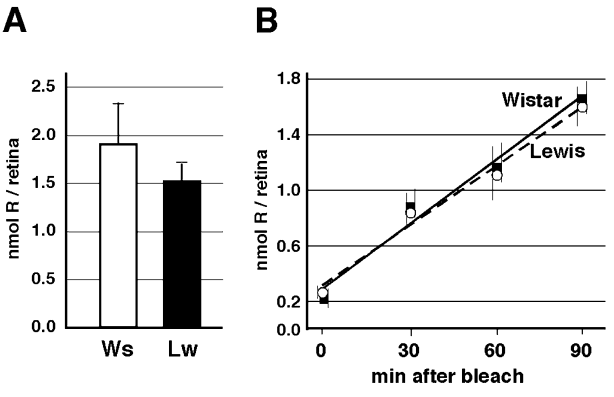

FIG. 3. Rhodopsin levels and rhodopsin regeneration in Wistar and Lewis rats. (A) Rhodopsin levels in dark adapted rat retinas. Average values ± S.D. of three retinas. Values are not statistically different ($P > 0·1$, unpaired t-test). Ws: Wistar, Lw: Lewis (B) Rhodopsin levels immediately after bleaching (0 min) or after a recovery period of 30, 60 or 90 min in darkness. Shown are average values ± S.D. of four to six retinas. Solid squares and solid line: Wistar. Open circles and dotted line: Lewis.

4. Discussion

Genetic factors determining LDS in mice have been postulated already in 1987 (LaVail et al., 1987a). One of these factors has been identified in a sequence variation in the *Rpe65* gene by a quantitative trait loci (QTL) analysis (Danciger et al., 2000). The sequence variant which causes the substitution of a leucine residue by a methionine at position 450 of the RPE65 protein has been suggested to influence levels of RPE65 protein by a post-transcriptional mechanism (Wenzel et al., 2001). Levels of RPE65 protein correlated with rhodopsin regeneration rate and light damage susceptibility in mice (Wenzel et al., 2001). Here, we analyzed RPE65 protein and LDS in different rat strains. In contrast to mice, levels of RPE65 protein did not correlate with LDS.

Two albino and two pigmented rat strains were analyzed for their RPE65 content. One albino and one pigmented strain had elevated levels of the protein demonstrating that pigmentation did not influence RPE65 levels. When analyzed for IRBP, another component of the visual cycle, the two albino strains revealed no differences. Therefore, levels of RPE65 did not influence levels of IRBP and the different RPE65 amounts seem to represent a specific difference in the two strains. A sequence variation at position 450 of the RPE65 protein may post-translationally determine the protein levels in mice (Wenzel et al., 2001). Therefore, we tested whether the same mutation may be the reason for the different protein levels in rats as well. However, the sequence at position 450 of the RPE65 protein in all rat strains tested was the same and encoded a leucine residue. Furthermore, the complete *Rpe65* coding region of the two albinotic rat strains Wistar and Lewis did not reveal any sequence differences despite different levels of the protein. The only exception was a valine to isoleucine substitution at position 128 of the RPE65 protein, a variation that was found in two out of four Lewis rats tested. However, since we did not observe a variability of the RPE65 protein levels (Fig. 1) and/or LDS (Fig. 4) in Lewis rats, we conclude that this sequence alteration may not be of significance for the stability and/or function of the RPE65 protein.

Further analysis of the two albino strains Wistar and Lewis showed different levels of *Rpe65* mRNAs correlating with levels of the RPE65 protein. Although we cannot exclude post-transcriptional mechanisms, this suggests that the various levels of the RPE65 protein are primarily determined at the gene expression level in rats. The reasons for the different *Rpe65* mRNA levels are unclear and may be caused by yet unknown factor(s).

Even though Wistar and Lewis rats had different levels of RPE65 protein, a major determinant for the efficiency of rhodopsin regeneration in mice (Wenzel et al., 2001), rhodopsin regeneration (nmol rhodopsin regenerated per unit time) was not different in the two

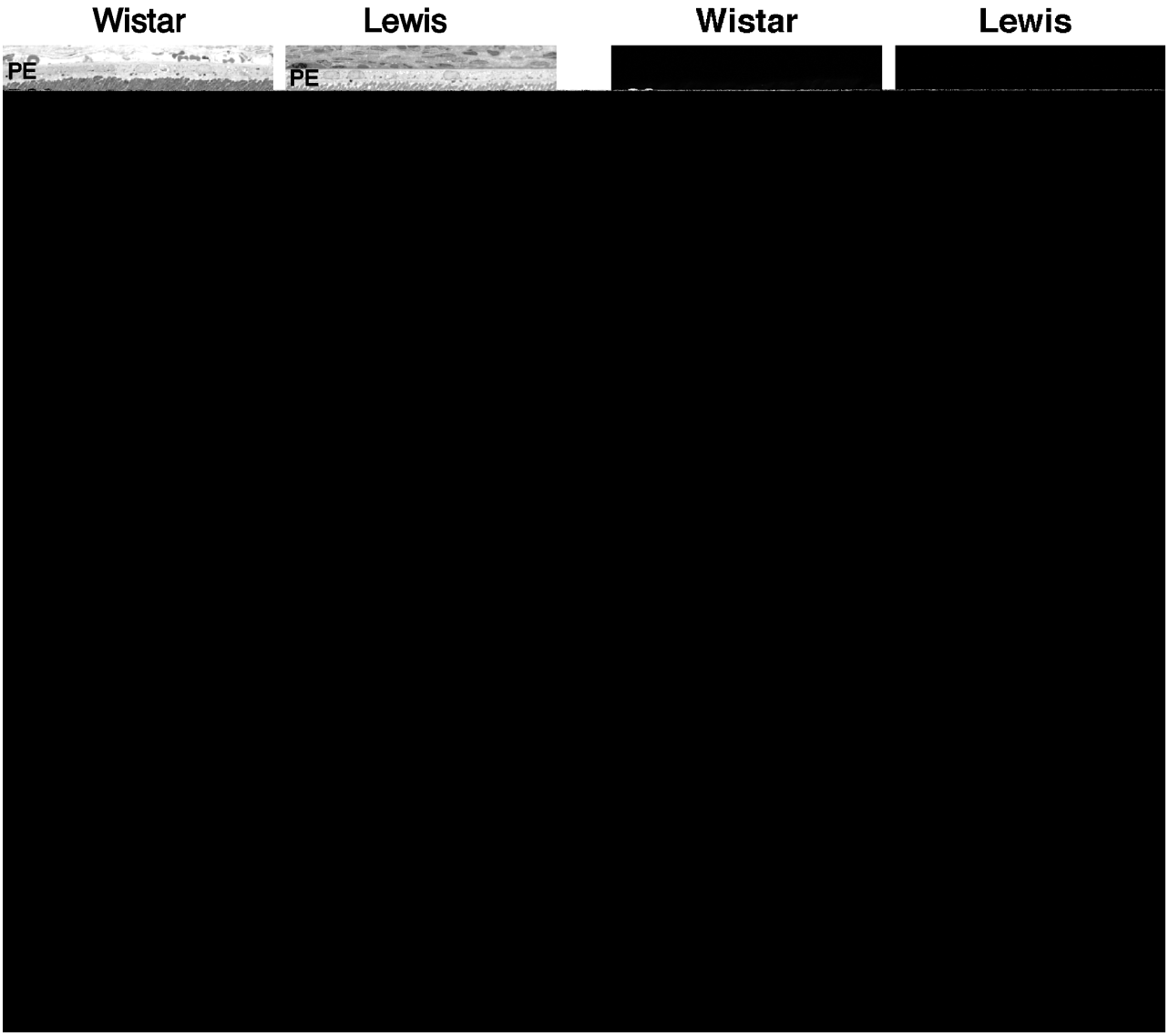

FIG. 4. Light damage susceptibility of Wistar and Lewis rats. (A–H) Light microscopy of control retinas not exposed to light (A, E), or of retinas exposed for 10 min (B, F), 20 min (C, G) or 30 min (D, H) to 3000 lux of white light. (I–Q) TUNEL staining of retinas not exposed to light (I, N), or of retinas exposed for 10 min (K, O), 20 min (L, P) or 30 min (M, Q) to 3000 lux of white light. PE: Pigment epithelium. ROS: rod outer segments. RIS: rod inner segments. ONL: outer nuclear layer. OS: outer segments. INL: inner nuclear layer. GCL: ganglion cell layer. Scale bar: 25 μm (A–H), 50 μm (I–Q).

rat strains. This suggests that RPE65 is not the limiting factor for regeneration of the visual pigment in rats.

In contrast to mice, rhodopsin regeneration did not strictly correlate with LDS. This, however, does not exclude that rhodopsin regeneration nevertheless may be an important factor for light damage also in rats. In addition, the tendency of higher rhodopsin dark values in Wistar compared to Lewis rats, may add to the higher LDS of Wistar since more photons can be absorbed in the initial bleaching step. However, this may only be of significance in conditions of similar rhodopsin regeneration as found here. Similar to mice, efficient photon absorption enabled by the photochemical reconstitution of bleachable rhodopsin is important for the severity of light damage. Blue light that causes photoreversal of bleaching by a fast photochemical process (Grimm et al., 2000a) is highly deleterious to the rat retina (Grimm et al., 2001). Green light, however, which is unable to photoreverse rhodopsin, does hardly damage photoreceptors in our system (Grimm et al., 2001). In a previous study, Lewis rats were more susceptible to photic injury than rats of the Wistar strain (Borges et al., 1990), a result that is in contrast to our findings here. However, Borges et al. (1990) used a 24 hr exposure to about 1800 lux (ca. 170 foot-candles) of a narrow banded light between 490 and 580 nm whereas in the present study, we exposed animals for 10–40 min to 3000 lux of broad white fluorescent light which also contained light in the blue range of the spectrum. As observed in previous studies, the wavelength of the used light can strongly influence light damage (Grimm et al., 2001) and different light exposure setups may lead to different results. The group of Organisciak, for example uses middle to long-term exposures to green light to induce photic injury

(Organisciak et al., 2000) a wavelength that does not induce photoreceptor apoptosis in our experimental setup (Grimm et al., 2001). Furthermore, Borges et al. (1990) analyzed the rat retinas after a recovery period of 6 days and found highest degree of damage in the superior retina, whereas we examined light damage 24 hr after illumination with most severe damage in the inferior part of the retinas. The reasons for these differences are unclear and need to be worked out in future experiments.

Although RPE65 and rhodopsin regeneration strongly influence light damage in mice, *Rpe65* is only one of several genetic factors determining LDS (Danciger et al., 2000). Since we observed different LDS in the albino strains Wistar and Lewis despite comparable rhodopsin regeneration kinetics, it is conceivable that also in rats, several endogenous (genetic) and exogenous (environmental) factors may contribute to a particular degree of LDS. It has been shown that within one strain of rats, the illuminance level in which the animals were born and raised contributes to LDS (Penn et al., 1986; Penn and Williams, 1987b). Depending on habitat illuminance, rats adjust rhodopsin content, phospholipid fatty acid composition, cholesterol as well as antioxidant levels in the retina (Penn and Anderson, 1987a), thus fine tuning the number of photon absorptions at a given light intensity. Similarly, within a given rat strain, LDS varies with the time of day (White and Fisher, 1987; Organisciak et al., 2000), a phenomenon that might be due to differential gene expression or be caused by the rhythmicity of retinal neurohormones and neuromodulators such as melatonin and dopamine (Pozdeyev and Lavrikova, 2000; Tosini, 2000). It remains to be seen whether different rat strains will display different capacities of LDS-regulation by the processes described above. Once identified in mice, it will be of importance to test the additional LDS factors also in the various rat strains in order to evaluate the general relevance of the identified genes in light enhanced retinal degeneration.

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References

Borges, J. M., Edward, D. P. and Tso, M. O. M. (1990). A comparative study of photic injury in four inbred strains of albino rats. *Curr. Eye Res.* **9**, 799–803.

Bush, R. A., Malnoe, A., Reme, C. E. and Williams, T. P. (1994). Dietary deficiency of N-3 fatty acids alters rhodopsin content and function in the rat retina. *Invest. Ophthalmol. Vis. Sci.* **35**, 91–100.

Bush, R. A., Reme, 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**, 741–52.

Chen, C. K., Burns, M. E., Spencer, M., Niemi, G. A., Chen, J., Hurley, J. B., Baylor, D. A. and Simon, M. I. (1999a). Abnormal photoresponses and light-induced apoptosis in rods lacking rhodopsin kinase. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3718–22.

Chen, J., Simon, M. I., Matthes, M. T., Yasumura, D. and LaVail, M. M. (1999b). Increased susceptibility to light damage in an arrestin knockout mouse model of Oguchi disease (stationary night blindness). *Invest. Ophthalmol. Vis. Sci.* **40**, 2978–82.

Cideciyan, A. V., Hood, D. C., Huang, Y., Banin, E., Li, Z. Y., Stone, E. M., Milam, A. H. and Jacobson, S. G. (1998). Disease sequence from mutant rhodopsin allele to rod and cone photoreceptor degeneration in man. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 7103–8.

Cruickshanks, K. J., Klein, R. and Klein, B. E. (1993). Sunlight and age-related macular degeneration. The beaver dam eye study. *Arch. Ophthalmol.* **111**, 514–8.

Cruickshanks, K. J., Klein, R., Klein, B. E. and Nondahl, D. M. (2001). Sunlight and the 5-year incidence of early age-related maculopathy: the beaver dam eye study. *Arch. Ophthalmol.* **119**, 246–50.

Danciger, M., Matthes, M. T., Yasumura, D., Akhmedov, N. B., Rickabaugh, T., Gentleman, S., Redmond, T. M., LaVail, M. M. and Farber, D. B. (2000). A QTL on distal Chr 3 that influences the severity of light-induced damage to mouse photoreceptors. *Mamm. Genome* **11**, 422–7.

Grimm, C., Reme, C. E., Rol, P. O. and Williams, T. P. (2000a). Blue light's effects on rhodopsin: photoreversal of bleaching in living rat eyes. *Invest. Ophthalmol. Vis. Sci.* **41**, 3984–90.

Grimm, C., Wenzel, A., Hafezi, F. and Reme, C. E. (2000b). Gene expression in the mouse retina: the effect of damaging light. *Molec. Vis.* **6**, 252–60.

Grimm, C., Wenzel, A., Hafezi, F., Yu, S., Redmond, T. M. and Reme, C. E. (2000c). Protection of Rpe65-deficient mice identifies rhodopsin as a mediator of light-induced retinal degeneration. *Nat. Genet.* **25**, 63–6.

Grimm, C., Wenzel, A., Williams, T., Rol, P., Hafezi, F. and Reme, C. (2001). Rhodopsin-mediated blue-light damage to the rat retina: effect of photoreversal of bleaching. *Invest. Ophthalmol. Vis. Sci.* **42**, 497–505.

Hafezi, F., Abegg, M., Grimm, C., Wenzel, A., Munz, K., Sturmer, J., Farber, D. B. and Reme, C. E. (1998). Retinal degeneration in the rd mouse in the absence of c-fos. *Invest. Ophthalmol. Vis. Sci.* **39**, 2239–44.

Keller, C., Grimm, C., Wenzel, A., Hafezi, F. and Reme, C. (2001). Protective effect of halothane anesthesia on retinal light damage: inhibition of metabolic rhodopsin regeneration. *Invest. Ophthalmol. Vis. Sci.* **42**, 476–80.

LaVail, M. M., Gorrin, G. M. and Repaci, M. A. (1987a). Strain differences in sensitivity to light-induced photoreceptor degeneration in albino mice. *Curr. Eye Res.* **6**, 825–34.

LaVail, M. M., Gorrin, G. M., Repaci, M. A. and Yasamura, D. (1987b). Light-induced retinal degeneration in albino mice and rats: strain and species differences. *Prog. Clin. Biol. Res.* **247**, 439–54.

LaVail, M. M., Gorrin, G. M., Yasumura, D. and Matthes, M. T. (1999). Increased susceptibility to constant light in nr and pcd mice with inherited retinal degeneration. *Invest. Ophthalmol. Vis. Sci.* **40**, 1020–4.

Manes, G., Leducq, R., Kucharczak, J., Pages, A., Schmitt-Bernard, C. F. and Hamel, C. P. (1998). Rat messenger

RNA for the retinal pigment epithelium-specific protein RPE65 gradually accumulates in two weeks from late embryonic days. *FEBS Lett.* **423**, 133–7.

Organisciak, D. T., Darrow, R. M., Barsalou, L., Kutty, R. K. and Wiggert, B. (2000). Circadian-dependent retinal light damage in rats. *Invest. Ophthalmol. Vis. Sci.* **41**, 3694–701.

Organisciak, D. T., Li, M., Darrow, R. M. and Farber, D. B. (1999). Photoreceptor cell damage by light in young Royal College of Surgeons rats. *Curr. Eye Res.* **19**, 188–96.

Penn, J. S. and Anderson, R. E. (1987a). Effect of light history on rod outer-segment membrane composition in the rat. *Exp. Eye Res.* **44**, 767–78.

Penn, J. S., Naash, M. I. and Anderson, R. E. (1987b). Effect of light history on retinal antioxidants and light damage susceptibility in the rat. *Exp. Eye Res.* **44**, 779–88.

Penn, J. S. and Williams, T. P. (1986). Photostasis: regulation of daily photon-catch by rat retinas in response to various cyclic illuminances. *Exp. Eye Res.* **43**, 915–28.

Pozdeyev, N. V. and Lavrikova, E. V. (2000). Diurnal changes of tyrosine, dopamine, and dopamine metabolites content in the retina of rats maintained at different lighting conditions. *J. Molec. Neurosci.* **15**, 1–9.

Redmond, T. M. and Hamel, C. P. (2000). Genetic analysis of RPE65: from human disease to mouse model. *Methods Enzymol.* **316**, 705–24.

Redmond, T. M., Yu, S., Lee, E., Bok, D., Hamasaki, D., Chen, N., Goletz, P., Ma, J. X., Crouch, R. K. and Pfeifer, K. (1998). Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. *Nat. Genet.* **20**, 344–51.

Reme, C. E., Grimm, C., Hafezi, F., Marti, A. and Wenzel, A. (1998). Apoptotic cell death in retinal degenerations. *Prog. Retin. Eye Res.* **17**, 443–64.

Reme, C. E., Malnoe, A., Jung, H. H., Wei, Q. and Munz, K. (1994). Effect of dietary fish oil on acute light-induced photoreceptor damage in the rat retina. *Invest. Ophthalmol. Vis. Sci.* **35**, 78–90.

Sanyal, S. and Hawkins, R. K. (1986). Development and degeneration of retina in rds mutant mice: effects of light on the rate of degeneration in albino and pigmented homozygous and heterozygous mutant and normal mice. *Vis. Res.* **26**, 1177–85.

Sieving, P. A., Chaudhry, P., Kondo, M., Provenzano, M., Wu, D., Carlson, T. J., Bush, R. A. and Thompson, D. A. (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 isotretinoin therapy. *Proc. Nat. Acad. Sci. U.S.A.* **98**, 1835–40.

Simons, K. (1993). Artificial light and early-life exposure in age-related macular degeneration and in cataractogenic phototoxicity. *Arch. Ophthalmol.* **111**, 297–8.

Smith, S. B., McClung, J., Wiggert, B. N. and Nir, I. (1997). Delayed rhodopsin regeneration and altered distribution of interphotoreceptor retinoid binding protein (IRBP) in the mi(vit)/mi(vit) (vitiligo) mouse. *J. Neurocytol.* **26**, 605–13.

Taylor, H. R., Munoz, B., West, S., Bressler, N. M., Bressler, S. B. and Rosenthal, F. S. (1990). Visible light and risk of age-related macular degeneration. *Trans. Am. Ophthalmol. Soc.* **88**, 163–73.

Tosini, G. (2000). Melatonin circadian rhythm in the retina of mammals. *Chronobiol. Int.* **17**, 599–612.

Wang, M., Lam, T. T., Tso, M. O. and Naash, M. I. (1997). Expression of a mutant opsin gene increases the susceptibility of the retina to light damage. *Vis. Neurosci.* **14**, 55–62.

Wenzel, A., Reme, C. E., Williams, T. P., Hafezi, F. and Grimm, C. (2001). The Rpe65 Leu450Met variation increases retinal resistance against light-induced degeneration by slowing rhodopsin regeneration. *J. Neurosci.* **21**, 53–8.

White, M. P. and Fisher, L. J. (1987). Degree of light damage to the retina varies with time of day of bright light exposure. *Physiol. Behav.* **39**, 607–13.