Rpe65−/− and *Lrat*−/− Mice: Comparable Models of Leber Congenital Amaurosis

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Abstract

Purpose—The *Rpe65*−/− mouse, used as a model for Leber congenital amaurosis, has slow rod degeneration and rapid cone loss, presumably due to the mistrafficking of cone opsins. This animal does not generate 11-*cis* retinal and both the loss of cones and the rod response are restored by 11-cis retinal administration. The *Lrat*−/− mouse likewise does not produce 11-*cis* retinal. We sought to determine if the same effects on rod and cone opsins in the *Rpe65*−/− mouse are also present in the *Lrat*−/− mouse, thereby establishing that these changes can be attributed to the lack of 11-*cis* retinal rather than some unknown function of RPE65.

Methods—Rod and cone opsins were localized by immunohistochemical methods. Functional opsin levels were determined by regeneration with 11-*cis* retinal. Isorhodopsin levels were determined from pigment extraction. Opsin phosphorylation was determined by mass spectrometry.

Results—Rods in both models degenerated slowly. Likewise, regenerable rod opsin levels were similar over the 6-month time course investigated, rod opsin was phosphorylated at a low level (~10%) and minimal 9-*cis* retinal was generated by a non-photic process giving a trace light response. In both models, both S-opsin and M/L-opsin failed to traffic to the cone outer segments appropriately, and rapid cone degeneration occurred. Cone opsin mistrafficking in both models was arrested upon 11-*cis* retinal administration.

Conclusions—Our data show that the *Lrat*−/− and *Rpe65*−/− mice are comparable models for studies of Leber congenital amaurosis and that the destructive cone opsin mistrafficking is due to the lack of 11-*cis* retinal.

Keywords

cones; opsin; retinal; rhodopsin; RPE65; LRAT; retinoid cycle; Leber congenital amaurosis

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INTRODUCTION

Vision is initiated by the absorption of a photon by photosensitive proteins located in rod and cone photoreceptors. These proteins (opsins) are G-coupled protein receptors and have as their ligand 11-*cis* retinal, which isomerizes to the all-*trans* form with light, initiating the visual transduction cascade. The rod photoreceptors and their opsin rhodopsin have been extensively studied (for a recent review see[1]) but it is the cones which are critical for normal human daylight vision.

The generation of 11-*cis* retinal is critical for visual pigment regeneration and maintaining normal visual function. The 11-*cis* retinal is generated in the retinal pigment epithelium (RPE) from the all-*trans* retinyl esters and is then transported to the photoreceptors for the regeneration of the functional pigment.[2] The process by which 11-*cis* retinal is generated (the retinoid cycle) has been the subject of study of many laboratories. The protein RPE65, a major protein of the RPE, has been shown to be critical for the production of 11-*cis* retinal[3] and recently has been identified as the long sought isomerohydrolase,[4–6] using the all-*trans* retinyl esters[7] as the substrate. The possibility of a separate process for the generation of 11-*cis* retinal for cones has been raised,[8–10] but this pathway does not appear to be a major pathway in rod dominant retinas.

The Rpe65 knockout mouse (*Rpe65$^{-/-}$*) has been extensively studied.[3] This animal has been shown to have no 11-*cis* retinal, but low levels of 9-*cis* retinal are generated, possibly by thermal isomerization, which leads to the formation of minimal levels of isorhodopsin (the photosensitive rod opsin/9-*cis* retinal pigment) and thus a minuscule visual response as measured by the electroretinogram (ERG).[11] The level of pigmentation of the animal affects the quantities of this isomer, less being formed in the pigmented mouse.[12] Large amounts of the all-*trans* esters accumulate as the conversion of the esters to the 11-*cis* retinal has been arrested.[3] Rod photoreceptor degeneration is slow and the opsin itself is found to be phosphorylated.[13,14] Several groups have shown that administration of 9- or 11-*cis* retinal can restore rod function, even in fully adult mice 18 months-old.[13,15]

However, unlike the rods, cone photoreceptors are found to degenerate rapidly in the *Rpe65$^{-/-}$* mouse.[16] The cone opsins (both the S-opsin and the M/L-opsin) are found to be mislocalized in *Rpe65$^{-/-}$* mice, but the repeated administration of 11-*cis* retinal at an early age (~postnatal day 10 (P10)) for several days results in opsin moving to the cone outer segment (COS), which prevents the rapid cone degeneration.[17] Recently, using adenovirus-expressing Rpe65 to efficiently deliver the Rpe65 gene to the RPE of *Rpe65$^{-/-}$* mice, it has been shown that rod function can be restored as expected, but also that the cone opsin mislocalization can be corrected.[18]

However, it has been demonstrated that RPE65 is expressed at very low levels in cone outer segments in several different species[19,20] (WB/JF preliminary unpublished observations, 2007). The function of RPE65 in cones is unknown. In order to confirm that the mislocalization of cone opsin was due to the lack of 11-*cis* retinal rather some unknown function of RPE65, it was necessary to examine another model in which the synthesis of 11-*cis* retinal is blocked.

Lecithin-retinol acyltransferase (LRAT) catalyzes the esterification of all-*trans*-retinol to all-*trans* retinyl esters[21] and is found in several tissues including the RPE.[22] In the RPE, LRAT has a key role in the retinoid cycle[23] as it is critical for the formation of the esters which is the essential substrate for the retinoid isomerase. The *Lrat$^{-/-}$* mouse generated only trace levels of all-*trans* retinyl esters and no 11-*cis* retinal was found, indicating the retinoid visual cycle was blocked at the esterification step. Both the cone and rod visual functions were found to be attenuated,[24] but the responsible chromophore for the residual visual function was not identified.

In this study, we have compared the rod and cone degeneration in both *Rpe65*^{−/−} and *Lrat*^{−/−} mouse models where the retinoid cycle is disrupted and no 11-*cis* retinal is synthesized. We conclude that the disruption of the retinoid cycle in these two models results in similar patterns of retinal degeneration: rapid cone degeneration with cone opsin mislocalization to the inner segment and relatively slow rod degeneration with correct rod opsin localization.

METHODS

Animals

Lrat^{−/−} mice were genotyped as described.[24] *Rpe65*^{−/−} mice[3] were provided by T. Michael Redmond (Laboratory of Retinal Cell and Molecular Biology, National Eye Institute). Unless otherwise noted, all experiments were conducted on cyclic light-reared, age-matched animals. For isorhodopsin accumulation measurements, animals 2 months-of-age were dark-reared for 8 weeks. Animal experiments were performed in accordance with the policy for the Use of Animals in Neuroscience research and conformed to the recommendations of the Association of Research for Vision and Ophthalmology. The procedures were approved by the Medical University of South Carolina Animal Care and Use Committee, and the Institutional Animal Care and Use Committee at the University of Utah. *Lrat*^{−/−} mice were housed in the dark starting at day P10. Experimental animals were injected intraperitoneally with 11-*cis* retinal (0.5 mg per dose) in 100 μL vehicle (1.0% ethanol, 10% bovine serum albumin, and 0.9% NaCl) on days P10, P13 and P17. Animals were sacrificed at age P20 for the cone opsin localization experiments. Littermate control mice were injected on the same schedule with vehicle alone.

Pigment Measurements

Tissue isolation and all subsequent steps were conducted under dim red light (Kodak GBX-2). The retina was dissected and homogenized in 1% dodecylmaltoside (buffered with 100 mM sodium phosphate buffer, pH 7.4). The sample was shaken at 4°C for 2 h, centrifuged (88,000*g* for 10 min), and measured in a Cary 300 spectrophotometer (Varian, Walnut Creek, CA). Difference spectra were determined from measurements before and after bleaching with white light in the presence of freshly prepared 20 mM hydroxylamine, pH 7.0. The isorhodopsin concentration was calculated based on the absorption at λ_{max} = 487 nm using the extinction coefficient: ε (isorhodopsin) = 43,000 $M^{-1}cm^{-1}$.[25] To determine the regenerable opsin present, homogenized retinas of age-matched animals were supplemented with 11-*cis* retinal (80 μM) for 2 h prior to obtaining difference spectra.[14] Samples were washed extensively (8X) with 100 mM phosphate buffer after the incubation with retinal which resulted in lower levels of pigment than reported from direct extraction techniques. Rhodopsin concentrations were calculated using the extinction coefficient of 40,000 M^{-1} cm^{-1}.[26]

Opsin Phosphorylation Measurements

Opsin phosphorylation was determined using mass spectrometry as described.[14,27] In brief, retinas were homogenized in 8 M urea and digested overnight with Asp-N (5 ng/200 μL, Sigma, St. Louis, MO) in 10 mM Tris buffer, pH 7.6 at 37°C. Supernatants were collected by centrifugation (120,000*g*) and analyzed online with a Finnigan LTQ ion-trap mass spectrometer (Thermo-Finnigan Instrument Systems, Inc., San Jose, CA). The values are slightly different than those previously published[14] due to improved instrumentation.

Immunohistochemistry

Age-matched mouse eyes were immersion-fixed for 2 h using freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and cryoprotected. Eyes were embedded, frozen and sectioned at 12–14 μm thickness prior to incubations for immunocytochemistry

(described in[17,28]). Affinity-purified primary antibodies were applied to each group of 2 to 4 sections in a humidified chamber overnight at 4°C. Propidium iodide (Invitrogen Corporation, Carlsbad, CA, 1:3000 dilution) was added to the solution containing FITC- or Alexa-488 (Molecular Probe, Eugene, OR) -conjugated secondary antibody. The sections were viewed using either a Zeiss LSM 510 inverted Laser Scan confocal microscope with a 40X-1.3 NA oil objective lens and optical slit setting of <0.9 μm, or a Zeiss fluorescence microscope. The following antibodies were used in this study: rhodopsin (1:1000; generously provided by Robert Molday, University of British Columbia), anti-S-opsin and anti-M/L-opsin (1:500; Chemicon/Millipore, Temecula, CA) and anti-S-opsin and anti-M/L-opsin (1:500; generously provided by Jeannie Chen; University of Southern California).

Peanut Agglutinin (PNA) Lectin Labeling

The RPE-choroid layer was separated from the retina-lens complex and fixed in 4% formaldehyde in phosphate buffered saline for 4 h at 4°C. After washing with buffer (30 min × 3, at 4°C), retinas were incubated with the 0.2 mg/mL PNA lectin FITC-conjugated (lectin *Arachi hypogaea*; Sigma, St. Louis, MO) overnight at 4°C. Retinas were washed (20 min × 3, at 4°C), mounted on a slide, and cover-slipped after application of an anti-fade solution (Prolong; Molecular Probes, Eugene, OR).[20] Samples were viewed with a Zeiss fluorescence microscope (Axioplan II; Carl Zeiss Inc., Jena, Germany) using a 100 W mercury light source and FITC filters. Cones were counted from the ventral field (n = 6 eyes).

RESULTS

Rod Degeneration Slow in Both *Rpe65*^{−/−} and the *Lrat*^{−/−} Mice

The rod photoreceptors in both the *Rpe65*^{−/−} and the *Lrat*^{−/−} mouse are reported to degenerate slowly.[3,24] A comparison of the two models with the *wt* mouse at P30 (Fig. 1A) shows the *Lrat*^{−/−} mouse and the *Rpe65*^{−/−} mouse to be comparable with rod outer segments decreased in length and abundance of rod nuclei when compared with *wt* mice. Rod opsin is present in the rod outer segments with no evidence of mislocalization. To determine that the opsin in the two models can form rhodopsin if the ligand 11-*cis* retinal is available, *in vitro* experiments were conducted, adding 11-*cis* retinal to retina homogenates and assaying for the formation of rhodopsin. Measurements at several ages demonstrate that the levels are very similar (Fig. 1B). Although the regenerated rhodopsin levels are certainly decreased from the *wt* animals, there was no significant variation between these two models and no significant decrease between 1 and 6 months-of-age in either model.

Isorhodopsin Formed in *Lrat*^{−/−} Mouse

In both the *Rpe65*^{−/−} and the *Lrat*^{−/−} mouse, a small light response is generated as measured by the ERG.[3,24] However, no 11-*cis* retinal has been detected in either animal. In the *Rpe65*^{−/−} mouse, it has been found that a small amount of 9-*cis* retinal is generated leading to the formation of the rod pigment, isorhodopsin. On dark-rearing over a number of weeks, this pigment accumulates to a significant level, indicating that the generation of the 9-*cis* retinal is by a non-photic process.[11] As it had been shown that 11-*cis* retinal is also lacking in the *Lrat*^{−/−} mouse,[24] we investigated if these animals also generated 9-*cis* retinal. Care was taken that the animals had similar coat pigmentation as this has been shown to have an effect on the levels of 9-*cis* retinal.[12] After 8 weeks of dark-rearing, isorhodopsin levels were found to be 12.6 ±0.8 pmol in the *Lrat*^{−/−} mouse retina, as compared with 27.3 ±0.6 pmol/retina in the age-matched *Rpe65*^{−/−} animals dark-reared for the same period (Fig. 2). Therefore we attribute the small light-induced ERG response in the *Lrat*^{−/−} mouse to this 9-*cis* retinal. The finding that less 9-*cis* retinal is generated in the *Lrat*^{−/−} animals than the *Rpe65*^{−/−} mice suggests that the 9-*cis* retinal may be arising from the ester form, which is elevated in the *Rpe65*^{−/−} animals, but the exact source of this isomer remains unknown.

Rod Opsin Phosphorylated in the *Lrat*$^{-/-}$ Mouse

The rod opsin in the *Rpe65*$^{-/-}$ mouse is known to be phosphorylated.[13,14] In order to determine if the opsin in the *Lrat*$^{-/-}$ mouse is likewise phosphorylated, analysis of the opsin from the animals was performed by mass spectrometry using known methodology.[14] As shown for the *Rpe65*$^{-/-}$ mouse, *Lrat*$^{-/-}$ opsin was phosphorylated, independent of light exposure. The opsin phosphorylation level in the light-adapted *Lrat*$^{-/-}$ mouse (13.1 ±0.7%) was slightly increased over that found in the light-adapted *Rpe65*$^{-/-}$ mouse (10.4 ±0.7%, *P* <0.05). As expected, there was no difference in the phosphorylation levels between dark- and light-adapted animals in either model, as there is only a minimal light response (Fig. 3). These results support the hypothesis that this low level of constitutive opsin phosphorylation may have a role in slowing rod degeneration.

Cone Photoreceptors Rapidly Degenerate

The cones of the *Rpe65*$^{-/-}$ mice are known to degenerate rapidly.[16] In the *Lrat*$^{-/-}$ mouse, the cones likewise degenerate very quickly. Figure 4 demonstrates that the M/L-opsin is almost totally absent at P28 for both models and S-opsin is totally gone at P42, demonstrating that the cones are rapidly lost in both models and the two phenotypes appear to be very comparable. When cones are counted in flat-mount PNA lectin preparations from P30 animals (ventral sections as shown in Fig. 4), the cones were found to be <5% of *wt* cones for both the *Rpe65*$^{-/-}$ and the *Lrat*$^{-/-}$ mice (*Lrat*$^{-/-}$: 2.1 ±0.7; *Rpe65*$^{-/-}$: 4.6 ±2.7; *P* =0.02).

Cone Opsin Mislocalizes

Mislocalization of both the S-opsin and the M/L-opsin has been shown to occur in the *Rpe65*$^{-/-}$ mouse. When studied in a mouse model lacking the rod opsin pool, the *Rpe65*$^{-/-}$ *Rho*$^{-/-}$ mouse, both the S-opsin and the M/L-opsin were observed to be distributed throughout the cone inner segment (CIS), cone cell body, axon and synaptic pedicle, while essentially absent in the COS.[17] At P28, COS disintegration appeared to be far advanced in the *Lrat*$^{-/-}$ mouse retina and M/L-opsin mislocalized to the inner segment, perinuclear region, axon, and synaptic pedicle (arrows, Figs. 4B and 5B, E).

Cone Opsin Mislocalization Corrected with Administration of 11-*cis* Retinal

Upon repeated administration of 11-*cis* retinal to very young *Rpe65*$^{-/-}$ *Rho*$^{-/-}$ animals, both opsins were targeted to the cone outer segment.[17] To study the effect of 11-*cis* retinal treatments in the *Lrat*$^{-/-}$ retinas, littermates were randomly assigned to the experimental or the control groups and kept in the dark starting at P10. The animals were injected intraperitoneally with 11-*cis* retinal [0.5 mg per dose in 100 μL vehicle (10% ethanol, 10% bovine serum albumin, and 0.9% NaCl)] or vehicle alone on days P10, P13 and P17. The animals were sacrificed at age P20. As shown in Figure 5, the mislocalization of cone opsins (both S-opsin and M/L-opsin) was partially corrected after the administration of 11-*cis* retinal. Compared with untreated retinae, the synaptic pedicles are less intensely stained, whereas more cone outer segments stained heavily for the cone opsins, indicating more cone opsin migrated properly into the outer segment after 11-*cis* retinal injections. The results in the *Lrat*$^{-/-}$ mouse are less dramatic than in the *Rpe65*$^{-/-}$*Rho*$^{-/-}$ mouse due to the large pool of rod opsin taking up the available 11-*cis* retinal. These results confirmed that the 11-*cis* retinal is critical to normal cone opsin trafficking.

DISCUSSION

The purpose of this study was to determine if the two mouse models lacking 11-*cis* retinal have similar patterns of rod and cone degeneration and are therefore both appropriate models for LCA. Even though both the Lrat and Rpe65 genes are candidate genes for LCA (e.g.,29,[30]),

the rationale for this comparison was the concern that the unexpected cone opsin mistrafficking observed in the *Rpe65**−/−* mouse might be related to some unknown function of RPE65, possibly within the cone photoreceptors. We therefore examined the *Lrat**−/−* mouse which likewise does not produce 11-*cis* retinal, but has normal RPE65 production and localization. The rod opsin levels in these two models is essentially identical, with some opsin phosphorylation which is not observed in *wt* animals. Both models show a minuscule level of 9-*cis* retinal production. The rods in both these models degenerate slowly and have no evidence of opsin mislocalization.

The cones of both the *Lrat**−/−* and the *Rpe65**−/−* mice degenerate rapidly, with eventual complete loss of cone function. Immunohistochemistry indicates that the cone opsins in both models are not trafficking to the outer segments appropriately and the remaining outer segments appear stunted. The degeneration and cone opsin mistrafficking can be arrested with administration of 11-*cis* retinal at an early age. We have confirmed functional cone recovery in the *Rpe65**−/− Rho**−/−* mouse, but were unable to confirm recovery of cone function in the *Lrat**−/−* mouse due to the interference of rods. We proposed that the lack of trafficking to the outer segments is the cause of cone degeneration. The cone opsin localization patterns look very similar in these two models lacking 11-*cis* retinal, with opsin found throughout the inner segment down to the pedicle. This mistrafficking appears to be a post-Golgi targeting disorder, as the opsin is exported from the endoplasmic reticulum, but cannot be transported to the outer segment.

In general, rod pigments are more stable than cone pigments, such as in their lability to hydroxylamine.[31] Another distinction between the rod and cone opsins are that cone opsins are known to be more highly phosphorylated than the rod opsins on light activation, having more serines and theonines in the C-terminus.[32] Also, the two mouse cone opsins lack the posttranslational modification of cysteine palmitylation, proposed to form the eighth "helix" on rod opsins.[33] Our data may suggest that the 11-*cis* retinal induces a conformational change that is necessary for recognition by a chaperone or transporting complex. Without this conformational change, the cone opsins cannot be transported to the outer segment, where it is required for disk (and therefore outer segment) stability, similar to the requirement for rhodopsin in rod outer segment formation.[34,35]

The trafficking of rod opsin has been studied in several species (for a recent review, see[36]). Our results indicate that rod opsin traffics normally in the absence of 11-*cis* retinal. There is some *in vitro* evidence that 11-*cis* retinal improves protein stability of certain rhodopsin mutants as well as movement from the endoplasmic reticulum to the cell membrane,[37] but there are no reports of 11-*cis* retinal availability affecting normal rhodopsin trafficking *in vivo*. The C-terminal amino acids of rhodopsin have been implicated in being essential for proper targeting,[38] and a general VXPX motif for targeting has been postulated[36] to be involved in sorting into post-Golgi carriers through binding with ARF-4.[39] Sorting mechanism for cone pigments are unknown, however, both cone pigment primary sequences carry a C-terminal sequence VSPA (M/L-opsin) or VGPH (S-opsin), each of which could qualify as a targeting sequence. Lack of correct targeting of cone pigments in the absence of 11-*cis* retinal could be explained by masking of the C-terminal targeting sequence or other targeting signals in the C-terminal region (or elsewhere in the cytoplasmic domain of cone pigments). It is conceivable that presence of 11-*cis* retinal enables cone pigments to assume a conformation in which the targeting signal(s) are exposed.

Acknowledgements

Abbreviations

CIS
> cone inner segment

COS
> cone outer segment

ERG
> electroretinogram

LRAT
> lecithin:retinol acyl transferase

S-opsin
> short wave cone opsin

M/L-opsin
> middle/long wave cone opsin

P
> postnatal

PNA
> peanut agglutinin

RPE
> retinal pigment epithelium

RPE65
> 65 kDa RPE-specific protein (retinoid isomerase)

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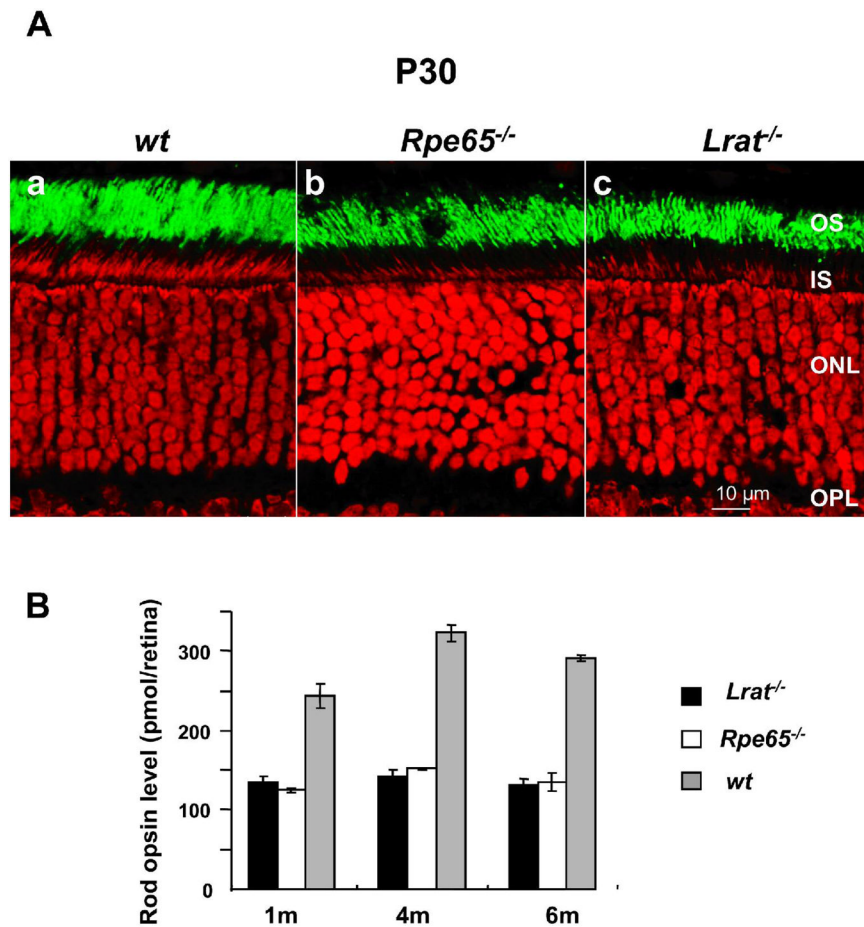
A

P30

B

Figure 1. Comparison of rod opsin in 1-month-old *wt, Rpe65*^{−/−} and *Lrat*^{−/−} retinae
(A) Immunocytochemistry Sections probed using antibody directed against rhodopsin (green). Nuclei were counterstained with propdium iodide (red); scale bar represents 10 μm. Abbreviations: OS, outer segments; IS, inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer. **(B) Relative regenerable opsin levels in *Lrat*^{−/−} and *Rpe65*^{−/−} retinae.**Levels of available opsin were determined by the incubation of homogenized retinae of age-matched 1-month, 4-month and 6-month-old animals reared in cyclic light with 11-*cis* retinal (80 μM) for 2 hours. Difference spectra were determined from measurements before and after bleaching in the presence of hydroxylamine (20 mM). Available opsin levels were calculated from regenerated rhodopsin concentrations using $\varepsilon = 40,000 \ M^{-1}cm^{-1}$ at λmax = 500 nm. Data are presented as means ±SEM, n = 3. White bars: *Rpe65*^{−/−} mice; black bars: *Lrat*^{−/−} mice, gray bars: *wt* mice.
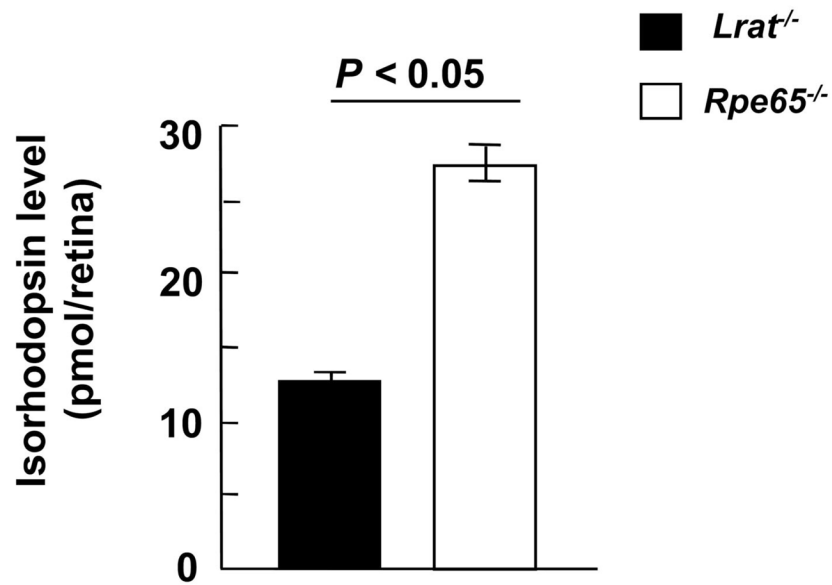
Figure 2. Relative isorhodopsin levels in *Lrat*^{−/−} and *Rpe65*^{−/−} retinae

Animals of 2-months-of-age were dark-reared for 8 weeks. Pigment levels were determined from difference absorption spectra. Data are presented as means ±SEM (n = 3) and analyzed by two-tailed Student *t* test, accepting a significant value of $P <0.05$. White bars: *Rpe65*^{−/−} mice; black bars: *Lrat*^{−/−} mice.
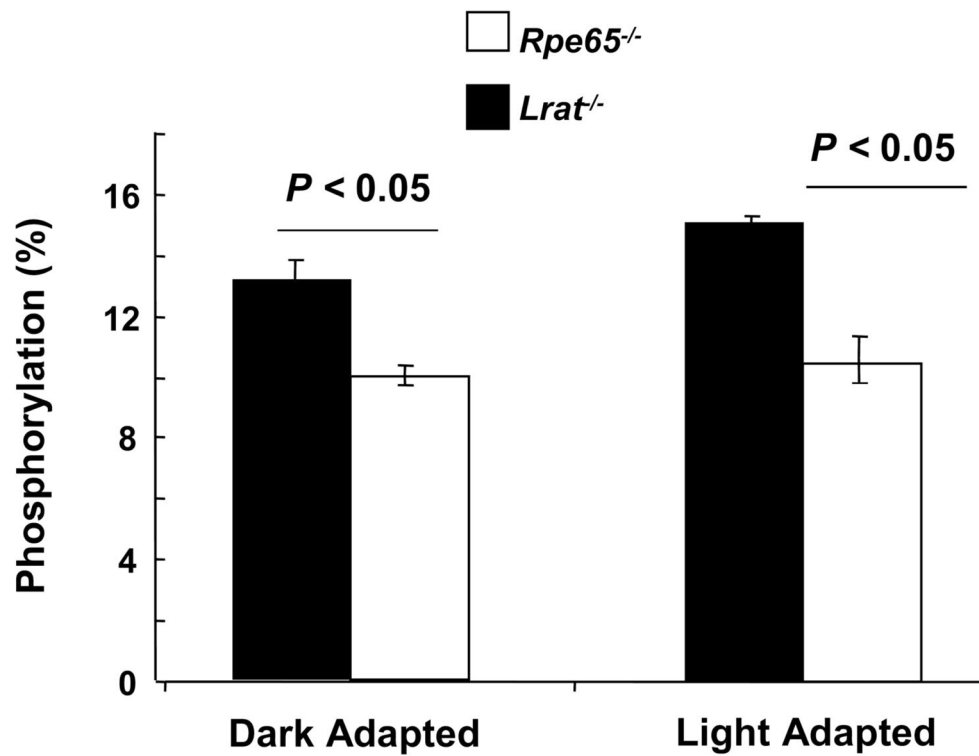
Figure 3. Opsin phosphorylation levels in *Lrat*^{−/−} and *Rpe65*^{−/−} mice

Retinae of 2-month-old *Rpe65*$^{-/-}$ and *Lrat*$^{-/-}$ mice were homogenized in 8 M urea and the pellet digested with Asp-N to cleave the opsin C-terminus. The peptides were analyzed by HPLC/MS/MS using a LTQ mass spectrometer. Data are presented as percentages of phosphorylated peptides (means ±SEM (n = 3)) and analyzed by the two-tailed Student *t* test, accepting a significant value of *P* <0.05. White bars: *Rpe65*$^{-/-}$ mice; black bars: *Lrat*$^{-/-}$ mice.
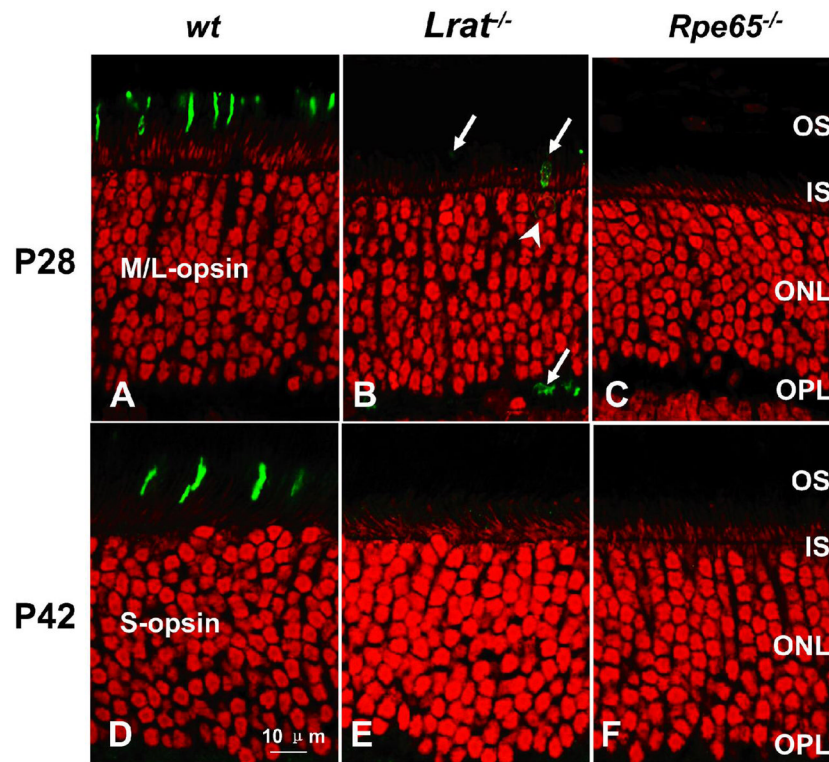
Figure 4. Confocal immunolocalization of cone opsins in *wt*, *Lrat*$^{-/-}$ and *Rpe65*$^{-/-}$ retinae
(A–C) P28 *wt* and mutant retinae are stained for M/L-opsin. The M/L-opsin is nearly absent at this stage. A perinuclear ring (**B**; arrowhead) represents the endoplasmic reticulum-region where M/L-opsin (arrow) is synthesized. **(D–F)** P42 *wt* and mutant retinae are stained for S-opsin. The S-opsin is completely missing in the *Lrat*$^{-/-}$ and *Rpe65*$^{-/-}$ retinae at this stage, whereas the *wt* cones appear healthy with the opsin in the outer segments. Nuclei are contrasted with propidium iodide (red); scale bar represents 10 μm. All retinae sections passed through the optic nerve, and photoreceptors were imaged ventral (inferior) to the nerve where the degeneration was most advanced. Abbreviations as in Figure 1A.
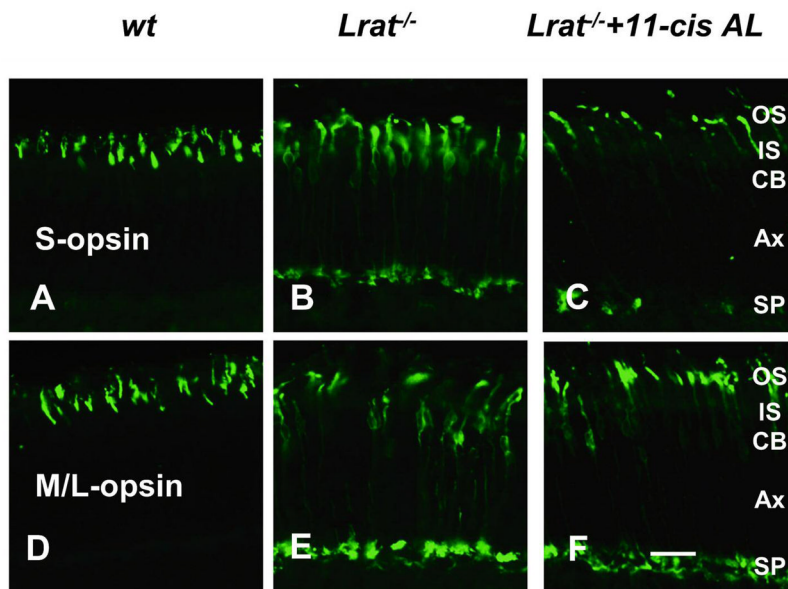
Figure 5. Effect of 11-*cis* retinal on cone opsin distribution
Retina sections (14 μm thickness, dorsal of the optic nerve) of P20 mice were stained with antibodies against S-opsin (**A–C**) and M/L-opsin (**D–F**) with and without 11-*cis* retinal injections: (**A, D**) C57BL/6 *wt* mice; (**B, E**) $Lrat^{-/-}$ mice; (**C, F**) $Lrat^{-/-}$ mice with11-*cis* injections. For the untreated knockout mice, the opsin was distributed throughout the entire cell (cone inner and outer segment, cell body, axon and synaptic pedicle). Upon 11-*cis* retinal administration, less cone opsin was localized inappropriately to the synaptic pedicle, whereas more cone opsin was appropriately localized to the outer segment. Abbreviations: OS, outer segment; IS, inner segment; CB, cell body; Ax, axon; SP, synaptic pedicle. Scale bar = 25 μm.