Absence of Functional and Structural Abnormalities Associated with Expression of EGFP in the Retina

May Nour,[1] *Alexander B. Quiambao,*[2] *Muayyad R. Al-Ubaidi,*[1,2] *and Muna I. Naash*[1,2]

Purpose. The present study was undertaken to evaluate the effect of uniform EGFP expression on retinal morphology and function.

Methods. Electroretinography (ERG) was used to evaluate the recovery of scotopic a- and b-wave amplitudes after a single 137-cd · sec/m^2 flash exposure. The cellular distribution of enhanced green fluorescent protein (EGFP) in the retina and its effect on retinal morphology were evaluated by fluorescence microscopy and histology, respectively. To evaluate its effect on retinal sensitivity to light, EGFP-expressing and control mice were exposed to constant light for 76 hours (3500 lux), and eyes were assessed functionally and structurally at 3 weeks after light exposure.

Results. Fluorescence microscopy showed a pronounced EGFP expression in the photoreceptor cell bodies and inner segments. ERG analysis revealed no significant differences in either a- or b-wave amplitudes or recovery between $EGFP^{+/-}$ and control mice under dark- or light-adapted conditions. Histologic assessment at as late as 4 months of age showed no difference in retinal morphology or photoreceptor nuclei count in $EGFP^{+/-}$ mice when compared with nontransgenic littermates. In addition, evaluation of animals, 3 weeks after constant light exposure, showed no difference between ERG amplitudes, recovery of the scotopic ERG response, or retinal morphology between $EGFP^{+/-}$ mice and control animals.

Conclusions. Functional and morphologic evidence shows that long-term, high, uniform levels of EGFP expression have no deleterious effect on the mouse retina. This data demonstrates the safety of EGFP use as an indicator of viral transduction in retinal gene therapy. (*Invest Ophthalmol Vis Sci.* 2004;45: 15–22) DOI:10.1167/iovs.03-0663

Since the cloning of green fluorescent protein (GFP) in 1992,[1] this exceptionally valuable molecule has been used as a versatile biomarker in a large number of biological systems. Unlike other previously available markers, jellyfish *Aequorea victoria*–derived GFP offers the advantage of acting as a powerful chromophore, without the need for additional cofactors or regulatory molecules.[2] When excited by blue or ultraviolet light, GFP emits its green light fluorescence at a wavelength of 510 nm.[2]

One of the experimental fields that has taken advantage of GFP and its enhanced variant EGFP, as fluorescent indicators, has been the field of gene therapy. In this field, GFP and EGFP have been particularly useful for testing the efficacy and site-specific targeting abilities of a number of nonviral and viral vector systems, including lenti-, retro-, herpes-, adeno-, and adeno-associated viruses.[3–11] Another important use of GFP that has contributed significantly to the advancement of gene therapy is its contribution to testing the activation, expression level, and cell specificity of numerous promoters intended for use to target gene therapies appropriately for the treatment of diseases.[12–16] Moreover, cell replacement therapy has also taken advantage of GFP/EGFP as fluorescent markers to track the localization of newly delivered cellular therapies.[17–22] The field of retinal gene therapy, in particular, has benefited equally by the use of GFP as an indicator of gene transfer and viral transduction in a variety of retinal cell types.[4,23–29] Gene therapy studies have also coexpressed GFP with the gene of interest to allow for a more direct indication of the number and regional localization of cells that were successfully transduced by the therapeutic vector.[30]

The expression of an exogenous protein in any biological system has the potential for resulting in an unfavorable disruption of biological processes. This may occur as a result of the initiation of an immune response against the foreign gene or simply an inability of the system to rid itself of the accumulation of this foreign protein. Many studies have effectively used GFP as a marker in different experimental settings and have shown normal development in EGFP-expressing transgenic mice.[31] However, other studies have reported unfavorable side effects. These deleterious effects range from indication of immune response against the expressed GFP in some systems[32,33] to a reported block of wanted cellular differentiation as a result of GFP expression in others.[34]

As prospects of retinal gene therapy in humans have become more promising with the most recent positive findings[35,36] and although the expression of GFP and its enhanced variant in the retina have been shown in several studies,[4,23–29] to date no study has thoroughly addressed the safety of this expression on retinal function and morphology. The phototransductory properties of the retina dictate a fine-tuned and well-regulated control of rhodopsin activation. Overexpression of rhodopsin and light-induced overactivation of this chromophore, leads to retinal degeneration and cell death.[37–42] As a fluorescent indicator, when excited by blue or ultraviolet light, GFP emits its green-light fluorescence at a wavelength of 510 nm.[2] This emission wavelength overlaps with the absorption spectrum for the rhodopsin chromophore,[43,44] the result of which may be rhodopsin overactivation. This effect may be similar to rhodopsin overexpression, which has been shown to result in retinal cell death.[37] For this reason, we investigated the effects of high and uniform EGFP expression on retinal morphology and function using β-actin–driven EGFP transgenic mice[45] in the backgrounds of pigmented C57BL/6, albino BALB/c, and two transgenic lines, each expressing a form of

From the [1]Oklahoma Center for Neuroscience, and the [2]Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma.

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Corresponding author: Muna I. Naash, University of Oklahoma Health Sciences Center, 940 Stanton L. Young Blvd., BMSB 781, Oklahoma City, OK 73104; muna-naash@ouhsc.edu.

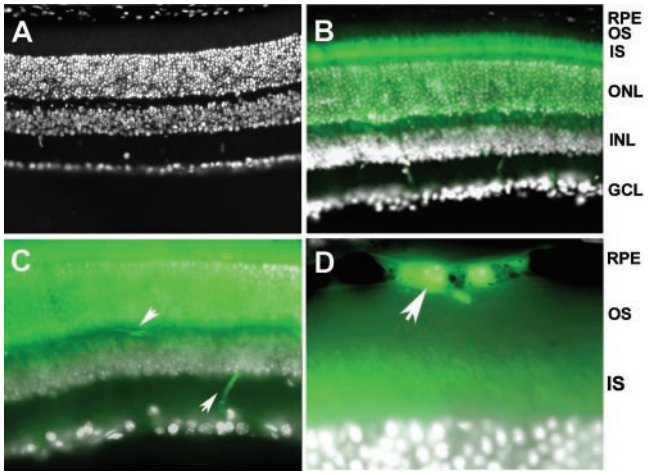


FIGURE 1. EGFP expression in the retina of β-actin EGFP-transgenic mice. All images represent an overlay of EGFP expression on DAPI nuclear stain (*white*). (**A**) No endogenous fluorescence was detected in $EGFP^{-/-}$ retinas. (**B**) In $EGFP^{+/-}$ samples, although cytoplasm of all retinal cell types showed EGFP expression, it was most evident in the IS and perinuclear space of photoreceptors in the ONL. (**C**) *Arrows*: EGFP expression in retinal blood vessels. (**D**) Physically removing endogenous pigment revealed an underlying, intense EGFP expression in the RPE cell layer. GCL, ganglion cell layer.

mutant opsin that has been found to associate with cases of retinitis pigmentosa (RP) in humans.

MATERIALS AND METHODS

Animals

EGFP-expressing mice (C57BL/6-TgN(ACTbEGFP)10sb) were purchased from the Jackson Laboratory (Bar Harbor, ME). A chicken β-actin promoter driving the expression of EGFP cDNA, followed by a cytomegalovirus enhancer, was used to generate this line of transgenic mice.[45] EGFP-expressing mice were kept in the heterozygous genetic background ($EGFP^{+/-}$), because the homozygotes of this line die in the embryonic stage of a site-of-integration effect. Both of the rhodopsin mutant mice (VPP and G90D) were generated in our laboratory, as described before (Naash MI, et al. *IOVS* 1996;37:ARVO Abstract 3189).[46] All mice were maintained in the breeding colony under cyclic light conditions (12 hours light–dark). Cage illumination was approximately 7 foot-candles during the light cycle. All procedures were approved by the local Institutional Animal Care and Use Committees and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Scotopic and Photopic Electroretinography

For assessment of the recovery pattern of rod function (scotopic ERG), the animals were dark adapted for at least 4 hours and anesthetized by an intramuscular (IM) injection of 85 mg/kg ketamine and 14 mg/kg xylazine. After pupil dilation with 5% phenylephrine, mice were placed on a regulated heating pad throughout the length of the experiment, and ERGs were recorded with a stainless-steel wire contacting the corneal surface through a layer of 2.5% methylcellulose. Responses were differentially amplified (half band-pass, 1–4000 Hz), averaged, and stored with a compact four-signal averaging system (Nicolet Instrument, Madison, WI). For the assessment of rod photoreceptor function, a strobe flash stimulus was presented to the dark-adapted, dilated eyes in a Ganzfeld (gs-2000; Nicolet) with a 137 cd · sec/m^2 flash intensity. The amplitude of the a-wave was measured from the prestimulus baseline to the a-wave trough. The amplitude of the b-wave was measured from the trough of the a-wave to the peak of the b-wave. The recovery of the scotopic ERG signal was assessed through a series of flashes: An initial flash was presented, followed by a second flash of the same intensity (137 cd · sec/m^2) at different time points (0, 10, 20, 30, 60, or 90 seconds). After a pair of flashes, the animal was allowed to recover fully for 60 seconds before the presentation of the next pair.

For the evaluation of cone function (photopic ERG), a strobe flash stimulus was presented to 5-minute light-adapted, dilated eyes in a Ganzfeld (gs-2000; Nicolet) with a 77-cd · sec/m^2 flash intensity. The amplitude of the cone b-wave was measured from the trough of the a-wave to the peak of the b-wave.

ERG recordings from 10 to 16 eyes were averaged for each experimental group. Analysis of variance and post hoc tests using the Bonferroni pair-wise comparisons were used to determine the significance of differences in the ERG responses of $EGFP^{+/-}$ and $EGFP^{-/-}$ mice (Prism 3.02; GraphPad, San Diego, CA). For each time point, four to six animals were used to evaluate retinal histology.

Dark Rearing and Continuous Light Exposure

For dark rearing, mice were born and raised until 60 days of age under dark conditions. For continuous light exposure experiments, mice (P60) were exposed to light (3500 lux) for 76 hours. Mice were then transferred to cyclic-light conditions for 3 weeks before retinal function and morphology were assessed. For each genetic background, three to six animals were used to evaluate retinal histology.

Histology and Fluorescence Microscopy

For histologic evaluation, after enucleation the posterior portion of each eye, containing the retina and retinal pigment epithelium was fixed for 24 hours in 0.1 M phosphate buffer (pH 7.4) containing 2% formaldehyde and 2.5% glutaraldehyde. The tissues were then osmicated, dehydrated through a graded ethanol series, embedded in paraffin, and processed for evaluation. The sections (0.5 μm thick) were cut along the horizontal meridian, passed through the optic nerve and stained with hematoxylin and eosin (H&E) in preparation for light microscopy. For each time point, three to six animals were used to evaluate retinal histology.

For the evaluation of the fluorescent signal produced by EGFP, eyes were processed in preparation for cryostat sectioning. After enucleation and a 1-hour fixation in 4% paraformaldehyde, the anterior segments were removed, and eyecups were fixed overnight at 4°C. The following day, eyecups were washed in PBS and transferred to a 30% sucrose solution for cryoprotection. On embedding in M-1 embedding matrix (Shandon, Pittsburgh, PA), sections of EGFP-positive and -negative retinas were cut at 10 μm and mounted with 4′,6′-diamino-2-phenylindole (DAPI) nuclear stain (Vector Laboratories, Inc., Burlingame, CA). Images represent an overlay of DAPI signal with endogenous EGFP fluorescence. Sections were examined by microscope (Axioscope; Carl Zeiss Meditec, Germany).

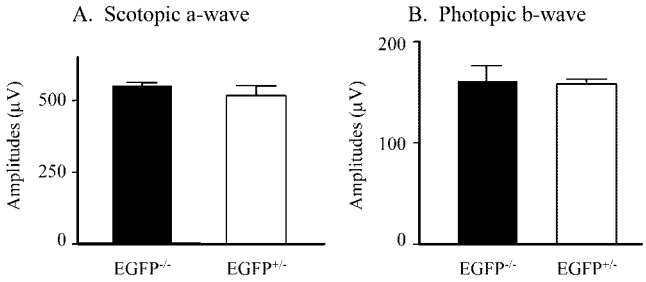
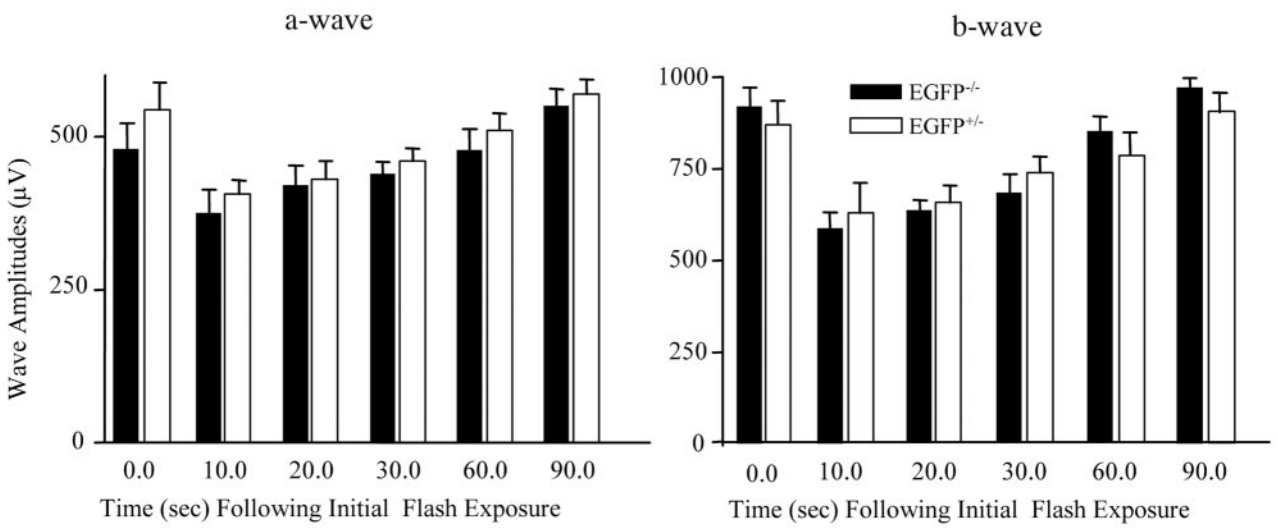


FIGURE 2. Effect of EGFP expression on retinal function under dark-reared conditions. Mice were born and raised for 60 days under complete dark-adapted conditions. Scotopic and photopic ERGs were used to assess rod and cone photoreceptor functions, respectively. (**A**) No statistically significant differences in amplitude of scotopic ERG a-wave were found between $EGFP^{+/-}$ and $EGFP^{-/-}$ mice. (**B**) Photopic ERG results also showed no statistically significant differences in b-wave amplitudes between EGFP transgenic mice and their nontransgenic littermates.



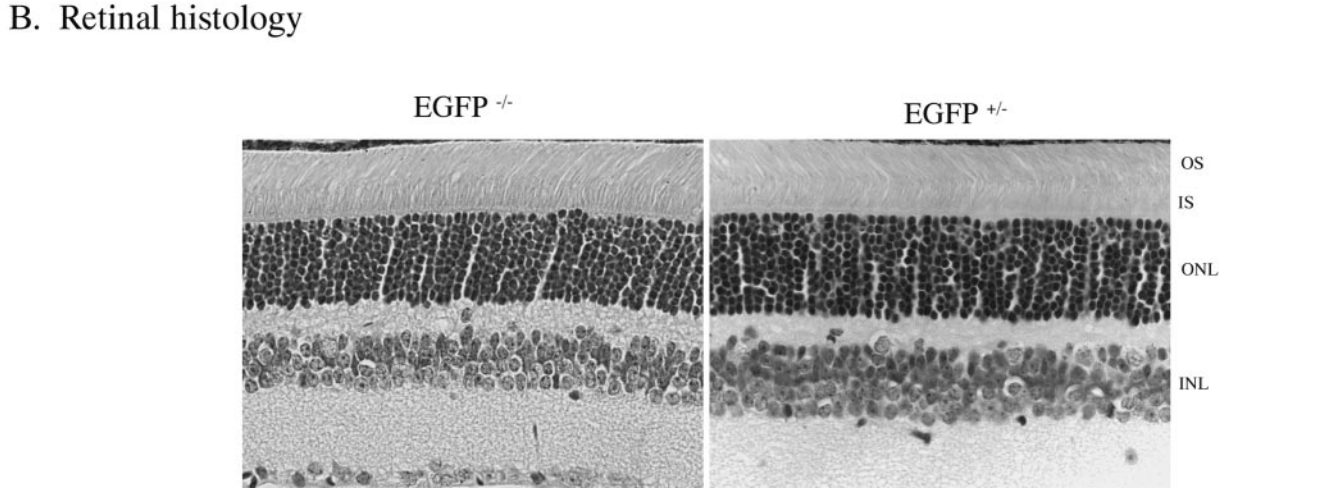


FIGURE 3. Effect of EGFP expression on retinal function and morphology under cyclic-light rearing conditions. (**A**) At 30 days of age, a series of flash pairs of the same intensity were presented to $EGFP^{+/-}$ and $EGFP^{-/-}$ mice at increasing time differences to monitor ERG recovery. Data showed no differences in the temporal pattern of scotopic ERG amplitude regeneration in $EGFP^{-/-}$ and $EGFP^{+/-}$ mice. (**B**) Histologically, no abnormalities were observed in retinal cross sections from EGFP-expressing mice.

RESULTS

Localization of EGFP in the Retina

As has been described,[45] the expression of the EGFP reporter gene in these transgenic mice is driven by the chicken β-actin promoter. As a result, EGFP expression in the retina was observed in the cytoplasm of all retinal cell types. Although no endogenous fluorescence was detected in nontransgenic ($EGFP^{-/-}$) retinal cross sections (Fig. 1A), the most intense fluorescent signal was detected in the inner segment (IS) and perinuclear space in the outer nuclear layer (ONL) of photoreceptors of transgenic mice (Fig. 1B). Expression of EGFP was also observed in retinal blood vessels (Fig. 1C, arrows) and the retinal pigment epithelial (RPE) cell layer (Fig. 1D, arrows).

Effect of EGFP Expression on Retinal Function and Morphology

To avoid any prior light-dependent photoactivation of rhodopsin by EGFP, we performed initial studies on 60-day-old $EGFP^{+/-}$ and $EGFP^{-/-}$ mice reared in dark-adapted conditions from birth. No statistically significant differences in amplitude of scotopic ERG a-wave were observed between $EGFP^{+/-}$ mice and their $EGFP^{-/-}$ littermates (Fig. 2A. Cone photoreceptor function, tested under light-adapted conditions, also revealed no statistically significant differences between $EGFP^{+/-}$ and nontransgenic littermates (Fig. 2B).

Similar results were observed in EGFP transgenic and nontransgenic mice reared in cyclic-light–adapted conditions (Fig.

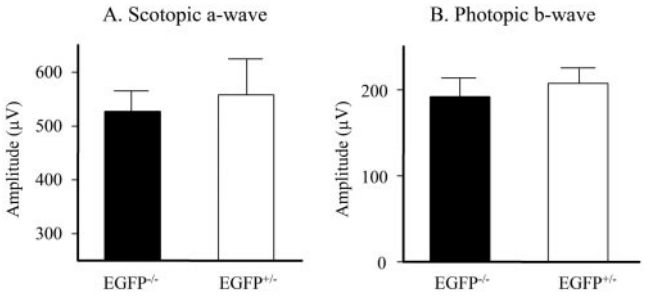


FIGURE 4. Effects of long-term EGFP expression on retinal function and morphology. (**A**) No statistically significant differences in scotopic ERG a-wave amplitudes were found between $EGFP^{+/-}$ and $EGFP^{-/-}$ mice at 4 months of age. (**B**) Cone photoreceptor function, represented by photopic ERG b-wave was also unaltered in EGFP-expressing mice when compared with their nontransgenic littermates. Mice were raised in cyclic light conditions before functional evaluation.

3). To evaluate the effect of EGFP expression on the temporal pattern of scotopic ERG regeneration, a single-intensity light stimulus was presented to mice in a series increasing in time difference from each other. This protocol was used to determine the effect of EGFP expression on the regeneration of full scotopic ERG signal in $EGFP^{+/-}$ and $EGFP^{-/-}$ mice. No statistically significant differences were detected between EGFP expressing mice and their nontransgenic littermates in a Bonferroni pair-wise comparison (Fig. 3A). The similarities between $EGFP^{+/-}$ and $EGFP^{-/-}$ mice were apparent in the initial scotopic ERG wave amplitudes, the rate of scotopic wave regeneration, and the overall temporal pattern of scotopic ERG recovery (Fig. 3A). In addition, no histologic abnormalities were observed on the evaluation of retinal cross sections from $EGFP^{+/-}$ mice (Fig. 3B). No differences in the ONL cell counts, inner nuclear layer (INL) thickness, or outer segment (OS) length were found between $EGFP^{+/-}$ and their $EGFP^{-/-}$ littermates (data not shown).

Functional and Morphologic Effects of Long-Term EGFP Expression on the Mouse Retina

The long-term effects of intense EGFP expression in the retina were tested in 4-month-old EGFP transgenic and nontransgenic mice that were raised in cyclic light conditions. One-way ANOVA and Bonferroni pair-wise comparison revealed no significant differences in scotopic ERG amplitudes between $EGFP^{+/-}$ and $EGFP^{-/-}$ mice (Fig. 4A). Consistent with normal rod function observed at 4-months of age in $EGFP^{+/-}$ mice, photopic ERG also revealed unaltered cone function when compared with ERGs in nontransgenic littermates (Fig. 4B).

Effect of EGFP Expression on the Albino Mouse Retina

Because the albino mouse retina has been shown to be more sensitive to light-induced retinal damage,[40,47] we generated $EGFP^{+/-}$ and $EGFP^{-/-}$ mice in the albino (BALB/c) background. These mice were used to evaluate the effects of EGFP expression on the albino retina. ERG analyses of 30-day-old $EGFP^{+/-}$ and $EGFP^{-/-}$ mice are shown in Figure 5. Results indicated that expression of EGFP in an albino background had no negative effect on rod or cone photoreceptor function as shown by scotopic and photopic ERG, respectively (Fig. 5).

Effect of EGFP Expression on the Mouse Retina under Constant Light Exposure

A condition of 76 hours of constant light exposure (3500 lux) was used to assess the potential deleterious effects of EGFP expression on light-induced retinal stress. This level was chosen to represent high, yet subthreshold, damaging light levels.[48] Pigmented $EGFP^{+/-}$ and $EGFP^{-/-}$ mice were placed in cyclic-light conditions for 3 weeks after continuous light exposure and then were evaluated according retinal function and morphology. Three weeks after exposure to light, representative scotopic ERG waves from $EGFP^{+/-}$ and $EGFP^{-/-}$ mice showed similar responses indicating rod photoreceptor function (Fig. 6A). One-way ANOVA and Bonferroni post hoc analysis reveal no statistically significant differences between $EGFP^{+/-}$ and $EGFP^{-/-}$ mice in either a- or b-wave amplitudes after light exposure (Fig. 6B). Furthermore, histologic evaluation of transgenic and nontransgenic retinas showed no differences in morphology after this period of constant light exposure (Fig. 6C). Under our experimental conditions, the 76 hours of constant exposure to 3500-lux light produced no

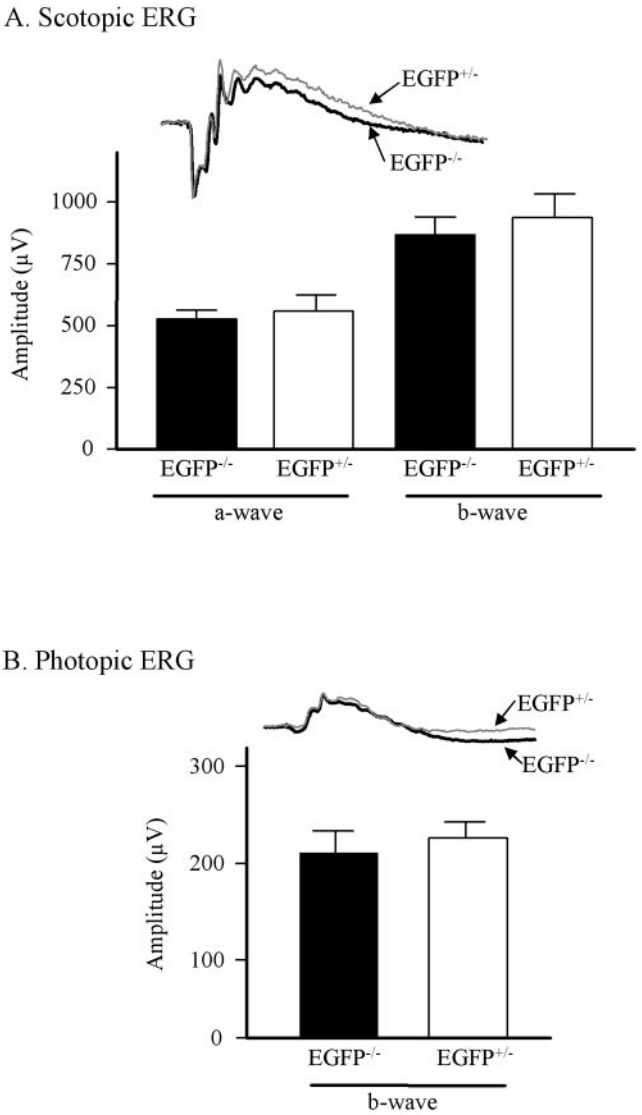


FIGURE 5. Effect of EGFP expression on rod and cone ERGs of the albino mouse retina. EGFP mice were crossed onto the albino BALB/c background, and results represent the ERG amplitudes of 30-day-old mice. (**A**) Scotopic ERG a-waves showed no difference between albino $EGFP^{+/-}$ and $EGFP^{-/-}$. Representative ERG waveforms (overlay shown *above* graph) also showed a very similar pattern of rod photoreceptor response. (**B**) Photopic ERG b-wave amplitudes also show comparable amplitudes generated from albino $EGFP^{+/-}$ and $EGFP^{-/-}$. This similarity in cone response is further demonstrated by representative ERG waveforms (overlay shown *above* graph).

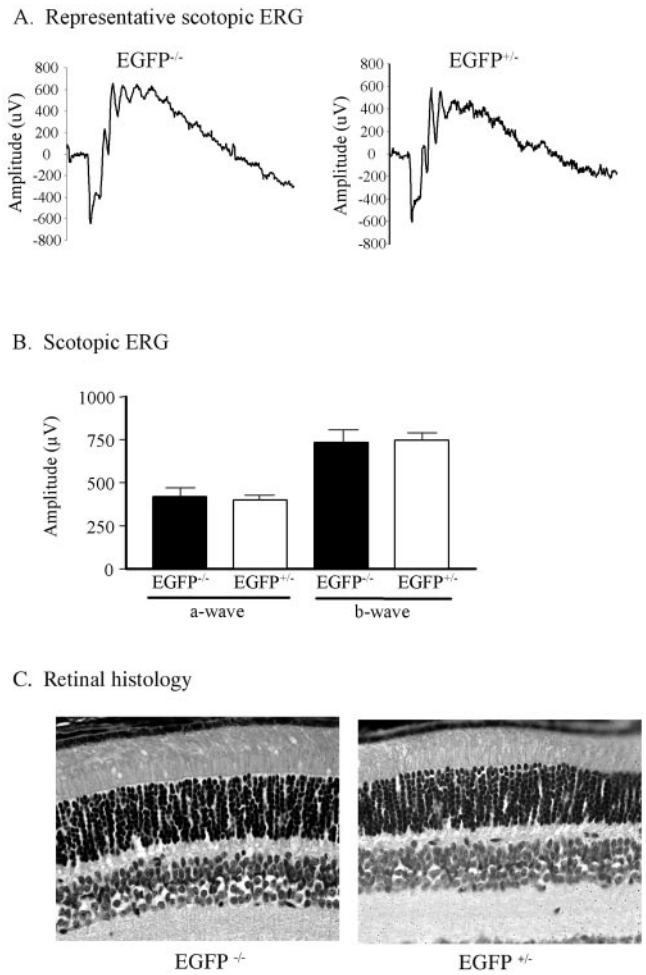


FIGURE 6. Effect of EGFP expression on retinal function and morphology under constant light exposure. Pigmented EGFP$^{+/-}$ and EGFP$^{-/-}$ mice were exposed to 76 hours of constant, 3500-lux light and were evaluated 3 weeks later. (**A**) Representative ERG waveforms showed a similar response in rod scotopic ERG function from EGFP-expressing, transgenic mice and their nontransgenic littermates. (**B**) No statistically significant differences were found between EGFP$^{+/-}$ and EGFP$^{-/-}$ mice in scotopic a- or b-wave amplitudes after constant light exposure. (**C**) No changes in retinal morphology were detected in either EGFP$^{-/-}$ or EGFP$^{+/-}$ retinas after the 76-hour period of constant light exposure.

deleterious effects on ERG function and retinal morphology in either mouse genotype.

Influence of EGFP Expression on the Course of Retinal Degeneration in Transgenic Mice Expressing the G90D Mutation in Rhodopsin

The dominant GLU90ASP (G90D) mutation in rhodopsin has been reported to cause congenital night blindness in humans.[49] It is believed to do so by constitutively activating rhodopsin.[50,51] Mice carrying this mutation, which interferes with phototransduction, have a late-onset form of slow retinal degeneration (Naash MI, et al. *IOVS* 1996;37:ARVO Abstract 3198).[50] G90D$^{+/-}$/EGFP$^{+/-}$ mice were used to evaluate the effect of EGFP expression on the function, morphology, and rate of retinal degeneration in mice expressing the G90D mutation in rhodopsin. At 30 days of age, a series of flash pairs were used to evaluate the temporal pattern of scotopic ERG regeneration. Results of ERG recovery analysis in G90D$^{+/-}$/EGFP$^{+/-}$ and G90D$^{+/-}$/EGFP$^{-/-}$ revealed a similar pattern of response in animals expressing EGFP and their non-EGFP littermates (Fig. 7A). One-way ANOVA and Bonferroni pair-wise comparison confirm that there are no statistically significant differences in ERG a-wave amplitudes or recovery caused in response to EGFP expression in the G90D transgenic retinas. At 90-days of age, transgenics expressing both the G90D mutation and EGFP continued to show similar scotopic ERG amplitudes when compared with G90D$^{+/-}$/EGFP$^{-/-}$ littermates (representative waves, Fig. 7A). Histologically, no differences in retinal morphology were detected between retinal cross sections of G90D$^{+/-}$/EGFP$^{+/-}$ and G90D$^{+/-}$/EGFP$^{-/-}$ mice, indicating that the expression of EGFP does not alter the time course of retinal degeneration in these mice.

Influence of EGFP Expression on Function, Morphology, and Time Course of Retinal Degeneration in Transgenic Mice Expressing the VPP Mutation in Rhodopsin

A second transgenic mouse model carrying a-three-point mutation near the N terminus of the rhodopsin molecule was used for further confirmation of the lack of deleterious effect of EGFP expression on degenerating retina. The VPP mutation involves a substitution of histidine for proline at position 23 (P23H), glycine for valine at position 20 (V20G), and leucine for proline at position 27 (P27L).[46] These mice express equal amounts of mutant and wild-type transcripts, and show progressive photoreceptor degeneration similar to that in humans with RP. Retinal degeneration in the VPP mouse line has an early onset and continues to degenerate in a light-dependent manner.[48,52] To assess the effect of EGFP expression on the severity and time course of retinal degeneration, mice were generated expressing both EGFP and the VPP mutant rhodopsin (VPP$^{+/-}$/EGFP$^{+/-}$). The results in Figure 8A produced from scotopic ERG analysis of mice at 30 days of age show no statistically significant differences in rod a- and b-wave amplitudes between VPP$^{+/-}$/EGFP$^{+/-}$ and VPP$^{+/-}$/EGFP$^{-/-}$ littermates (one-way ANOVA and Bonferroni pair-wise comparison). Furthermore, no differences in retinal morphology were detected between double transgenics expressing both the VPP mutation and EGFP when compared with their single transgenic VPP littermates (Fig. 8B). This indicates that EGFP does not accelerate the course of retinal degeneration.

DISCUSSION

The generation and characterization of transgenic animal models representative of human retinal diseases[37,48,50,53] has made it possible to test the therapeutic prospect of numerous experimental gene therapies.[11,25,36,54–56] Several relevant studies have relied heavily on GFP and EGFP to test the expression level and cell specificity of several promoters,[12–16] track the localization of newly delivered cellular therapies,[8,17–22] and indicate the targeting ability[3–11] and level of viral transduction afforded by viral vectors in the retina.[4,23–29] Bennett et al.,[28] described the lack of negative effects resulting from long-term GFP expression in the monkey retina. However, others have reported evidence of rejection resulting from lentivirus-mediated expression of GFP in RPE cells.[32] Aside from the prospects of immunologic response to the expression of an exogenous protein, the expression of GFP in the retina poses another challenge. The overlap in emission wavelength of GFP (510 nm) with the absorption wavelength of rhodopsin raises a concern regarding the potential for GFP-induced overactivation of the chromophore. Several studies to date have shown, the devastating effects of rhodopsin overactivation and overexpression in the retina, which in severe cases, led to photoreceptor cell death.[37–42] For these reasons, mutations that alter the phototransductory function of rhodopsin may be most

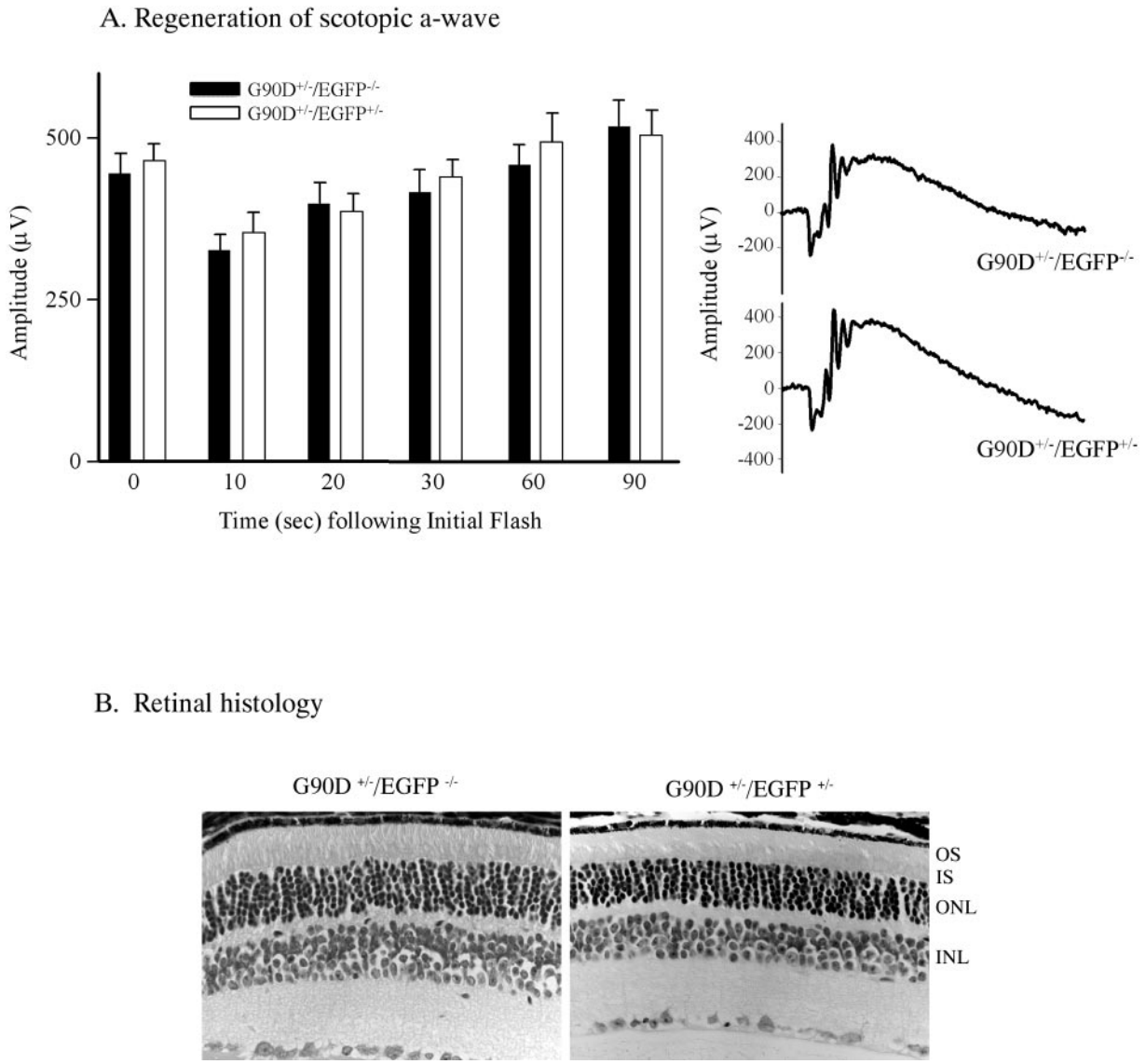


FIGURE 7. Effect of EGFP expression on the course of retinal degeneration in transgenic mice carrying a G90D mutation in rhodopsin. Data shown were generated from 30-day-old G90D$^{+/-}$/EGFP$^{-/-}$ and G90D$^{+/-}$/EGFP$^{+/-}$ transgenic mice. (**A**) A single intensity of light stimulus was presented to mice in a series of pairs, increasing in time difference from one another. Results from scotopic ERG a-wave recovery of G90D$^{+/-}$/EGFP$^{+/-}$ and G90D$^{+/-}$/EGFP$^{-/-}$ mice reveal a similar pattern of response (*left*). At 90 days of age, the double transgenics expressing both the G90D mutation and EGFP continued to show similar scotopic ERG amplitudes when compared with their littermates expressing only the G90D transgene (representative waves, *right*). (**B**) Histologically, no differences in retinal morphology were seen in retinal cross sections between G90D$^{+/-}$/EGFP$^{-/-}$ and G90D$^{+/-}$/EGFP$^{+/-}$ mice, indicating that the expression of EGFP does not alter the time course of retinal degeneration in these mice.

vulnerable to GFP-induced damage. Several of these mutations have been linked to retinal diseases, and their prevalence makes rhodopsin transgenic models particularly attractive for the testing and application of gene therapy, which may also make them subject to GFP expression. For this reason, we investigated the effect of retinal EGFP expression on both the normal and, more significantly, the diseased retina of transgenic mice expressing mutations in the opsin gene. The chosen two transgenic models of retinal diseases carry either the G90D or the VPP mutations in rhodopsin have been shown to cause human congential stationary night blindness and RP, respectively. Using both of these mouse models serves to assess the safety of EGFP under conditions of slow, late-onset (G90D) (Naash MI, et al. *IOVS* 1996;37:ARVO Abstract 3198),[50] as well as fast, early-onset, light-sensitive patterns of retinal degeneration (VPP).[48]

In the normal retina, although EGFP was abundantly expressed, neither retinal function nor histology was deleteriously affected (Figs. 2, 3, 4). In terms of the temporal pattern of retinal functional recovery after activation of phototransduction, the expression of EGFP in the normal retina caused no alteration in ERG recovery. Even at later stages, up to 6 months of age (data not shown), in both pigmented and albino backgrounds, transgenic and nontransgenic β-actin EGFP mice displayed comparable rod and cone ERG amplitudes and virtually indistinguishable retinal morphology. Furthermore, under conditions of constant light exposure, which has been shown to accelerate the rate of degeneration in rhodopsin mutant,[48] EGFP expression did not increase the susceptibility of the retina to light-induced damage. The effect of EGFP expression on the retina of disease-affected mice was also similar to the results obtained from nontransgenic littermates. There was no alteration in either rod or cone photoreceptor function or in retinal morphology and cell count. Moreover, no changes in the rate of retinal degeneration were detected in either of the transgenic lines carrying the G90D or VPP mutation in opsin.

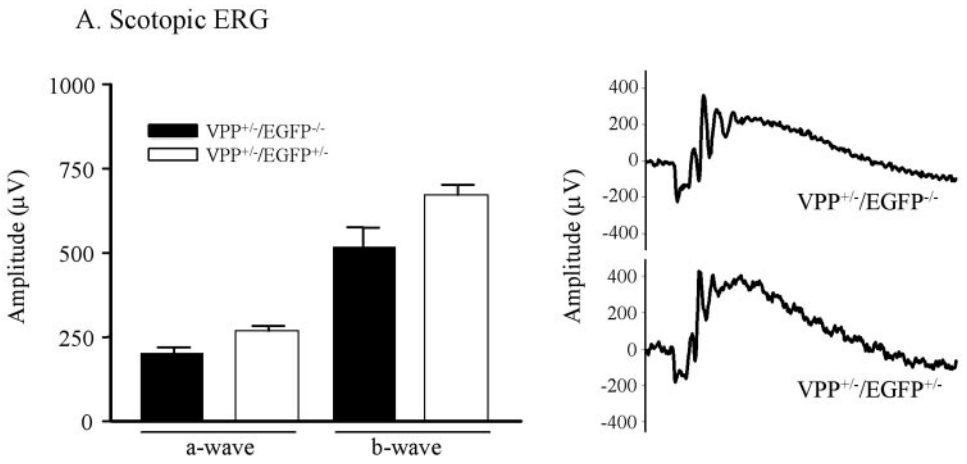


FIGURE 8. Effect of EGFP expression on the course of retinal degeneration in transgenic mice expressing the VPP mutation in rhodopsin. (**A**) At 30 days of age, scotopic ERG analysis showed no statistically significant differences in rod a- or b-wave amplitudes between $VPP^{+/-}/EGFP^{+/-}$ when compared with $VPP^{+/-}/EGFP^{+/-}$ mice (*left*). Representative waveforms also show the similarity in rod ERG response between animals expressing EGFP and those who do not (*right*). (**B**) No differences in retinal morphology were detected between double transgenics expressing both the VPP mutation and EGFP when compared with their single transgenic VPP littermates.

In summary, the present study has isolated and evaluated the effects of uniform, abundant EGFP expression in all retinal cell types and reports no deleterious effects on retinal function or morphology in response to high levels of EGFP expression in the normal and degenerating mouse retinas. When taken together, this validates the safety of EGFP expression in the retina and supports its use as a powerful, noninvasive means of monitoring viral transduction during therapeutic gene delivery to the retina.

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