The Human cGMP-PDE β-Subunit Promoter Region Directs Expression of the Gene to Mouse Photoreceptors

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Purpose. We previously demonstrated that 350 bp of the human rod cGMP phosphodiesterase β-subunit (β-PDE) gene promoter are sufficient to direct high levels of gene expression in human Y-79 retinoblastoma cells in vitro. In this study the cell specificity and expression pattern conferred by the short β-PDE 5′ flanking sequence in vivo were examined.

Methods. A construct containing the bacterial LacZ gene driven by a fragment of the β-PDE 5′ flanking region (−297 to +53) was used to generate transgenic mice. Gene expression was analyzed by measuring β-galactosidase activity in tissue homogenates or visualizing enzymatic activity or protein production at a cellular level by in situ histochemistry or immunocytochemistry.

Results. Three independently derived transgenic lines were generated carrying the −297 to +53 β-PDE 5′ flanking region fragment. Within the retina, the reporter gene was specifically expressed in photoreceptors, consistent with the localization of endogenous β-PDE. Significant expression of LacZ was not observed in other ocular or peripheral tissues.

Conclusions. Photoreceptor-specific reporter gene expression is driven in vivo by a 350-bp segment of the β-PDE 5′ flanking sequence. This study demonstrates the utility of the human β-PDE promoter for directing the expression of foreign genes to photoreceptors and suggests that the −297 to +53 β-PDE 5′ flanking region fragment may have important implications for therapeutic gene delivery to the visual cells. (*Invest Ophthalmol Vis Sci.* 2000;41:4059–4063)

The human phosphodiesterase β-subunit (β-PDE) gene encodes one of two catalytic subunits present in cGMP phosphodiesterase, a key enzyme in the rod phototransduction cascade.[1] Primary lesions in this gene have been shown to cosegregate with retinal degeneration affecting humans,[2–7] mice,[8,9] and dogs.[10,11] In light of its participation in the development of retinal abnormalities, it is relevant to understand the events that direct expression of the β-PDE gene in rod photoreceptors.

In previous studies, we cloned the 5′ flanking region of the human β-PDE gene and examined the elements controlling its rod-specific expression in vitro.[12] We were able to delineate sequences involved in transcriptional activation by transiently expressing various lengths of the 5′ flanking region in human Y-79 retinoblastoma cells.[13] Here, we have extended these studies using transgenic technology to elucidate the participation of regulatory elements on β-PDE gene expression in vivo. We have evaluated three independent transgenic mouse lines carrying the −297/+53 β-PDE 5′ flanking region fused to the LacZ reporter gene. Biochemical and immunocytochemical analyses suggest that this sequence contains the information necessary for the appropriate expression of β-PDE in photoreceptor cells.

Materials and Methods

β-PDE–LacZ Fusion Gene Constructs

A fragment of the 5′ flanking region of the human β-PDE gene extending from −297 to +53 was generated by PCR using sequence-specific primers.[13] The 5′ primer, complementary to residues −297 to −279 (5′ AGCAGAAAGCGTCATGCTG 3′) contained a *Kpn*I linker. The 3′ primer complementary to nucleotides +34 to +53 (5′ GTGGCTGCCTGTCCCTGG 3′) contained an *Xba*I linker. The PCR product was digested with *Kpn*I and *Xba*I and directionally subcloned into the pLacF vector[14] upstream of the β-galactosidase reporter gene to generate plasmid −297LacZ. The insert was fully sequenced in both directions by the dideoxy chain-termination method[15] to assure 100% identity with the original template. Primers were synthesized by the phosphoramidite method on an ABI DNA synthesizer (Foster City, CA) according to the manufacturer's specifications.

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Generation of Transgenic Mice

Linear DNA of the heterologous construct −297LacZ (free of vector sequences) was purified and microinjected into the pronuclei of fertilized one-cell C57BL/6J mouse embryos using standard procedures.[16] Animals were screened for inheritance of the transgene by Southern blot hybridization and PCR. For Southern blot analysis, 10 μg of tail genomic DNA was digested to completion with *Hin*dIII and electrophoresed in 1% agarose gels (Promega, Madison, WI). Gels were then blotted onto Hybond-N+ membranes (Amersham, Arlington Heights, IL) in 20× standard saline citrate (SSC). The probe used to detect the chimeric construct was a 1-kb *Eco*RI/*Sac*I fragment corresponding to the 3′ end of the LacZ coding sequence. DNA was labeled as previously described[17] using the Klenow fragment of DNA polymerase (USB, Cleveland, OH) and [α-^{32}P]dCTP (NEN, Boston, MA). Overnight hybridization was performed using 2×10^7 cpm of labeled probe in 7% SDS, 0.5 M phosphate buffer, pH 7.0, 1 mM EDTA, and 1% BSA at 65°C. Blots were washed at a final stringency of 0.2× SSC, 0.1% SDS at 60°C and then visualized by autoradiography after overnight exposure at −80°C. For PCR analysis, a 5′ primer (5′ GGGCTAGCGGGTTCCTAATCTCACTAA3′) complementary to the human β-PDE promoter residues −47 to −28 and a 3′ primer (5′ATGTGCTGCAAGGCGATTAA 3′) complementary to LacZ nucleotides +71 to +90 were used to span the chimeric constructs. PCR products were electrophoresed on 4% agarose gels and visualized after ethidium bromide staining.

Transgene Expression Assays

Eyes were enucleated immediately after euthanasia, and the corneas, lenses, and vitreous were removed. Retinas were dissected and processed for protein analysis, in situ histochemistry, and immunocytochemistry.

β-Galactosidase Activity Measurements

A colorimetric assay using *o*-nitrophenyl-β-D-galactopyranoside (ONPG; Sigma, St. Louis, MO) was used to detect β-galactosidase expression.[18] Tissues were homogenized using lysis reporter buffer (Promega), cell fragments were pelleted, and the supernatant was then mixed with a 0.1:22:130 mixture of 0.1 M $MgCl_2$ in 4.5 M β-mercaptoethanol, ONPG (4 mg/ml in 0.1 M sodium phosphate buffer, pH 7.5), and 0.1 M sodium phosphate buffer, pH 7.5. The samples were incubated at 37°C for 30 minutes and the reaction was stopped with 500 μl 1 M Na_2CO_3. The specific activity of β-galactosidase in each sample (normalized for cell protein) was measured from the optical density reading at 420 nm. Protein concentration was determined as described by Peterson.[19] Background β-galactosidase enzymatic activity in wild-type mouse tissues was subtracted from the activity of the corresponding transgenic tissue.

Histochemistry

Retinal flat mounts from nontransgenic (control) and transgenic mice were placed on glass slides with the photoreceptors up. Retinas were washed in PBS (0°C) for 15 minutes and stained in situ with 2 mM fluorescein-di-β-galactopyranoside (FDG; Molecular Probes) in an 8:1:1 mixture of water, ethanol, and DMSO for 2 minutes at 20°C. Retinas were rinsed in PBS and examined by fluorescence microscopy using the standard fluorescein-isothiocyanate filter set (excitation, 490 nm; emission, 525 nm).

For histologic examination, eyes were placed into OCT compound (VWR Scientific, Cerritos, CA), immediately frozen in liquid nitrogen, and sectioned. Sections were labeled with FDG as described above.

Immunocytochemistry

Retinal samples were prepared for immunocytochemistry by the technique of Hale and Matsumoto.[20] Briefly, posterior eyecups were fixed in 4% formaldehyde for 15 minutes and rinsed in PBS, and the retinas dissected from the eyecup and embedded in 5% agarose in PBS. Sections (100 μm) were prepared on a vibratome and incubated with a rabbit anti–β-galactosidase (1:50) antibody (5′Prime-3′Prime, Boulder, CO) for 12 hours. Sections were then rinsed in PBS (three times) and incubated with a secondary antibody conjugated to the Cy3 fluorochrome (1:2000 dilution, Sigma) for 2 hours. Sections were rinsed, mounted in anti-fading agent (Molecular Probes), and viewed by confocal laser microscopy.

All animals were handled according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Results

Screening of Transgenic Mice

Our group has previously characterized in vitro the potential *cis*-acting elements located in the proximal 5′ upstream region of the rod β-PDE gene that might confer photoreceptor specificity (Fig. 1A). Here, we have further investigated the functionality and specificity of the −297 to +53 fragment using transgenic mice. Three founder mice for the −297/+53 LacZ construct were identified by Southern blot analyses of tail DNA (Fig. 1B). These mice were bred with C57Bl/6J mice to generate three independent heterozygous lines. Copy number was estimated by comparing hybridization signals of probe (pLacF vector) to transgene and to serial dilutions of a known quantity of the LacF plasmid (not shown). Lines TgN1, TgN2, and TgN3 carried 10, 2, and 3 copies of the −297/+53 LacZ transgene, respectively.

β-Galactosidase Activity

Measurements of β-galactosidase activity in homogenates from retina and other tissues from transgenic mice were used to estimate the transcriptional efficiency of the β-PDE 5′ flanking region construct (Table 1). The highest β-galactosidase activity was observed in retina, and it correlated well with the number of copies of the −297/+53 LacZ transgene present in the tissue. Thus, retinas from TgN1 animals had more enzyme activity than those of TgN3, and these had in turn higher activity than retinas from the TgN2 line. Reporter gene activity was very low or undetectable in brain, heart, and muscle of all transgenic lines expressing the −297/+53 promoter construct. All other tissues, including the nonretinal ocular tissues, had very low β-galactosidase activity.

In situ Transgene Expression in the Retina

Retinal Whole Mounts. We used an in situ staining procedure for whole mounts of mouse retinal tissues to deter-

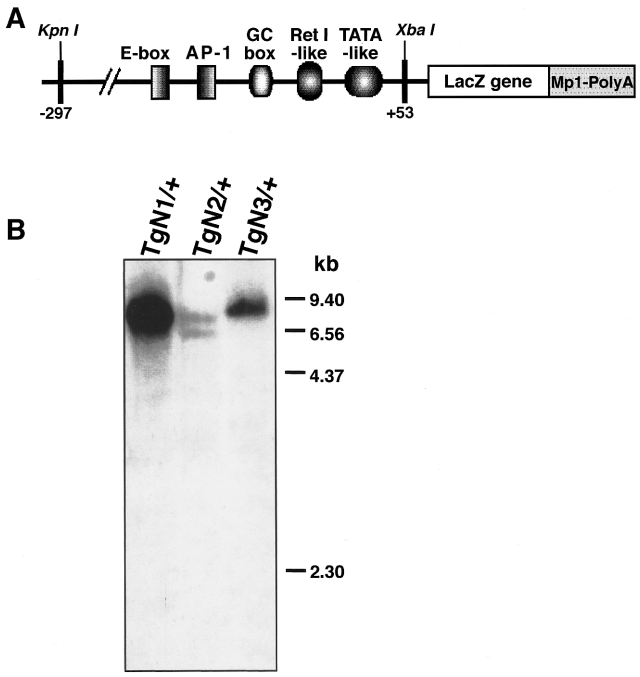

FIGURE 1. Diagram of the transgene construct and Southern blot analysis of transgenic mice. (**A**) A PCR fragment of the β-PDE promoter region (−297 to +53) was cloned immediately upstream of the LacZ gene. A polyadenylation signal from the mouse protamine gene (Mp1) was inserted at the 3′ end of the construct. The consensus *cis*-acting elements that might be conferring tissue-specificity are detailed. (**B**) Approximately 10 μg of *Hin*dIII-digested tail DNA of the different founder animals were loaded onto a 1% agarose gel. After electrophoresis, the DNA was transferred to a Hybond-N + membrane and hybridized to a radiolabeled LacZ probe.

mine whether the β-PDE 5′ flanking region fragment directed the LacZ gene to the photoreceptors (Fig. 2). LacZ-positive cells were identified by fluorescence microscopy using the fluorogenic β-galactosidase substrate, FDG. This method is several-fold more sensitive than standard X-Gal methods. Nonfluorescent FDG is hydrolyzed in the presence of β-galactosidase enzyme to produce UV-excitable fluorescein. Retinal whole mounts (visualized with transmitted light in Fig. 2A) exhibited a slight red background fluorescence in the absence of the FDG substrate (Fig. 2B). After the addition of FDG, slight green background fluorescence was observed in control retinas from nontransgenic mice (Fig. 2C). A highly fluorescent signal indicative of significant β-galactosidase activity was detected in whole mount retinas from −297/+53 LacZ transgenic mice (Fig. 2D).

Retinal Sections. Cell types expressing the LacZ reporter in the retina were examined in FDG-labeled cryosections (Fig. 3). Mice carrying the −297/+53 LacZ construct exhibited photoreceptor cell–specific LacZ expression with the same relative expression level as found in whole-mount preparations. A conventionally processed histologic cross section of a normal mouse retina is shown for orientation in Figure 3A. LacZ staining in −297TgN mice (Fig. 3B) was localized primarily in the outer segments (OS), inner segments (IS), and synaptic terminals of photoreceptors in the outer plexiform layer (OPL). A section of a nontransgenic (control) retina is shown for comparison of nonspecific background fluorescence (Fig. 3C). As can be seen, there is no green-yellowish or green fluorescence indicative of the presence of the bacterial β-galactosidase in this tissue.

Immunocytochemistry. Immunocytochemical studies using a polyclonal antibody against β-galactosidase that was conjugated to the Cy3 fluorochrome on transgenic mice retinas carrying the β-PDE 5′ flanking region −297/+53 (Fig. 4A) revealed the same pattern of β-galactosidase expression as that seen in the FDG-labeled cryosections. Labeling in photoreceptors was observed in the outer segments, inner segments, cell bodies (ONL), and synaptic terminals (OPL). Control animals did not show immunostaining, but the autofluorescence of their outer segments can be clearly observed. Also notice the difference in fluorescence intensity scale between the experimental and control panels (Fig. 4B).

DISCUSSION

The appropriate expression and function of cGMP phosphodiesterase in rod photoreceptors is a requirement for normal visual function. In the present study, we used the protein product of the LacZ reporter gene to examine the cell specificity and expression levels driven by the β-PDE promoter in vivo. A construct containing the −297 to +53 nt region of the β-PDE gene (Fig. 1A) integrated into the genome of transgenic mice directed expression of the reporter gene to the retinal photoreceptors. These results demonstrate a clear correlation with our previous results obtained in vitro.[13] Furthermore, they indicate that the elements necessary for photoreceptor-specific expression of the β-PDE gene in vivo seem to be contained within a 350-nt fragment (−297 to +53 nt) of the proximal 5′ flanking region of the human β-PDE gene.

Analysis of β-galactosidase activity in several tissues of the transgenic mice showed that β-PDE−driven expression is highest in the retina and minimal in other tissues (Table 1). These low levels of β-galactosidase activity may be indicative of "leaky" transcription, because it is possible that some expression of LacZ in nonretinal tissues may have occurred in cells

TABLE 1. Distribution of β-Galactosidase Activity in Different Tissues of Transgenic Mice

Tissue	β-Galactosidase Activity (nmol of ONPG Hydrolyzed/ min · mg Protein)
Brain	0.5 ± 0.02
Heart	ND
Kidney	4 ± 0.5
Liver	1.3 ± 0.2
Muscle	ND
Spleen	1.2 ± 0.16
Retina-less eye	2 ± 0.05
Retina	26 ± 3.6*
	17 ± 1.2†
	19.8 ± 1.0‡

β-Galactosidase activity was examined in brain, heart, kidney, liver, muscle, spleen, and nonretinal ocular tissues dissected from heterozygous −297 to +53 LacZ mice. Enzyme activity was normalized for total protein content and values are mean optical density ±SE of three independent samples, each measured in triplicate. ND, not detectable levels over background.

β-galactosidase levels found in * TgN1, in † TgN2, and in ‡ TgN3 transgenic animals.

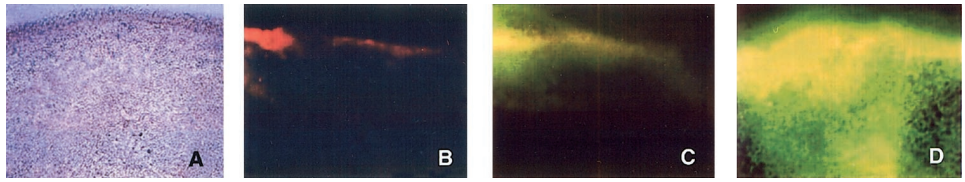

FIGURE 2. In situ reporter gene expression in whole retina. (**A**) Retinal whole mount visualized with transmitted light; (**B**) background fluorescence (no FDG staining); (**C**) wild-type retina stained with FDG; (**D**) −297LacZ-transgenic mouse retina after FDG staining. Magnification, ×200.

that do not normally express β-PDE. However, it is unlikely that the photoreceptor cell–specific expression pattern observed was caused by the integration site of the transgene because similar results were obtained with all the transgenic lines generated with different founder mice. In addition to measuring enzymatic activity in bulk pieces of mouse tissues, we have studied by an in situ histochemical reaction the localization of our reporter gene at the cellular level in the retina. A simple staining on an entire or sectioned retina provided a sensitive, rapid, and reliable method for analyzing cell-type-specific expression (Figs. 2, 3). This technique allowed us to obtain valuable histologic data consistent with the results observed after a conventional and laborious immunologic staining (Fig. 4). Our results demonstrated that the −297 to +53 fragment of the β-PDE gene efficiently directs expression of the reporter gene to the photoreceptors.

Several years ago, transgenic mouse studies aimed at introducing into *rd* mouse rod photoreceptors normal copies of the β-PDE gene (the *rd* mouse has a mutation in the β-PDE gene that causes retinal degeneration) used fragments of the 5′ flanking region of the rod opsin gene to drive its expression.[21] Although opsin promoter fragments had proven to be very effective in generating high rod-specific expression of transgenes, rescue of photoreceptors was never permanent, neither for the *rd* mouse nor for other animals affected with retinal degenerations resulting from different gene abnormalities.[22] A possible explanation for these results was that the regulatory elements present in the promoters of the specific genes to be replaced were not available on the rod-opsin promoter, and therefore, this promoter supported only transiently the expression of the therapeutic gene product. An alternative interpretation was that because the opsin promoter is a strong promoter, after some time it might have caused an accumulation of overexpressed transgene and an enhancement of the already occurring cell death. Viral-mediated gene transfer strategies have also used rod opsin promoters to direct expression of the β-PDE gene to the visual cells with only temporary rescue of the photoreceptors.[23,24] Because the results that we have presented in this article indicate that photoreceptor-specific gene expression can be achieved in vivo using the −297/+53 fragment of the human β-PDE promoter, it is likely that future

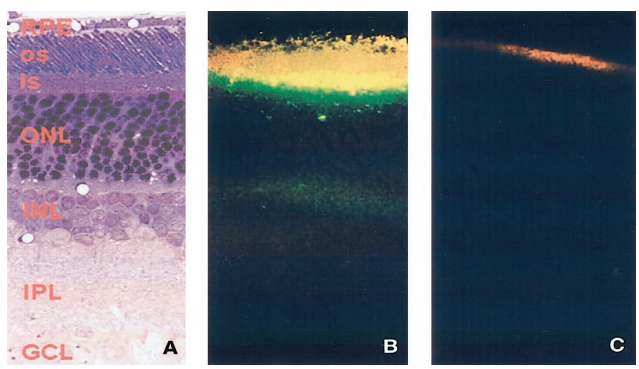

FIGURE 3. Localization of FDG staining in retina sections. Retinal cell types expressing β-galactosidase were identified in 8-μm cryosections labeled with 2 mM FDG. (**A**) Plastic embedded section of normal retina shown for reference; (**B**) −297LacZ-transgenic; (**C**) wild-type mouse retina. Sections were exposed to 5 seconds of UV illumination (excitation, 490 nm; emission, 525 nm) in a conventional fluorescence microscope. Final magnification, ×200.

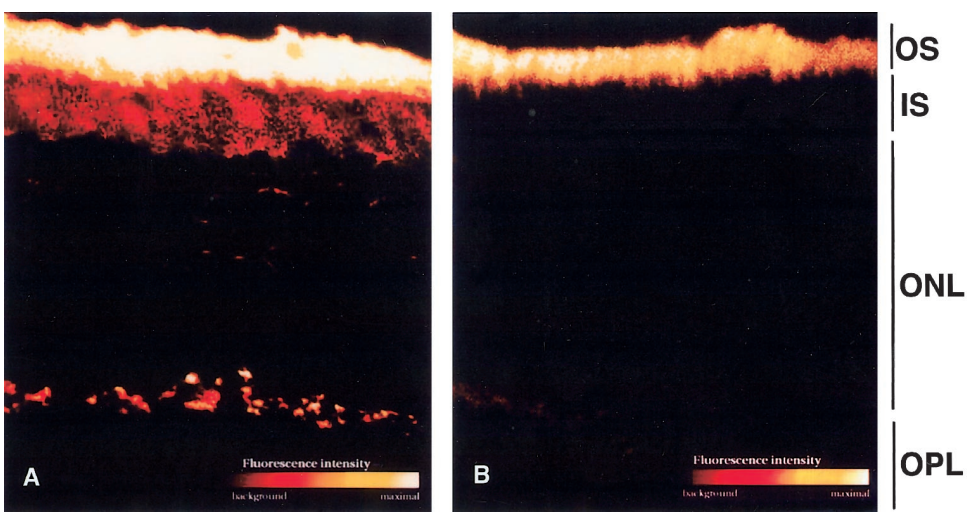

FIGURE 4. Immunolocalization of β-galactosidase in retinal sections. Confocal microscopy of retinal sections (100 μm) immunostained with rabbit anti–β-galactosidase and then with a secondary antibody conjugated to the Cy3 fluorochrome. LacZ expression is indicated by a signal with intensity increasing from *red* to *white* fluorescence. Reporter gene expression is significantly above background throughout the photoreceptor layer in the −297LacZ-transgenic mouse retina (**A**). Signal in photoreceptor outer segments contains background autofluorescence, as seen in the nontransgenic control retina (**B**).

applications requiring lower expression levels of transgenes in the visual cells may use promoters such as the one in the β-PDE gene. In fact, we have recently reported the delivery of this same fragment of the β-PDE 5′ flanking region fused to the β-PDE cDNA by means of subretinal injection of EAMs (encapsidated adenoviral minichromosomes) to the retina of 5-day-old *rd* mice.[25] In these experiments, we found that we could obtain prolonged β-PDE transgene expression and rescue of rod photoreceptor cells in the mutant animals.

In summary, our results suggest that the −297/+53 fragment of the β-PDE gene can be used to direct expression of therapeutic genes to the photoreceptors. Additional studies will help elucidate in more detail the specific elements responsible for the functional regulation of the β-PDE gene expression in vivo.

References

1. Stryer L. Visual excitation and recovery. *J Biol Chem.* 1991;266: 10711–10714.
2. McLaughlin ME, Sandberg MA, Berson EL, Dryja TP. Recessive mutations in the gene encoding the beta-subunit of rod phosphodiesterase in patients with retinitis pigmentosa. *Nat Genet.* 1993; 4:130–134.
3. Danciger M, Blaney J, Gao YQ, et al. Mutations in the PDE6B gene in autosomal recessive retinitis pigmentosa. *Genomics.* 1995;30: 1–7.
4. McLaughlin ME, Ehrhart TL, Berson EL, Dryja TP. Mutation spectrum of the gene encoding the beta subunit of rod phosphodiesterase among patients with autosomal recessive retinitis pigmentosa. *Proc Natl Acad Sci USA.* 1995;92:3249–3253.
5. Bayes M, Giordano M, Balcells S, et al. Homozygous tandem duplication within the gene encoding the beta-subunit of rod phosphodiesterase as a cause for autosomal recessive retinitis pigmentosa. *Hum Mutat.* 1995;5:228–234.
6. Danciger M, Heilbron V, Gao YQ, Zhao DY, Jacobson SG, Farber DB. A homozygous PDE6B mutation in a family with autosomal recessive retinitis pigmentosa. *Mol Vis.* 1996;2:10.
7. Gal A, Orth U, Baehr W, Schwinger E, Rosenberg T. Heterozygous missense mutation in the rod cGMP phosphodiesterase beta-subunit gene in autosomal dominant stationary night blindness. *Nat Genet.* 1994;7:64–68.
8. Bowes C, Li T, Danciger M, Baxter LC, Applebury ML, Farber DB. Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase. *Nature.* 1990;347: 677–680.
9. Pittler SJ, Baehr W. Identification of a nonsense mutation in the rod photoreceptor cGMP phosphodiesterase beta-subunit gene of the rd mouse. *Proc Natl Acad Sci USA.* 1991;88:8322–8326.
10. Farber DB, Danciger JS, Aguirre G. The beta subunit of cyclic GMP phosphodiesterase mRNA is deficient in canine rod-cone dysplasia 1. *Neuron.* 1992;9:349–356.
11. Suber ML, Pittler SJ, Qin N, et al. Irish setter dogs affected with rod/cone dysplasia contain a nonsense mutation in the rod cGMP phosphodiesterase beta-subunit gene. *Proc Natl Acad Sci USA.* 1993;90:3968–3972.
12. Di Polo A, Bowes C, Farber DB. Isolation and initial characterization of the 5′ flanking region of the human and murine cyclic guanosine monophosphate phosphodiesterase β-subunit genes. *Invest Ophthalmol Vis Sci.* 1996;37:551–560.
13. Di Polo A, Lerner LE, Farber DB. Transcriptional activation of the human rod cGMP-phosphodiesterase beta-subunit gene is mediated by an upstream AP-1 element. *Nucleic Acids Res.* 1997;25: 3863–3867.
14. Peschon JJ, Behringer RR, Brinster RL, et al. Spermatid-specific expression of protamine 1 in transgenic mice. *Proc Natl Acad Sci USA.* 1987;84:5316–5319.
15. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA.* 1977;74:5463–5467.
16. Hogan B, Costantini F, Lacy E. *Manipulating the Mouse Embryo: A Laboratory Manual.* Plainview, NY: Cold Spring Harbor Laboratory Press; 1986.
17. Feinberg AP, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem.* 1983;132:6–13.
18. Rosenthal N. Identification of regulatory elements of cloned genes with functional assays. *Methods Enzymol.* 1987;152:704–720.
19. Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal Biochem.* 1977;83: 346–356.
20. Hale IL, Matsumoto B. Resolution of subcellular detail in thick tissue sections: immunohistochemical preparation and fluorescence confocal microscopy. *Methods Cell Biol.* 1993;38:289–324.
21. Lem J, Flannery JG, Li T, Applebury ML, Farber DB, Simon MI. Retinal degeneration is rescued in transgenic rd mice by expression of the cGMP phosphodiesterase beta subunit. *Proc Natl Acad Sci USA.* 1992;89:4422–4426.
22. Travis GH, Groshan KR, Lloyd M, Bok D. Complete rescue of photoreceptor dysplasia and degeneration in transgenic retinal degeneration slow (*rds*) mice. *Neuron.* 1992;9:113–119.
23. Jomary C, Vincent KA, Grist J, Neal MJ, Jones SE. Rescue of photoreceptor function by AAV-mediated gene transfer in a mouse model of inherited retinal degeneration. *Gene Ther.* 1997;4:683–690.
24. Bennett J, Tanabe T, Sun D, Zeng Y, Kjeldbye H, Gouras P, Maguire AM. Photoreceptor cell rescue in retinal degeneration (*rd*) mice by in vivo gene therapy. *Nat Med.* 1996;2:649–654.
25. Kumar-Singh R, Farber DB. Encapsidated adenovirus mini-chromosome mediated delivery of genes to the retina: application to the rescue of photoreceptor degeneration. *Hum Mol Genet.* 1998;7: 1893–1900.