Selective Degradation of Nonsense
β-Phosphodiesterase mRNA in the Heterozygous
rd Mouse

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PURPOSE. To investigate the molecular mechanism relating phenotype and genotype in the rd mouse, mRNA and pre-mRNA levels derived from the wild-type and position-347 nonsense mutant β-phosphodiesterase (β-PDE) genes were determined and compared with the corresponding gene copy ratios.

METHODS. Total RNA and genomic DNA was isolated from the retinas of three heterozygous rd/+ mouse strains. For each, quantitative reverse transcription-polymerase chain reaction (RT-PCR) was used to determine the ratio of wild-type and rd β-phosphodiesterase pre-mRNA and mature mRNA. The gene copy ratio between wild-type and rd β-PDE was also determined by quantitative PCR.

RESULTS. The pre-mRNA ratio of wild-type versus nonsense mutant was close to 1:1, whereas the corresponding mRNA ratio was greater than 3:1, even though the gene copy ratio was confirmed to be 1:1.

CONCLUSIONS. The equivalence of pre-mRNA ratio level for wild-type and nonsense mutant in the rd/+ retina indicates that both genes were transcribed at similar levels. Thus, neither the nonsense mutation at position 347 nor the intron 1 retroviral insertion also present in the rd gene seem to have affected gene transcription. In contrast, the strain-independent bias favoring wild-type mature mRNA in vivo suggests a specific degradation of mutant transcript during or after pre-mRNA splicing. This allele-specific degradation serves to decrease mutant transcript levels dramatically in all rd strains, and suggests that photoreceptor cells have the capacity to reduce the level of an mRNA containing a nonsense mutation. (Invest Ophthalmol Vis Sci. 1998;39:2529–2536)

The rd mouse is a classic model for autosomal recessive retinal degeneration. The homozygous rd/rd mouse has early-onset rod inner segment defects followed by outer segment disorganization at postnatal day 8, with nearly complete rod loss by postnatal day 30.1,2 Although the heterozygous rd/+ mouse has no apparent anatomic abnormalities and does not undergo rod degeneration, the cyclic guanosine monophosphate (cGMP) level in the light-adapted rd/+ mouse retina is 40% lower than that in the normal mouse,3,4 and the electroretinogram shows an altered sensitivity to light stimulation.5

A nonsense mutation at codon 347 and a retroviral insertion (Xmv-28) in the first intron of the β-subunit of cGMP phosphodiesterase (β-PDE) are the causes for the rd mouse's retinal degeneration.6–9 cGMP PDE is a key player in photoreceptor signal transduction and consists of one α-subunit, one β-subunit, and two γ-subunits.10 When rhodopsin is excited by a photon, photoisomerized rhodopsin activates the G-protein transducin that binds the inhibitory γ-subunit of phosphodiesterase,11 showing the cGMP phosphodiesterase activity of the α/β-heterodimer. This serves to lower intracellular levels of cGMP, which causes the closure of cation-specific channels in the plasma membrane of rod outer segments, hyperpolarizes the plasma membrane, and produces a neural signal.12 The gene structure of β-PDE is complex, consisting of at least 21 introns and 22 exons encoding an 853-amino-acid protein with several functional domains:6–7 A six-amino-acid domain at the carboxyl terminus involved in membrane binding can be proteolyzed, methylated, or isoprenylated14,17; a region conserved among several mammalian species (amino acids 554–750) is important for catalytic activity16; two regions (amino acids 92–125 and 296–264) are cGMP binding domains17,18; and the N-terminus contains the γ-subunit-binding site. The nonsense mutation in codon 347 of the rd gene could produce in theory a truncated protein that retains the inhibitory γ-subunit-binding site. Thus far, however, the predicted truncated protein has not been detected,19 and the mechanism relating the phenotype to the genotype remains unclear.

Wild-type and rd β-subunit mRNA levels have been studied in normal, rd/+, and rd/rd mice.20,21 Although β-subunit transcripts are reduced in the rd/+ mouse, the key question of whether this is because of a specific elimination of the rd transcript or a nonspecific reduction of wild-type and rd
mRNAs remains unanswered. Transcripts of both alleles must be simultaneously and specifically detected for a conclusion to be drawn regarding mutant-specific mRNA elimination. For this reason, we used a reverse transcription–polymerase chain reaction (RT-PCR) approach with intron- or exon-specific primers coupled with an allele-specific pair of restriction enzyme cleavages of the PCR products. We found a strain-independent, posttranscriptional, mutant-specific mRNA reduction.

**MATERIALS AND METHODS**

**Animals and Retinal Nucleic Acid Isolation**

Three strains of 4-week-old female heterozygous rd mice (B6C3 × C3H fl, B6CBA/J fl, and C57L/J fl) were purchased from the Jackson Laboratory (Bar Harbor, ME). Until death, animals were kept in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. After mice were killed by CO₂ inhalation, two to six retinas from each strain were dissected and pooled. Total RNA and genomic DNA were purified by extraction (Trizol; Gibco, Gaithersburg, MD), according to the manufacturer’s instructions. Total RNA and genomic DNA were then extracted with phenol-chloroform ethanol, precipitated with 0.3 M sodium acetate (pH 5.3), and resuspended in RNase-free H₂O. The concentration of total RNA and genomic DNA was determined spectrophotometrically and the integrity of total RNA verified by electrophoresis on formamide–formaldehydeagarose (1%) gels.

**RNA and DNA Analyses**

All primers used for RT-PCR reactions were synthesized by the University of Florida’s DNA Synthesis Core (Fig. 1). Oligo WH741 (5’ GGCAGGGAAATGGTCCTCTAC 3’) is a sense primer containing exon 5 and 6 sequences and corresponding to β-PDE cDNA nucleotides 921 to 943; Oligo WH742 (5’ CCCAGGAACGTGTGGCAGAGA 3’) is an antisense primer con-
taining exon 9 and 10 sequences and corresponding to β-PDE cDNA nucleotides 1258 to 1279; Oligo WH770 (5' TCTCTTC-GACCTCTGTTCTTTTCCC 3') is a sense primer within β-PDE intron 9 and Oligo WH769 (5' GGAGCTTCAGGTGGCAAATGGA 3') is an antisense primer within β-PDE intron g. To detect β-PDE mRNA, a 20-μl reaction was set up using 1 μg total retinal RNA from each mouse strain, 25 picomoles primer WH742, first-strand buffer (Superscript; Gibco), 1 mM dithiothreitol (DTT), and 0.5 mM deoxyribonucleoside triphosphates. The reaction mix was denatured at 80°C for 2 minutes and then annealed at 56°C for 20 minutes. Two hundred units reverse transcriptase (Superscript II; Gibco), 1 mM dithiothreitol (DTT), and 0.5 mM deoxyribonucleoside triphosphates. The reaction mix was denatured at 94°C for 5 minutes and then run for 30 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. After the reaction, the PCR product was denatured at 94°C for 10 minutes and slowly cooled to room temperature for 3 hours. The PCR products were purified using a kit (Wizard; Promega, Madison, WI), according to the manufacturer's protocol; ethanol precipitated; and resuspended in 20 μl H2O.

To detect the β-PDE pre-mRNA, 1 μg total retinal RNA was incubated with 2 units RNase-free DNase (Ambion, Austin, TX) and 1 unit RNase inhibitor in first-strand buffer (Superscript; Gibco) at 37°C for 15 minutes. The DNase was deactivated at 94°C for 5 minutes, and the DNase-treated total RNA then used for RT-PCR as described.

To detect the gene copy ratio between wild-type and mutant β-PDE, 1 μg genomic retinal DNA was used in a 50-μl PCR with 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 10 picomoles WH741, 10 picomoles WH742, 2 mM MgCl2, 0.2 mM deoxyribonucleoside triphosphates, and 5 μCi α-32P deoxythymidine triphosphate (3000 Ci/mmol; NEN-DuPont, Boston, MA). The PCR mix was denatured at 94°C for 4 minutes and then run for 30 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. After the reaction, the PCR product was denatured at 94°C for 10 minutes and slowly cooled to room temperature for 3 hours. The PCR products were purified using a kit (Wizard; Promega, Madison, WI), according to the manufacturer's protocol; ethanol precipitated; and resuspended in 20 μl H2O.

After each PCR reaction, 5 μl of 20 μl was used for restriction enzyme digestion. PCR products were digested with 20 units BsaAI (New England Biolabs, Beverly, MA) in 100 mM NaCl, 50 mM Tris HCl, 10 mM MgCl2, pH 7.9, buffer 3 at 37°C overnight, then ethanol precipitated at −70°C with 0.1 volume 3 M sodium acetate (pH 5.3) and 2.5 volume absolute ethanol for 30 minutes using 1 μl glycerol (5 mg/ml) as a carrier. The precipitate was centrifuged at 14,000 g for 15 minutes, washed with 1 ml 70% ethanol, and resuspended in 10 μl H2O. For Ddel digestion, 5 μl of each PCR product was incubated with 20 units Ddel (Gibco) in restriction buffer (BRL buffer 3; Gibco) at 37°C overnight and ethanol precipitated, as for BsaAI digestion. For each digested and undigested product, 4 μl of the reaction was mixed with 1 μl of fivefold concentrated agarose dye (50% sucrose, 50 mM EDTA, 0.2% bromphenol blue, and 0.2% xylene cyanol) and run on an 8% nondenaturing polyacrylamide gel at 600 volts for 3 hours. The digestion product bands were quantified by commercial software (ImageQuant ver. 3.2) using a phosphorimager (PhosphorImager; Molecular Dynamics, Sunnyvale, CA). The phosphorimager has a linear range of between five and six orders of magnitude, determined by scanning a serially diluted sample with a known 32P activity (data not shown). All bands analyzed were within this experimentally determined range.

**RESULTS**

In the rd/rd mouse there is approximately 33% mutant β-PDE mRNA left at postnatal day 10, whereas in the heterozygote rd/+ animal approximately 66% of the normal message level is present at this time.21 However, in the rd/+ retina, the fraction of β-PDE mRNA that is mutant versus wild-type has not been clearly defined. To determine whether wild-type and mutant mRNA levels were equivalent, we analyzed a series of rd/+ mice in which both transcripts were simultaneously and specifically measured. The strategies for using RT-PCR and PCR to determine the ratio between the wild-type and mutant mRNAs, pre-mRNAs, and gene copies are shown schematically in Figure 1. Briefly, the rd nonsense point mutation in codon 347 creates a Ddel restriction site23 and removes a BsaAI site from the wild-type sequence. Digesting mRNA RT-PCR products with BsaAI or Ddel therefore yields two diagnostic fragments of 120 bp and 239 bp and the uncut PCR product of 359 bp (Fig. 1, left column). PCR of genomic DNA or RT-PCR of pre-mRNA (Fig. 1, middle and right columns) uses an intron primer pair to distinguish them from mRNA products. These products contain a second Ddel site common to wild-type and mutant products and the mutation-generated polymorphic Ddel-BsaAI site. Ddel digestion detects mutant DNA strands and yields four fragments, two diagnostic fragments of 110 bp and 72 bp and the common fragments of 182 bp and 30 bp. BsaAI digestion detects wild-type products and yields two diagnostic fragments of 140 bp and 72 bp in addition to the uncut 212-bp fragment.

To produce uniform final conditions for each restriction digestion, after the last PCR cycle, the DNA products were denatured and renatured. This yielded a fraction of PCR products forming heteroduplexes between one wild-type DNA strand and one mutant DNA strand, creating a single-base mismatch at nucleotide 1047. These heteroduplexes cannot be cleaved by BsaAI or Ddel. However, they can be accounted for by adopting the simple approach of Apostolakos et al.22 on the basis that the final renaturing step randomly associates wild-type (W) and mutant (M) strands giving duplex wild-type (WW), duplex mutant (MM), and heteroduplex (WM) forms. Because only WW products will be cut by BsaAI and only MM products will be cut by Ddel, the fractions of WW (fWW), and MM (fMM) are experimentally determined. The values of interest, fW and fM, can be then directly calculated, because fWW = fW (fW is the fraction of wild-type single-strand product) and fMM = fM (fM is the fraction of mutant single-strand product). For example, if fW is 0.8 and fM is 0.2, the expected fraction of input DNA that is digestible by BsaAI is fWW = fW = 0.64, and that digestible by Ddel is fMM = fM = 0.04. The remaining fraction (0.32) is heteroduplex, undigestible by either enzyme. If it is found experimentally that fW + fM = 1, then both restriction digestions are complete, thus eliminating partial restriction enzyme reaction as an experimental variable.

This approach is essentially a competitive PCR23,24 between wild-type and mutant templates. It is therefore impor-
tween 15 and 20 cycles, the wild-type transcript amount was progressed sufficiently to quantitate PCR products accurately or because the effect of background signal was too high for an accurate ratio to be determined. The linear range of this RT-PCR reaction was therefore from 15 to 40 cycles, and we chose 30 cycles as our standard condition in all experiments. In this initial experiment, we found that the \( \text{rd} \) mutant mRNA bearing the nonsense mutation was significantly underrepresented compared with the wild-type. As a fraction of the total \( \beta \)-PDE transcript in the heterozygous \( \text{rd}/+ \) mouse retina, approximately 24% was derived from the mutant gene, and the remaining 76% was the wild-type sequence.

**\( \beta \)-Phosphodiesterase mRNA \( \text{rd}/+ \) Ratio in Three Strains of Heterozygous \( \text{rd}/+ \) Mice**

To ensure that this unequal ratio between the wild-type and mutant mRNAs was not because of the genetic background of the strain of the mouse tested (i.e., a non-\( \beta \)-PDE gene effect), the same RT-PCR was performed in triplicate using total RNA from three heterozygous \( \text{rd}/+ \) mouse strains as templates (Fig. 3). These three strains have different genetic backgrounds for one or both of the wild-type and the \( \text{rd} \) alleles. Using the relative \( ^{32} \text{P} \) counts in each band and normalizing for the cytidine content of each fragment, the fractions of mutant and wild-type mRNAs were calculated according to the method described. The 359-bp bands in Figure 3 have been overexposed to make the 239-bp and 120-bp bands visible in all lanes. We found that the unequal mRNA ratio was maintained in all three strains (Table 1). In each, the ratio of wild-type to mutant was 3:1 to 4:1. Therefore, this seemed to be a general phenomenon intrinsic to mutant \( \beta \)-PDE expression and metabolism in the heterozygous \( \text{rd}/+ \) mouse, not an effect of the genetic background of the mouse strain carrying the mutant allele.

**\( \beta \)-Phosphodiesterase \( \text{rd}/+ \) Gene Copy Ratio in Three Strains of Heterozygous \( \text{rd}/+ \) Mice**

Even though we used the total RNA of heterozygous \( \text{rd}/+ \) mouse retinas as RT-PCR templates, it was worth formally testing whether the unequal gene expression ratio was caused by unequal gene copy numbers between the wild-type and mutant \( \beta \)-PDE genes. A pair of intron-specific primers flanking exon 7 were designed, and \( \text{rd}/+ \) retinal genomic DNA was used as the PCR template. The PCR products were then digested with \( \text{BsaAI} \) or \( \text{Ddel} \) (Fig. 4) to distinguish \( \text{rd} \) from wild-type gene sequences. In all three strains, wild-type gene sequences comprised approximately 50% of the total PCR products determined by \( \text{BsaAI} \) or \( \text{Ddel} \) digestion (Table 1). The sum of the fraction of wild-type (\( f_w \)), determined as described, from \( \text{BsaAI} \) digestion and the fraction of mutant (\( f_m \)) from \( \text{Ddel} \) digestion was always close to 1.0. Therefore, both digestions were complete and, because the same digestion conditions were used in the RT-PCR assay for mRNAs, our estimates of mRNA fraction are not compromised by incomplete restriction enzyme digestions. We conclude that the gene copy number of

![Figure 2](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933204/)

![Figure 3](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933204/)

![Figure 4](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933204/)
TABLE 1. Amounts of Wild-Type and Mutant rd β-PDE Genomic DNA, mRNA, and pre-mRNA in the rd/+ Mouse Retina

<table>
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<tr>
<th>rd/+ strains</th>
<th>B6C3 × C3H fl</th>
<th>B6CBA fl</th>
<th>CSJL/J fl</th>
<th>B6C3 × C3H fl</th>
<th>B6CBA fl</th>
<th>CSJL/J fl</th>
<th>B6C3 × C3H fl</th>
<th>B6CBA fl</th>
<th>CSJL/J fl</th>
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</thead>
<tbody>
<tr>
<td>BsaAI cleavage (%)</td>
<td>22.1 ± 3.2</td>
<td>22.2 ± 1.4</td>
<td>22.0 ± 1.0</td>
<td>24.4 ± 3.6</td>
<td>33.5 ± 5.3</td>
<td>25.5 ± 4.6</td>
<td>58.1 ± 0.4</td>
<td>61.2 ± 1.1</td>
<td>62.9 ± 1.9</td>
</tr>
<tr>
<td>Wild-type amount (%)</td>
<td>45.9 ± 3.5</td>
<td>47.2 ± 1.4</td>
<td>47.0 ± 1.0</td>
<td>49.3 ± 3.8</td>
<td>57.7 ± 4.9</td>
<td>50.3 ± 4.7</td>
<td>76.2 ± 0.3</td>
<td>78.2 ± 0.7</td>
<td>79.3 ± 1.2</td>
</tr>
<tr>
<td>Wild-type-to-mutant ratio</td>
<td>0.87 ± 0.20</td>
<td>0.90 ± 0.07</td>
<td>0.89 ± 0.03</td>
<td>0.98 ± 0.21</td>
<td>1.40 ± 0.39</td>
<td>1.03 ± 0.27</td>
<td>3.2 ± 0.10</td>
<td>3.59 ± 0.21</td>
<td>3.85 ± 0.40</td>
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DISCUSSION

In this study we tested the hypothesis that the specific degradation of nonsense mRNA can protect the heterozygous carrier of the rd gene. The ratios of wild-type to rd mutant for gene copy number, pre-mRNA, and mRNA averaged over three strains of rd/+ mice were 0.90 ± 0.10, 1.14 ± 0.29, and 3.55 ± 0.24, respectively. The unequal mRNA ratio between the wild-type and mutant gene could have been caused by unequal transcription or by specific factors affecting the stability of the mRNA.

FIGURE 5. Pre-mRNA levels of wild-type and mutant β-phosphodiesterase in three strains of rd/+ mouse. (A) B6C3 × C3H fl rd/+ strain. Lane M: 100-bp marker. The sizes of the markers are indicated on the left side of the gel (refer to Fig. 1). (A) B6C3 × C3H fl rd/+ strain. Lane 1: polymerase chain reaction (PCR) products digested with BsaAI. Lane 2: RT-PCR products digested with DdeI. Lane 3: uncut PCR products. (B) B6CBA/J fl rd/+ strain. The lane arrangement is the same as (A). (C) CSJL/J fl rd/+ strain. The lane arrangement is the same as (A).

FIGURE 4. Levels of genomic wild-type and mutant β-phosphodiesterase gene copies in three rd/+ strains. Lane M: 100-bp marker. The sizes of the markers are indicated on the left side of the gel. (A) B6C3 × C3H fl rd/+ strain. Lane 1: polymerase chain reaction (PCR) products digested with BsaAI. Lane 2: RT-PCR products digested with DdeI. Lane 3: uncut PCR products. (B) B6CBA/J fl rd/+ strain. The lane arrangement is the same as (A). (C) CSJL/J fl rd/+ strain. The lane arrangement is the same as (A).
degradation of nonsense rd transcript. Because pre-mRNA levels were equivalent, transcription from the wild-type and mutant β-PDE alleles must have been the same. This suggests that the retroviral insertion in the first intron did not affect transcription of the mutant β-PDE gene and that a posttranscriptional process caused loss of the rd transcript. Although specific degradation of nonsense mRNA is well documented in some eukaryotic cells (see later), we cannot exclude the possibility that the retroviral insertion interfered with β-PDE pre-RNA splicing and caused degradation independent of any nonsense-mediated process.

Studies, primarily on the yeast Saccharomyces cerevisiae, have shown that transcripts with nonsense mutations can be specifically recognized by a translation-linked RNA degradation system. On translation initiation, the ribosomes scan the mRNA and initiate transcript degradation when a nonsense mutation is encountered. Although the exact triggering mechanism remains to be resolved, nonsense-mediated degradation depends on cis-mRNA elements and occurs independently of the normal 3' polyA shortening mechanism causing turnover of full-length mRNA. In both processes, however, removal of the 5' cap structure is rate limiting. The exonuclease Xrn1 then degrades the de-capped mRNA in the 5' → 3' direction. Although a precisely rate limiting. The exonuclease Xrn1 then degrades the de-capped mRNA in the 5' → 3' direction. Although a precisely rate limiting. The exonuclease Xrn1 then degrades the de-capped mRNA in the 5' → 3' direction. Although a precisely rate limiting. The exonuclease Xrn1 then degrades the de-capped mRNA in the 5' → 3' direction. Although a precisely rate limiting. The exonuclease Xrn1 then degrades the de-capped mRNA in the 5' → 3' direction.

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Nonsense mutations cause nucleus-associated degradation of transcripts after splicing in several mammalian cell types, and this degradation is also related to translation initiation. More limited evidence also exists for a protein synthesis-independent downregulation of nonsense message by an RNA-sparing inhibition mechanism.

The best studied examples of nonsense-mediated mRNA degradation in mammals are for some forms of human β-thalassemia, an autosomal recessive disease of the red blood cells. Nonsense or frameshift mutations that cause the premature termination of translation in exon 1 or exon 2 of the β-globin gene are responsible for approximately half of the defect causing the disease. It has been shown that specific degradation of the mutant mRNA dramatically decreases the mutant β-globin transcript level and that this may well protect the heterozygous carrier. Therefore, it seems that degradation of nonsense mRNA is important in this protection. In some cases of β-thalassemia, if the nonsense mutant and wild-type mRNA levels are equivalent in the heterozygote, people will be symptomatic, formally exhibiting dominant disease. We have shown a similar specific mRNA degradation for a nonsense β-PDE allele that may have an analogous protective effect for the rd/+ heterozygous mouse.

A variety of mutations are known to be responsible for degenerative retinal diseases such as retinitis pigmentosa. Changes in the rod opsin coding region are a major cause, accounting for approximately one fourth of cases of RP, and nonsense mutations are an important subclass of such mutations. Some mutant alleles such as E247Ter, a glutamic acid 247 to stop codon change, cause an autosomal recessive phenotype, whereas others, such as Q344Ter, a glutamine 344 to stop codon change, cause the autosomal dominant RP. Why one nonsense mutation causes dominant disease and another causes recessive disease remains unclear. One possibility is that the nonsense gene protein product may interfere with the wild-type protein, either by producing a dominant negative truncated protein or by interfering at the RNA level with mRNA metabolism. In these cases, nonsense codon-induced mRNA degradation pathways could play an important role in determining retinal phenotype. It has been shown that the degradation efficiency of a nonsense mRNA will increase if the mutation is close to the 5' end of the message or to a cis-element for degradation. This should lead to little or no phenotype in the heterozygous carrier and therefore to a "recessive" designation. This may explain why the E247Ter mutation is autosomal recessive. However, if degradation of the nonsense mRNA is inefficient, the mutant transcript level would remain sufficiently high to cause dominant disease, such as in the Q344Ter mutation.

The rd β-PDE mutation fits this hypothesis well. Because the point mutation is at codon 347, located at the site only 40% of the way through the coding sequence, mRNA degradation in the retinal photoreceptor may be efficient. In fact, depending on the specific kinetics of formation and decay of β-PDE mRNA, a small increase in nonsense-specific mRNA degradation could easily produce the observed 3:1 to 4:1 ratio. The remaining mutant mRNA may not be sufficient to cause disease in the heterozygous rd/+ mouse, even though it may be capable of interfering with mRNA metabolism or reducing PDE activity. This effect of a truncated mRNA may also explain the enigmatic results of attempts to rescue rd/rd retinal degeneration by creating a transgenic rd/rd mouse with an extra copy of β-PDE cDNA. In that experiment, a wild-type copy of the β-PDE gene driven by a 4.4-kb mouse opsin promoter was added to the rd/rd mouse. This mouse line showed rescued of photoreceptors for 3 months. However, photoreceptor degeneration ensued, and at 4 months, most of the photoreceptors had been lost. Introduction of a wild-type copy of the β-PDE gene by viral-mediated gene delivery also fails to rescue photoreceptors permanently. There are several possible reasons for these results. One is that the rod opsin promoter may not be able to support long-term expression outside its normal chromosomal context. However, transgene expression for a prolonged period (>20 months) has been recently observed (Weiming Yan, John Flannery, and William Hauswirth, unpublished observations, April 1998) using even a relatively small rod opsin promoter. This suggests that at least in some cases, "promoter turnover" may not occur. Another possibility is that even though the rd/+ heterozygote has no apparent retinal phenotype, the rd gene may not be phenotypically neutral. Because there are two copies of the mutant β-PDE gene in the rd/rd/+ transgenic mouse, the level of mutant transcript is likely to be higher than that in the heterozygous rd/+ mouse. This could interfere with correct function of the wild-type transgene product, manifested as slow retinal degeneration in the rd/rd/+ transgenic animal, thus explaining the absence of permanent photoreceptor rescue.

We have shown that a β-PDE nonsense mRNA is specifically degraded in heterozygous rd/+ mouse retinas. Targeted message degradation, a general mechanism for protecting heterozygous carriers from nonsense mutations, occurs in photoreceptors, suggesting that nonsense mutants may effect the photoreceptor phenotype only when expressed at levels equivalent to the wild-type. Such degradation of nonsense mutant mRNA, if sufficiently general, may be an important factor in
Acknowledgment

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