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# STATIONARY NIGHT BLINDNESS OR PROGRESSIVE RETINAL DEGENERATION IN MICE CARRYING DIFFERENT ALLELES OF PDE GAMMA

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## 1. ABSTRACT

A challenge in genetics is to understand the molecular basis of genetic and allelic heterogeneity. Divergent phenotypes caused by different variants of the same gene determine allelic heterogeneity. In the past few years, we have been studying an allelic series of mutations in the gamma-subunit of the cGMP phosphodiesterase gene *(Pdeg)* that resulted in visual defects ranging from stationary night blindness to progressive retinal degeneration. Here we describe the morphology and physiology of the retina in mice carrying four different *Pdeg* alleles: *Pdeg*$^{tm}$, Del 7C, Y84G, and W70A and the effect that these mutations of PDE gamma have on components of the activation and deactivation phases of phototransduction.

## 2. INTRODUCTION

For several years, we have been interested in understanding the involvement of the inhibitory gamma-subunits of rod-specific cGMP-phosphodiesterase (PDE) in the regulation of different activation and deactivation steps in phototransduction. Initially, we cloned PDE gamma cDNA from mouse retina (1) and mapped its gene (*Pdeg*) to mouse chromosome 11 (2). We then isolated and characterized the human PDE gamma cDNA and mapped it to human chromosome 17q 21.1 (3). PDE gamma is encoded by three exons that correspond to amino acids 1-49 (exon 2), 50-62 (exon 3) and 64-87 (exon 4). Interestingly, the amino acid sequences of the PDE gamma from mouse, bovine and human differ by only two amino acids.

### 2.1. PDE gamma and the phototransduction cascade

Neurotransmitters and hormones signal their target cells through G-protein-coupled receptors. Light acts similarly in the retinal photoreceptor cells and starts a cascade of events that results in hyperpolarization of the photoreceptors' plasma membrane leading to cell signaling. Figure 1 depicts the main molecular components of this phototransduction process and the sequence of activation/deactivation reactions in which they are involved. The first step in the visual response to a single photon begins with the activation of rhodopsin (R R*) (4-6), which causes transducin [a heterotrimeric G-protein (T alpha-beta-gamma)] to exchange its bound guanosine diphosphate (GDP) for guanosine triphosphate (GTP) (7). Activated transducin molecules, T alpha* - GTP, then dissociate from T beta-gamma molecules and bind to the two inhibitory gamma-subunits of PDE (a heterotetrameric enzyme, PDE alpha-beta-gamma-gamma), thereby relieving the inhibition that they exert on the catalytic PDE alpha- and beta-subunits (7-11). Activated PDE (PDE*) is required for the final step in phototransduction: PDE alpha-beta hydrolyzes cGMP and the reduction in cGMP levels causes the closure of cGMP-gated channels on the rod outer segment plasma membrane (4, 5, 12). This prevents the entry of $Na^+$ and $Ca^{2+}$ into the rod outer segment and hyperpolarizes the entire cell. Since $Ca^{2+}$ continues to go out the outer segment through the $Na^+/K^+/Ca^{2+}$ exchangers (also located on the plasma membrane), its concentration at the end of the activation phase of phototransduction has decreased considerably.

The termination of the photoresponse requires inactivation of the photoexcited rhodopsin and transducin molecules, as well as inhibition by PDE gamma of the PDE alpha-beta catalytic core; it also involves the activation of guanylate cyclases, RetGC-1 and RetGC-2, (by the

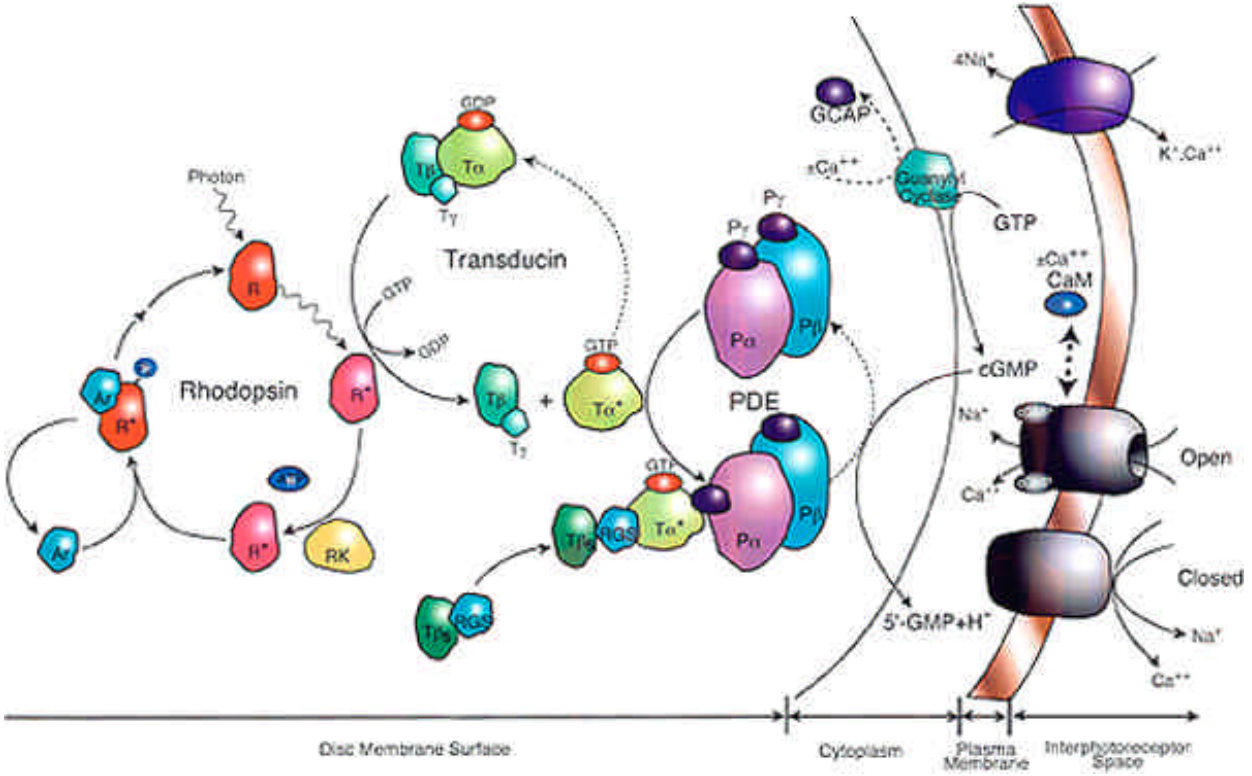


**Figure 1.** Diagram of the biochemical cascade of events involved in the activation and inactivation of rod-specific cGMP-PDE. [Modified from Farber, 1995, (51)].

reduction in $Ca^{2+}$ levels) for the synthesis of cGMP (13). Photoactivated rhodopsin is inactivated with the phosphorylation of serine and threonine residues at its carboxyl tail (R-Pi) by a specific rhodopsin kinase (RK, Figure 1) (14). Phosphorylated rhodopsin binds arrestin (AR, also known as S-antigen) more efficiently than T αlpha (15). Arrestin inhibits the removal of phosphates from rhodopsin by phosphoprotein phosphatase 2A (PPP2A). [It has been shown that mouse lines lacking rhodopsin kinase (16) or arrestin (17) have a delay in the deactivation of the photoresponse. This is the result of an increase in the amount of active (dephosphorylated) rhodopsin available to activate T alpha]. Inactivation of transducin is mediated by its own GTPase activity, which hydrolyzes T alpha* - GTP to T alpha - GDP. The latter then reassociates with T beta-gamma. Separation of T alpha - GTP from PDE gamma facilitates the inactivation of PDE* by allowing PDE gamma to re-inhibit the catalytic PDE alpha-beta (10).

The regions of PDE gamma required for its various functions have been previously studied in reconstituted systems. PDE gamma contains a central lysine-rich region, spanning Arg24 to Gly45, in which 10 of 13 amino acids are basic. These residues have one site for interaction with T alpha (18) and are essential for binding to the PDE catalytic core (19). T alpha binding site is found in Trp70-Leu76. The region involved in inhibiting PDE alpha-beta catalytic activity is thought to lie at the PDE gamma carboxyl terminus, including Gly85-Ile86-Ile87, and it is different from the region required for binding (18). This would explain why, in experiments reported in the literature, a C-terminus-deleted PDE gamma, and also a Y84G mutant PDE gamma, did not inhibit PDE activity *in vitro* but did bind to the PDE alpha-beta core (20). Similarly, other deletions and point mutations in the carboxyl-terminus did not affect the binding of PDE gamma to PDE alpha-beta, but decreased inhibition of PDE activity (21, 22). Further, peptides corresponding to the carboxyl-terminus inhibited trypsin-activated PDE (23).

### 2.2. PDE gamma and light adaptation

Light adaptation desensitizes a photoreceptor by increasing the amount of light needed to generate a photoresponse. This process is especially prominent in cones but is also exhibited by rods (24). The light-induced drop in $Ca^{2+}$ influx that occurs at the end of the activation phase in phototransduction leads to adaptation of the photoreceptor to constant light (25). In the dark, intracellular $Ca^{2+}$ inhibits the guanylate cyclases Ret GC-1 and Ret GC-2 (13), but when $Ca^{2+}$ levels are reduced, these two enzymes are stimulated to form more cGMP, thus counteracting the reduction in cGMP caused by PDE alpha-beta. This stimulation is mediated by guanylate cyclase-activating proteins GCAP-1 and GCAP-2 that possess $Ca^{2+}$-binding sites (26). $Ca^{2+}$ also appears to influence the half-life of photoexcited rhodopsin (25) as well as its catalytic action. The rate of rhodopsin phosphorylation is

inversely proportional to $Ca^{2+}$ concentration; this $Ca^{2+}$ sensitivity of rhodopsin kinase phosphorylation is mediated by recoverin [Figure 1, (27)]. Prolonged light not only causes a decrease in cytoplasmic $Ca^{2+}$ concentration, but also a cessation in recoverin inhibition of rhodopsin kinase. Another function of $Ca^{2+}$ is to regulate the cGMP-gated channels that are essential for maintaining the influx of $Na^{+}$ and $Ca^{2+}$ into the photoreceptors. These channels are composed of three functional alpha-subunits and a structurally similar beta-subunit. At high levels of $Ca^{2+}$, the beta-subunit binds to calmodulin, which then increases the channel affinity for cGMP (28).

PDE gamma plays a role in termination of the photoresponse by providing another mechanism of negative feedback. There are two sites in the phototransduction cascade where PDE gamma has been suggested to influence adaptation: one is in the regulation of the photoexcited state of transducin and the other is in the catalytic action of PDE. In the former, PDE gamma appears to influence the intrinsic GTPase activity of T alpha. As mentioned, during the recovery of the photoresponse, T alpha is deactivated by hydrolysis of its bound GTP. A long-standing puzzle that has not been completely solved as yet rotates around the fact that GTP hydrolysis by transducin, *in vitro*, is much slower than the recovery of the photoresponse. This suggests that one or more GTPase activators are responsible for the rapid deactivation of transducin, *in vivo.* Initially, the effector itself, PDE gamma, was proposed to be the activator since the GTPase activity of transducin was found to be accelerated by the addition of PDE gamma to reconstituted photoreceptor membranes (29, 30). However, it soon became clear that this effect of PDE gamma required the presence of another membrane-associated photoreceptor protein. This protein has recently been identified as a member of the RGS family, RGS9 [Regulator of G-protein Signaling 9, (31)]. Other than in the retina, RGS9 is also expressed in the caudoputamen, nucleus accumbens, olfactory tubercle, and medial hypothalamus (32). Interestingly, a fragment of RGS9 containing the RGS homology domain, as well as other RGS proteins, substantially accelerates the GTPase activity of transducin in the absence of PDE gamma, but in its presence the catalytic action of RGS9 is further enhanced (31). Rods from mice lacking RGS9 hydrolyze GTP more slowly than those from wild-type mice, and their flash responses turn on normally but recover more slowly **(**33**)**. In addition, it has been found that, *in vivo*, RGS9 is always associated with another G-protein beta-subunit, G-beta5. This complex is an essential determinant of T alpha GTPase activity (34, 35).

The second site in the activation phase of the phototransduction cascade in which PDE gamma could influence light adaptation is at the level of PDE. PDE gamma complexed with transducin-alpha-GTP dissociates from the catalytic PDE subunits and, after GTP hydrolysis, becomes phosphorylated. In the frog, phosphorylation of PDE gamma has been shown to occur at threonine 22 (by frog PDE gamma kinase, also known as CKD5) as well as at threonine 35 [by protein kinase C, PKC (36) and protein kinase A, PKA (37)]. It seems that PDE gamma ribosylation at arginine 33 and arginine 36 by the endogenous ADP-ribosyltransferase of rod outer segment membranes may be required for the phosphorylation of threonine 35 (38). Once PDE gamma is released from transducin alpha-GTP it again binds the PDE catalytic subunits. Free PDE gamma is a better substrate than PDE alpha/beta/$(gamma)_2$ for the kinase (36); in other words, the phosphorylatable sites appear to be less accessible to the enzyme when PDE gamma is attached to PDE alpha/beta. The kinase phosphorylates transducin-bound PDE gamma or free PDE gamma (about 10% of the total PDE gamma pool) rather than PDE alpha/beta/$(gamma)_2$. Since the phosphorylation of PDE gamma contributes to inhibit PDE, it raises the levels of cGMP in the dark. Higher cGMP means more channels open and more inward current resulting in increased influx of $Ca^{2+}$ and, therefore, higher cytoplasmic $Ca^{2+}$ concentration $[Ca^{2+}]_i$. If $[Ca^{2+}]_i$ increases, several targets of $Ca^{2+}$-dependent processes become affected, e.g. guanylate cyclase activity and recoverin-dependent inhibition of rhodopsin kinase.

Light adaptation, therefore, acts to increase the free pool of PDE gamma and, consequently, phosphorylated PDE gamma, increasing inhibition of PDE. If PDE gamma is phosphorylated before it re-inhibits PDE alpha/beta, a pool of PDE alpha/beta/$(gamma)_2$ with PDE gamma phosphorylated may be created, the size of which will depend on the intensity of the background illumination. This PDE will have higher activation energy and will be relatively resistant to transducin alpha GTP-dependent (light-dependent) removal of PDE inhibition.

In contrast to the results described above, other researchers have found that phosphorylation of T22 decreased by about 3-fold, rather than increased, the ability of PDE gamma??to inhibit either purified PDE alpha/beta or T αlpha-activated PDE (39). On the other hand, phosphorylation of T35 increased by about 2-fold cGMP binding to the non-catalytic domain and subsequent binding of PDE gamma to PDE catalytic subunits. Consistent with previous reports, Paglia *et al.* (39) also have shown that phosphorylation of either T22 or T35 impaired the ?ability of PDE γamma to bind to activated transducin and that phosphorylated PDE γamma could then regulate other signaling molecules such as arrestin, PDE5 and dynamin II (39).

## 3. A NOVEL ROLE OF PDE GAMMA: STUDIES OF PDE GAMMA KNOCKOUT MICE

To examine the *in vivo* function of the inhibitory PDE gamma subunits, we generated a mouse line, $Pdeg^{tm1}/Pdeg^{tm1}$, carrying a disruption of the PDE gamma gene (40). The expectation was that these mice would exhibit a constitutively active PDE catalytic core free of the restraint of the regulatory gamma subunits and, thus, give insights into mechanisms of light-induced

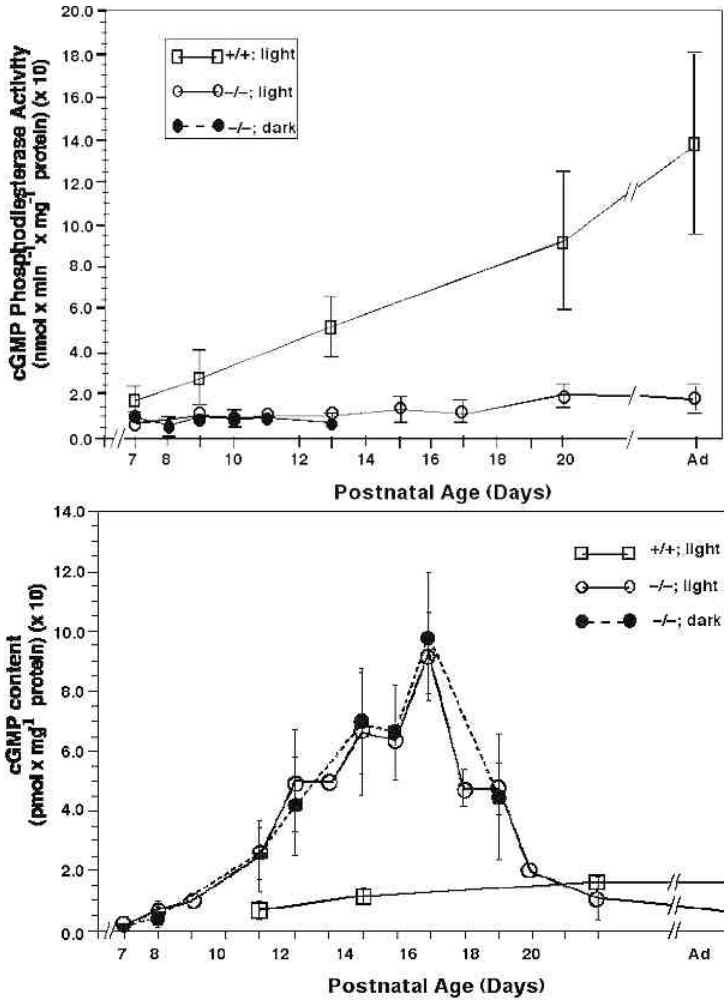


**Figure 2.** Developmental PDE activity and cGMP content in the retinas of control and mutant mice. **A**. PDE activity in freshly dissected retinas of *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mutant and wild-type mice. Light failed to activate PDE in these retinas. **B**. cGMP content in the freshly dissected posterior poles of *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ and wild-type animals during postnatal development. Shown are the means ± SEM of three to nine samples. The increase in cGMP levels preceded photoreceptor degeneration. Light- (open circles) or dark-adapted (closed circles) *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ (-/-) mutant retinas and light-adapted, wild-type (+/+) retinas (open squares) are indicated. [From Tsang and collaborators, 1996, (40)].

damage. However, the data surprisingly showed that the loss of the inhibitory PDE gamma subunit reduced PDE function (Figure 2A) and elevated rather than depressed cGMP levels in the developing photoreceptors (Figure 2B), even though the PDE alpha and beta subunits were present (Figure 3). In addition, this mutation resulted in a rapid retinal degeneration resembling that observed in human retinitis pigmentosa (Figure 4). Furthermore, we found that the PDE of *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mice was not trypsin- or light-activatable, suggesting that an interaction between the gamma subunit and PDE alpha-beta is essential for the proper function of PDE. In the normal retina, light and trypsin may induce an alteration of PDE gamma structure instead of its complete removal from the PDE alpha-beta-gamma-gamma complex, and this could play a critical role in the proper activation of the enzyme, as well as in the proper folding or conformation of the catalytic sites of the PDE alpha-beta core. This positive participation of PDE gamma in the formation of an active PDE complex was unexpected.

*Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ homozygous mice appeared healthy, and bred well; therefore, PDE gamma is not essential for prenatal development. Retinal physiology was characterized by examination of light-evoked responses generated by the photoreceptors and second order retinal cells. The electroretinogram (ERG) was grossly abnormal and showed that *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mice, when compared to wild type (+/+) controls, have a severely diminished response including a delay in b-wave implicit time. The loss in response was much greater in 8-week-old than in 2-week-old animals. The ERG became almost undetectable at later times (Figure 5). Thus, the targeted mutation led to a progressive loss of photoreceptor function (40).

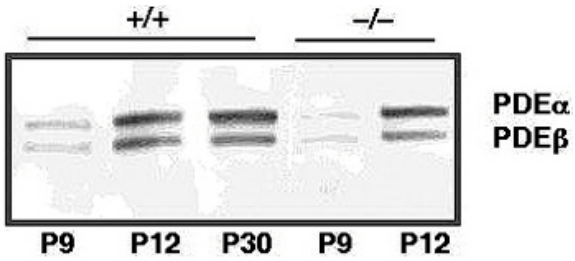


**Figure 3.** Immunoblot of PDE alpha and PDE beta subunits. Proteins from retinal homogenates were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and incubated with a polyclonal antibody against PDE. Both alpha and beta subunits of PDE are present in the *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ animals. [Adapted from Tsang and collaborators, 1996, (40)].

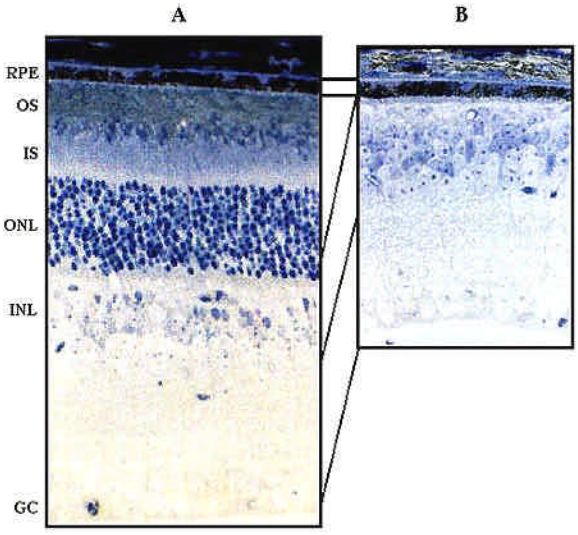


**Figure 4.** Morphological characterization of *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ homozygous mutant mice. Light micrographs of the retina from 8-week-old control mouse (**A**) and *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mutant mouse (**B**). OS, outer segments; IS, inner segments; ONL, outer nuclear layer; INL, inner nuclear layer; GC, ganglion cell layer. The retina of the *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mutant mouse has lost the complete photoreceptor layer (OS, IS, ONL) by 8-weeks of age. The INL and GC layers appear to be unaffected. [Modified from Tsang and collaborators, 1996, (40)].

We have now shown that incubation of the *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ retinal extract with purified bovine PDE gamma subunit restores the trypsin-induced PDE activity of the mutants (Yamashita, C.K., and Farber, D.B., unpublished results). These results imply that the PDE alpha and beta subunits are able to assemble in a functional complex in the *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mutant retina. In other words, the genetic loss of the PDE gamma subunit does not appear to affect the assembly, folding or stability of the PDE alpha-beta complex.

## 4. INVOLVEMENT OF VARIOUS REGIONS OF PDE GAMMA IN REGULATION OF PHOTOTRANSDUCTION STEPS REVEALED BY STUDIES OF MICE CARRYING DIFFERENT PDE GAMMA MUTATIONS

Our studies on the *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mice demonstrated a novel role for PDE gamma and indicated the need to investigate more subtle PDE gamma mutations in order to elicit constitutive PDE activity. With this purpose, we introduced in the *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ background mutations that according to *in vitro* results would restore the PDE catalytic core, but would fail to inhibit its activity. We hoped these mutants would allow us to determine the results of continuous photoexcitation on photoreceptor development and survival, and that some of them would not respond to light, possibly producing a stationary rod dysfunction but without rod degeneration. In addition, these animals would not only provide important information about mechanisms of light adaptation in response to constitutive signaling or reveal the action of structural components of PDE gamma on the photoresponse, but also would allow us to study the pathogenesis of retinal degeneration caused by deficient PDE gamma.

### 4.1. The role of PDE gamma in the formation of a functional PDE enzyme: effects of a transgene with a deletion of the last seven amino acids of PDE gamma

Our initial study was designed to test whether the PDE gamma domain that supports the function of the PDE catalytic core, *in vivo*, is in the carboxyl terminus. For this, we constructed various mutant and wild-type PDE gamma cDNAs under the control of the opsin promoter and used them to produce transgenic mice by conventional means (41). The transgenes were then transferred to the *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mice by breeding, and animals of the appropriate genotype were identified by PCR and Southern blot analysis of genomic DNA. Deletion of the last seven amino acids (Del7C) of PDE gamma had previously been shown in *in vitro* experiments to decrease the inhibitory potential of the subunit (19, 42), but not to affect binding of PDE gamma to PDE alpha-beta (18); its effects on binding to transducin were not determined. Thus, we predicted that mutant mice carrying the *Del7C* transgene would have a restored, functional, constitutively active core enzyme and, as a consequence, produce low levels of retinal cGMP.

Surprisingly, PDE activity in the retina of dark-adapted, 12-day-old transgenic *Del7C* (18.3 ± 1.3 nmol x min$^{-1}$ x mg$^{-1}$ total protein) and *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ (10.9 ± 1.4 nmol x min$^{-1}$ x mg$^{-1}$ total protein) mice was lower than that measured in the +/+ control retina (40.0 ± 9.0 nmol x min$^{-1}$ x mg$^{-1}$ total protein) (43). The discrepancy between *in vitro* and *in vivo* results can be explained by the 90% reduction in the expression of the catalytic PDE alpha and beta subunits that we observed in the transgenic *Del7C* animals (Figure 6).

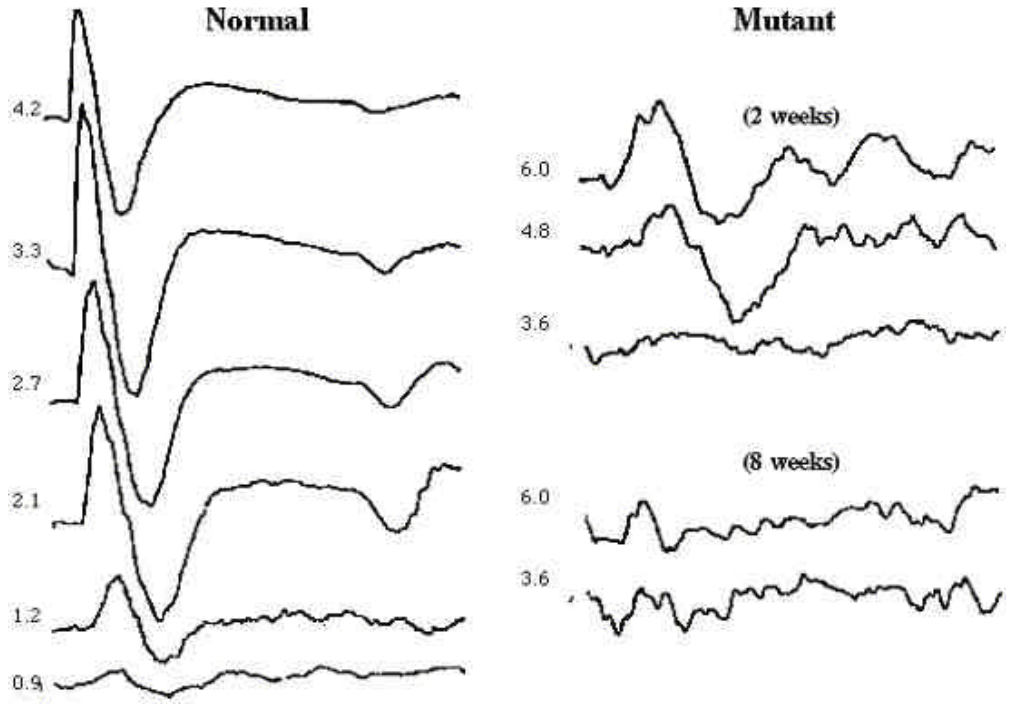


**Figure 5.** Corneal ERGs of a normal mouse compared to those of the PDE gamma knockout mouse at 2 and 8 weeks after birth. Each trace is 0.7 sec in duration and represents the average of 100 responses of a 1 Hz square-wave flickering stimulus of equal duty cycle. The numbers on the left indicate the log relative energy of the light stimulus. Vertical calibration: 25 microvolts for the upper trace, 12 microvolts for the second and third traces, 8 microvolts for the fourth trace, and 6 microvolts for the lower two traces on the left and all traces on the right. Horizontal calibration: 75 milliseconds for all traces. [From Tsang and collaborators, 1996, (40)].

Mice expressing the *Del7C* mutant allele did not exhibit a substantial photoresponse as judged by their ERGs. However, the response was more sensitive and its amplitude larger than that of the $Pdeg^{tm1}/Pdeg^{tm1}$ mice (43). The retinal function of transgenic *Del7C* mutants decayed gradually over time, similar to what happens in individuals with retinitis pigmentosa. Furthermore, the *Del7C* transgene only slowed down for a few days the process of degeneration, as determined by comparing the *Del7C* animals with the parental $Pdeg^{tm1}/Pdeg^{tm1}$ mice (Figure 7). In other words, the *Del7C* transgene could not complement the $Pdeg^{tm1}/Pdeg^{tm1}$ mutant for photoreceptor survival. Our findings suggested that a novel positive role of PDE gamma in promoting PDE activity might be found within its carboxyl tail. Therefore, this region of the protein may be essential for expression of the PDE catalytic core and for the maintenance of the proper function and integrity of the photoreceptors.

### 4.2. The role of PDE gamma in the formation of a functional PDE enzyme: studies of mice carrying a point mutation (Y84G) in PDE gamma

We also examined the substitution of the tyrosine at residue 84 of PDE gamma for a glycine (Y84G), previously shown to decrease the inhibitory potential of the PDE gamma subunit (18). Again, just as with the carboxyl deletion mutant, the PDE gamma transgene with the Y84G mutation would be expected to result in the formation of a constitutively active PDE and thus to produce low levels of cGMP in the animals carrying it. These mice would also be expected to show little or no rod response and normal cone responses in ERG measurements.

After introduction of the Y84G point mutation into the PDE gamma cDNA by standard PCR-based site-specific mutagenesis, a construct containing this cDNA, 4.4 kb of the mouse opsin promoter and the polyadenylation signal of the mouse protamine gene was injected into the male pronuclei of oocytes from superovulated F1 (DBA X C57BL6) females mated with homozygous $Pdeg^{tm1}/Pdeg^{tm1}$ males. The microinjected oocytes, transferred to the oviducts of pseudopregnant F1 females and carried to term, resulted in transgenic $Pdeg^{tm1}/+$ mice that were backcrossed to $Pdeg^{tm1}/Pdeg^{tm1}$ animals to place the transgene into the knockout background.

Contrary to our expectations, the newly engineered animals showed normal inhibition of rod-specific PDE and cGMP levels similar to those of control retinas (44). Furthermore, the degeneration of the parental $Pdeg^{tm1}/Pdeg^{tm1}$ mice was rescued by the mutant transgene (Figure 8).

It had been previously shown *in vitro* that trypsin-induced PDE activity, which reflects the maximal activity of the PDE alpha-beta core, requires for its function micromolar concentrations of T alpha and is inhibited by

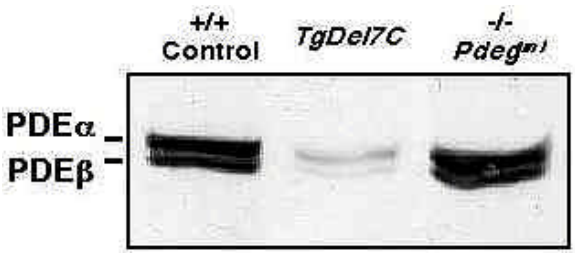


**Figure 6.** Immunoblot showing PDE alpha and PDE beta expression in control (+/+), transgenic *Del7C* and *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ (-/-) mouse retinas. The polyclonal peptide antibody used recognizes both PDE subunits. 10 micrograms of protein were loaded in each lane. [Adapted from Tsang, Yamashita and collaborators, 2002, (43)].

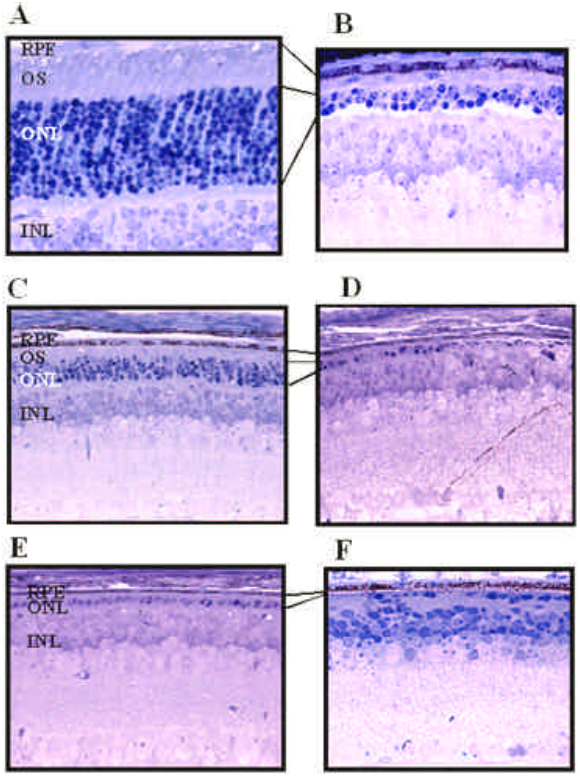


**Figure 7.** Light micrographs of retinal sections from *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mice with (A, C and E) or without (B, D and F) the *Del7C* transgene at different times during postnatal development. A and B: 14-day-old mice; C and D: 17-day-old mice; E and F: 21-day-old mice. Abbreviations as in Figure 4. [Adapted from Tsang, Yamashita and collaborators, 2002, (43)].

nanomolar concentrations of PDE gamma (45). This is consistent with our *in vivo* findings in that the affinity of the PDE alpha-beta core for PDE gamma is much higher than for T alpha-GTP in retinal homogenates from control mice. However, in the mutant Y84G retinas T alpha-GTP gammaS could only activate PDE to a level about 5-fold lower than that in control retinas (Figure 9); similarly, GTP gammaS in conjunction with light was less effective in activating the mutant PDE complex (Figure 9 inset). Thus, these results indicate that it is more difficult for T alpha to remove the mutant Y84G PDE gamma than the wild-type PDE gamma from the PDE alpha-beta core (44).

In addition, since PDE gamma accelerated the GTPase activity of T alpha in the presence of rod outer segment membranes *in vitro* (46), we tested if the Y84G mutation altered the interaction of PDE gamma with T alpha *in vivo* by measuring the rod outer segment GTPase activity as a function of time in control and mutant retinas. The rate constants obtained were similar for both samples, suggesting that the Y84G mutation does not alter the interaction of PDE gamma and T alpha-GTP (44).

Also contrary to our expectations, although the Y84G mutation caused a significant biochemical defect in PDE activation by transducin, the transgenic mice showed a significant photoresponse, with only a 10-fold decrease in the amplitude of the a-wave and a 1.5-fold reduction in the b-wave. Thus, the Y84G mutation produced a much milder phenotype *in vivo* than that predicted from the biochemical assays *in vitro*.

### 4.3. The role of PDE gamma in the regulation of Transducin alpha - GTPase activity: studies of mice carrying the W70A mutation in PDE gamma

A substitution of tryptophan at position 70 of PDE gamma for alanine (W70A) not only reduced the affinity of PDE gamma for transducin but also completely abolished this effector-mediated acceleration of T alpha-GTPase in reconstituted systems (47, 48). Thus, the *in vivo* effect of this mutation was tested by introducing a W70A PDE gamma transgene into the *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ genetic background (49). This transgene rescued the *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ retinal degeneration (Figure 10). In addition to PDE gamma, the levels of other components of the phototransduction cascade, namely RGS9, PDE alpha-beta, T alpha, and rhodopsin, were all essentially normal in the retinas of the transgenic W70A mice, but T alpha-GTPase hydrolysis was 3 times slower than in control mice (Figure 11), the rods were remarkably insensitive to light (Figure 12) and the time needed for the recovery of the photoresponse was also much slower than in control animals. Consequently, this W70A mutation prolonged the duration of the light response, indicating that the wild-type PDE gamma is essential for the termination of the photoresponse in mammalian rods and, furthermore, that RGS9 alone is not sufficient for the acceleration of GTPase activity (49). The decrease in T alpha-GTPase activity in the transgenic W70A mice also showed that tryptophan at position 70 in PDE gamma does influence T alpha binding or activation *in vivo*, even though this site does not lie in the positively charged region (amino acids 24-45) which is thought to be the major interaction domain of PDE gamma with T alpha.

To determine whether the ability of T alpha to activate PDE was different in transgenic W70A and control mice, various amounts of GTP gammaS were added to homogenates of bleached rod outer segments from these animals. In control mice the increase in PDE hydrolytic

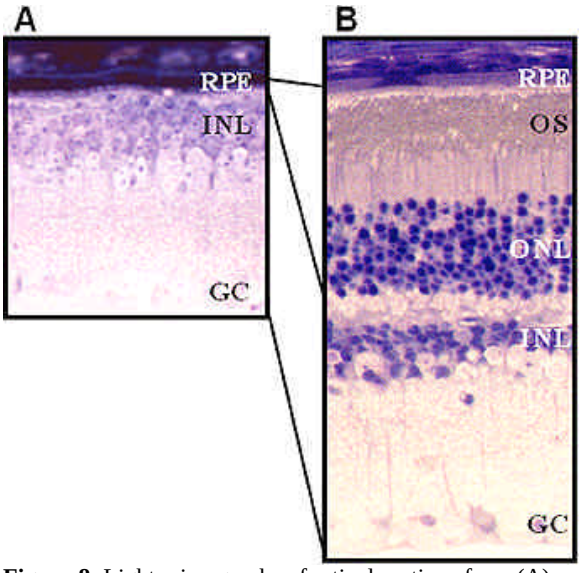


**Figure 8.** Light micrographs of retinal sections from (A) an adult homozygous *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mouse and (B) a homozygous *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mouse with the Y84G transgene at 4 months of age. Abbreviations as in Figure 4. [Adapted from Tsang, Yamashita and collaborators, 2001 (44)].

activity was proportional to the amount of GTP gammaS added to the rod outer segments. On the contrary, in rod outer segments of transgenic W70A mice PDE activity remained at a basal rate despite of increasing concentrations of GTP gammaS (Figure 13). Since the transducin content (as determined by GTP gammaS binding) and total activatable PDE (as determined by trypsin activation) in transgenic W70A and control rod outer segments were essentially the same (49), the results suggest that the W70A allele prolongs T alpha life by uncoupling the activated transducin from the W70A PDE.

The introduction of the W70A transgene uniquely altered the ERG of *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mice (50). Dark-adapted transgenic W70A mice had no a-wave and a significantly decreased sensitivity to light (Figure 14). The b-wave obtained with bright flashes showed a prolonged time to peak; its amplitude, however, remained stable for at least 13 months, suggesting lack of retinal degeneration and corroborating the morphological observations. Furthermore, the b-wave implicit time remained virtually constant with flash intensity, failing to show the increased delay characteristic of a sensitive rod response. In order to assess cone function, the ERGs of transgenic W70A mutant mice were examined in the light-adapted state. These ERGs were identical to those of normal mice. Thus, the mutation has no effect on cone function but it only affects the rods (50).

Therefore, the introduction of the W70A transgene into the *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mice produced drastic changes in the ERG desensitizing and delaying their photoreceptors, but prevented their degeneration. We believe that as a consequence of these effects, a new stationary form of nyctalopia was created in the W70A transgenic strain.

## 5. PERSPECTIVE

Mutant alleles of PDE gamma introduced into the *Pdeg*$^{tm1}$/*Pdeg*$^{tm1}$ mice have revealed the importance of some amino acids or domains of this subunit for maintaining the morphological integrity of the retina. Moreover, these mutant alleles have produced new animal models of retinal degeneration and have also contributed to our understanding of the role of PDE gamma, one of the major players in phototransduction and in *in vivo* physiology. In the future, learning about how the PDE gamma-RGS9 complex modulates the turn-off of signals in phototransduction will provide a mechanism for critically tuning the photoresponse without affecting the levels of the primary signaling components, rhodopsin and transducin. Additionally, the generation of a more extensive allelic series of mutant mice may further provide important links between stationary night blindness and retinitis pigmentosa.

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