Cone phosphodiesterase-6α' restores rod function and confers distinct physiological properties in the rod phosphodiesterase-6β-deficient rd10 mouse

Wen-Tao Deng
University of Florida

Keisuke Sakurai
Washington University School of Medicine in St. Louis

Saravanan Kolandaivelu
West Virginia University

Alexander V. Kolesnikov
Washington University School of Medicine in St. Louis

Astra Dinculescu
University of Florida

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

Recommended Citation

Deng, Wen-Tao; Sakurai, Keisuke; Kolandaivelu, Saravanan; Kolesnikov, Alexander V.; Dinculescu, Astra; Li, Jie; Zhu, Ping; Liu, Xuan; Pang, Jijing; Chiodo, Vince A.; Boye, Sanford L.; Chang, Bo; Ramamurthy, Visvanathan; Kefalov, Vladimir J.; and Hauswirth, William W., "Cone phosphodiesterase-6α' restores rod function and confers distinct physiological properties in the rod phosphodiesterase-6β-deficient rd10 mouse." The Journal of Neuroscience. 33,29. 11745-11753. (2013).
https://digitalcommons.wustl.edu/open_access_pubs/1629

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.
Authors
Wen-Tao Deng, Keisuke Sakurai, Saravanan Kolandaivelu, Alexander V. Kolesnikov, Astra Dinculescu, Jie Li, Ping Zhu, Xuan Liu, Jijing Pang, Vince A. Chiodo, Sanford L. Boye, Bo Chang, Visvanathan Ramamurthy, Vladimir J. Kefalov, and William W. Hauswirth
Cone Phosphodiesterase-6α’ Restores Rod Function and Confers Distinct Physiological Properties in the Rod Phosphodiesterase-6β-Deficient rd10 Mouse

Wen-Tao Deng, Keisuke Sakurai, Saravanan Kolaanivelu, Alexander V. Kolesnikov, Astra Dinculescu, Jie Li, Ping Zhu, Xuans Liu, Jiaying Fang, Vince A. Chiiodo, Sanford L. Boye, Bo Chang, Visvanathan Ramamurthy, Vladimir J. Kefalov, and William W. Hauswirth

1Department of Ophthalmology, University of Florida, Gainesville, Florida 32610, 2Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, Missouri 63110, 3Departments of Ophthalmology and Biochemistry, Center for Neuroscience, West Virginia University, Morgantown, West Virginia 26506, 4Beijing Tsinghua Hospital, North First quatianyuan, Changping District, Beijing 102218, China, and 5The Jackson Laboratory, Bar Harbor, Maine 04610

Phosphodiesterase-6 (PDE6) is the key effector enzyme of the vertebrate phototransduction pathway in rods and cones. Rod PDE6 catalytic core is composed of two distinct subunits, PDE6α and PDE6β, whereas two identical PDE6α’ subunits form the cone PDE6 catalytic core. It is not known whether this difference in PDE6 catalytic subunit identity contributes to the functional differences between rods and cones. To address this question, we expressed cone PDE6α’ in the photoreceptor cells of the retinal degeneration 10 (rd10) mouse that carries a mutation in rod PDE6 subunit. We show that adenoviral-associated virus-mediated subretinal delivery of PDE6α’ rescues rod electroretinogram responses and preserves retinal structure, indicating that cone PDE6α’ can couple effectively to the rod phototransduction pathway. We also show that restoration of light sensitivity in rd10 rods is attributable to assembly of PDE6α’ with rod PDE6βγ. Single-cell recordings revealed that, surprisingly, rods expressing cone PDE6α’ are twofold more sensitive to light than wild-type rods, most likely because of the slower shutoff of their light responses. Unlike in wild-type rods, the response kinetics in PDE6α’-treated rd10 rods accelerated with increasing flash intensity, indicating a possible direct feedback modulation of cone PDE6α’ activity. Together, these results demonstrate that cone PDE6α’ can functionally substitute for rod PDE6β in vivo, conferring treated rods with distinct physiological properties.

Introduction

Rod and cone photoreceptor cells share a similar phototransduction pathway but exhibit strikingly different physiological properties. Rods, responsible for scotopic vision, are highly light sensitive. Cones, responsible for photopic vision, are intrinsically less sensitive, have faster response kinetics, and adapt to a wider range of light intensities (Pugh and Cobb, 1986; Fu and Yau, 2007). One of the key unresolved questions is how the physiological differences between rods and cones can be correlated with the distinctive properties of their phototransduction proteins. Previous studies have shown that the lower thermal stability of cone pigments is likely to contribute to the lower sensitivity of cones but, once activated, rod and cone pigments can couple equally efficiently to rod or cone transducin (Kefalov et al., 2003, 2005; Shi et al., 2005, 2007; Fu et al., 2008). Thus, consistent with our previous research (Deng et al., 2009) and other studies (Ma et al., 2007), the signaling properties of rod and cone transducin α-subunit do not contribute to the difference in light sensitivity between rods and cones (but see Chen et al., 2010). As a result, the expression levels and molecular properties of phototransduction components downstream of transducin are likely to play an important role in defining the distinctive physiological properties of rods and cones.

The photoreceptor cyclic nucleotide phosphodiesterase-6 (PDE6) plays an essential role in phototransduction by regulating the GMP levels in rods and cones (Fu and Yau, 2007). The most obvious distinction between rod and cone PDE6 is that rod PDE6 is composed of two distinct catalytic subunits α, β (PDE6A, PDE6B) and two inhibitory subunits γ (PDE6G), whereas cone PDE6 is composed of two identical catalytic subunits α' (PDE6C) plus two cone-specific inhibitory subunits γ' (PDE6H) (Gillespie et al., 1999; Li et al., 2002; Deng et al., 2009).
and Beavo, 1988; Hamilton and Hurley, 1990; Li et al., 1990). Each of the catalytic subunits of PDE6 consists of two N-terminal regulatory cGMP-binding GAF (for cGMP-specific phosphodiesterases, adenylyl cyclases, and FlH) domains (GAFa and GAFb) and a catalytic domain located in the C-terminal region. The catalytic domains are highly conserved among rod and cone PDE6 subunits and exhibit equivalent enzymatic activities (Mou and Cote, 2001; Muradov et al., 2010). Among the GAF domains, rod PDE6 A GAF displays a higher affinity toward cGMP than cone PDE6 (Gillespie and Beavo, 1989). It has been suggested that the differences in GAF binding affinities toward cGMP and PDE6 may contribute to the higher efficiency of cone PDE6 activation by transducin α-subunit (Muradov et al., 2010).

In this study, we tested whether PDE6 catalytic subunit identity contributes to the functional differences between rods and cones by expressing cone PDE6α in the retinal degeneration 10 (rd10) photoreceptor cells, which carry a mutation in the β-subunit of rod PDE6 (Chang et al., 2007). We show that cone PDE6α can restore rd10 rod function by assembling with rod PDE6α. Furthermore, it confers rods with distinct physiological properties.

Materials and Methods

Animals. rd10 mice and wild-type (WT) C57BL/6J controls were obtained from The Jackson Laboratory. The mice of either sex were bred and maintained in the University of Florida Health Science Center Animal Care Services Facilities in a continuously dark room, except for husbandry at ~400 lux illuminance. All experiments were approved by the local Institutional Animal Care and Use Committees at the University of Florida and Washington University and conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and National Institutes of Health regulations.

Construction and packaging of adenovirus vectors. PDE6α cDNA was purchased from Invitrogen. The adenovirus vector (Ad5) was constructed in the p-collection system (Invitrogen). Ad5::PDE6α cDNA is under the control of a small chicken β-actin (smCBA) promoter. Ad5::PDE6α was packaged in Ad5::smCBA vector (24,550 × 10^6 vector genomes/ml) by transfection of HEK293 cells according to previously published methods (Zolotukhin et al., 1999).

Subretinal injections. Postnatal day 14 (P14) rd10 pups raised in the dark were brought to a normal illuminated room for injection and then returned back to dark. A total volume of 1 μl of Ad5::PDE6α or Ad5::PDE6α cDNA under the control of a small chicken β-actin (smCBA) promoter was packaged in Ad5::smCBA vector and injected subretinally into the left eyes and the right contralateral eyes served as control eyes. Subretinal injections were performed as described previously (Pang et al., 2006, 2008). Briefly, a 33-gauge blunt needle mounted on a 5 μl Hamilton syringe was introduced through the corneal opening made by a 30-gauge needle, and injections were visualized by fluorescein-positive subretinal bleb. One percent streptomycin eye drops and neomycin-polymyxin B/kanamycin ophthalmic ointment were given after injection.

Electroretinogram analyses. At 5 weeks after injection, rod- and cone-mediated electroretinograms (ERGs) were recorded separately using a UTAS Visual Diagnostic System equipped with Biolight Ganzfeld (LKC Technologies) according to protocols described previously with minor modifications (Pang et al., 2010). Scotopic rod recordings were performed with three increasing light intensities at −1.6, −0.6, and 0.4 log cd/s/m². Ten responses were recorded and averaged at each light intensity. Scotopic cone recording were taken after mice were adapted to a white background light of 30 cd/m² for 5 min. Recordings were performed with four flash intensities at 0.1, 0.7, 1.0, and 1.4 log cd/s/m² in the presence of 30 cd/m² background light. Fifty responses were recorded and averaged at each intensity. Scotopic and photopic b-wave amplitudes from untreated, rd10, and WT controls at each intensity were averaged and used to generate an SD. The differences between recordings from untreated and treated eyes were analyzed by the paired t test.

Morphology and immunohistochemistry. Treated rd10 mice were killed and enucleated 24 hr after ERG recordings for morphological and immunohistochemical analysis. The eyes were fixed in a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde for 3 hr at room temperature and then paraformaldehyde and sectioned at 4 μm through the optic nerve for hematoxylin and eosin (H&E) staining. Retinal sections for immunohistochemistry were prepared according to previously described methods (Deng et al., 2009, 2012). Briefly, eyes were fixed in 4% paraformaldehyde. Cornea, lens, and vitreous were removed from each eye without disturbing the retina. The remaining eye cup was rinsed with PBS and then cryoprotected by placing it in 30% sucrose in PBS for 4 hr. Eye cups were then embedded in cryostat compound (Tissue Tek OCT; Sakura Finetek) and frozen at −80°C. Retinal tissue cryosections were sectioned at 12 μm thickness, rinsed in PBS, and blocked in 2% normal goat serum and 0.3% Triton X-100 in 1% BSA in PBS for 1 hr at room temperature. Anti-PDE6α (3184P) (Kirschman et al., 2010), rhodopsin, or red/green-cone opsins (Millipore Bioscience Research Agents) antibodies (all 1:1000 dilutions) were diluted in 0.1% Triton X-100 and 1% BSA in PBS and incubated with sections overnight at 4°C. The sections were then washed three times with PBS incubated with IgG secondary antibody tagged with Alexa Fluor-594 (Invitrogen) at 1:500 dilution and lectin peanut agglutinin (PNA) conjugated to Alexa Fluor-488 (Invitrogen) at 1:200 dilution in PBS at room temperature for 1 hr, and washed with PBS. Sections were mounted with Vectashield Mounting Medium for Fluorescence (H-1000; Vector Laboratories) and coverslipped. Sections were analyzed with a Carl Zeiss Axio Observer microscope equipped with Axiovision release 4.6 software.

Western blot analyses. Untreated, Ad5::PDE6α-treated rd10 and WT eyes (five eyes each) were carefully dissected, and the eyecups were pooled and homogenized by sonication in a buffer containing 0.23 M sucrose, 5 mM HCl, pH 7.5, and protease inhibitors (Roche Complete). After centrifugation, aliquots of the experimental
**Figure 2.** ERG responses, retinal morphology, immunohistochemistry, and scotopic visual acuity of rd10 mice after AAV8 Y7335-F-smCBA-PDE6β' delivery at 5 weeks after injection (5-wk-pj). A, B. Representative examples of dark-adapted ERG traces (A) and light-adapted ERG traces (B) from an rd10 mouse 5 weeks after injection. C. Dark-adapted ERG was partially restored in injected rd10 eyes. Statistical analysis demonstrated a significant difference between uninjected and fellow vector-treated eyes for dark-adapted a-waves at -1.6, -0.6, and 0.4 log cd/m² (p < 0.01). D. Light-adapted ERG responses were improved in treated rd10 eyes compared with their contralateral controls as a result of rod function rescue and rod cell survival (p < 0.02). Error bars are mean ± SEM. E. Comparison of ERG responses between FosBβ-treated and PDE6β'-treated rd10 eyes 5 weeks after injection. There were no significant differences between dark-adapted a-waves amplitude at three light intensities tested between FosBβ-treated and PDE6β'-treated rd10 eyes (4 p > 0.1). Error bars representing the mean ± SEM. F. Bar graph representing the mean ± SEM. B-Wave amplitude at indicated flash intensities was compared by repeated-measures ANOVA, with the Bonferroni's post hoc test for ANOVA (p < 0.1) used to compare means at individual flash intensities. F. Restoration of scotopic visual acuity in PDE6β'-treated rd10 mice 5 weeks after injection. Data were derived from mouse optometer responses to rotating gratings under background monitor luminance of 0.45 log cd/m². Error bars are mean ± SEM.

**Immunoprecipitation.** Frozen retinal eyecups from untreated, AAV8 Y7335-F-smCBA-PDE6β' treated rd10 and WT (five each) were homogenized in 400 µl of immunoprecipitation (IP) buffer (in mM: 10 Tris-HCl, pH 7.5, 100 KCl, 20 NaCl, and 1 MgCl₂) containing protease and phosphatase inhibitors and 100 µg/mL phenylmethylsulfonyl fluoride (PMSF). Supernatants were collected by centrifugation at 10,000 g (Eppendorf 5414C) for 5 min at 4°C. IP was performed with supernatants (400 µl) using mouse monoclonal ROS-1 antibodies. We used 1.5 µg of ROS-1 antibody for each pull-down experiment. Bound proteins were eluted by boiling with 50 µl of 1X Laemmli's sample buffer and separated by 4-20% SDS-polyacrylamide gel (Bio-Rad) and transferred to Immuno-Blot PVDF membrane (Bio-Rad). Immunoblot analyses were performed with individual rod PDE6α, PDE6β, and PDE6γ subunits and cone PDE6β' (3184p)-specific primary antibodies according to our previously published method (Kolandaivel et al., 2011).

**Single-cell recordings.** Mice kept in darkness for at least 12 h were killed by CO₂, and the eyes were removed under dim red light. Under infrared light, the retina was cut into small pieces and then finely chopped. Isolated pieces of retina were stored in Locke's solution at 4°C until use. The perfusion Locke's solution (in mM: 112 NaCl, 3.6 KCl, 2.4 MgCl₂, 1.2 CaCl₂, 10 HEPES, 20 NaHCO₃, 3 NaH₂PO₄, 0.5 Na-glutamate, and 10 glucose, pH 7.4) was equilibrated with 95% O₂/5% CO₂, bubbled and heated to 34–37°C. Glass capillaries were pulled and heat-polished to fit the rod outer segment (ROS) diameter and then filled with electrode solution containing the following (in mM): 140 NaCl, 3.6 KCl, 2.4 MgCl₂, 1.2 CaCl₂, 10 HEPES, 10 glucose, pH 7.4. A rod photoreceptor was drawn into the electrode to record the inward current of the outer segment (OS). The dark current was amplified by a current-to-voltage converter (Axopatch 200B, Molecular Devices), low-pass filtered by an eight-pole Bessel filter with a cutoff frequency of 30 Hz (Krein-4-Trite), digitized at 1 kHz, and recorded with pClamp 8.2 software (Molecular Devices). Ten-millisecond flashes were delivered from a calibrated light source via computer-controlled shutters. Light intensity and wavelength were changed with neutral density and interference (Amax = 500 nm) filters (Edmund Optics). Intensity–response data were fit by the Hill equation: 

\[
R = R_{\text{max}} \left( \frac{I}{I^* + I_{\text{h}}} \right)^n
\]

where R is the transient peak amplitude of response, Rmax is maximal response amplitude, I is flash intensity, and Ih is flash intensity to generate half-maximal response.

**Visual acuity test.** Scotopic visual acuity of 2-month-old mice was determined using a two-alternative forced-choice protocol (Umin(0,6),(989,991)
The Optometry system (Cerebral Mechanics) consisted of a square array of four computer monitors with a pedestal in the center where the mouse was placed. An infrared-sensitive television camera and a round array of six infrared light-emitting diodes mounted above the animal were used to observe the mouse but not the monitors. Using a staircase paradigm, rotating sine-wave vertical gratings were applied on the monitors where they formed a virtual cylinder around the animal (Fuske et al., 2004). The mice responded to the stimuli by reflexively rotating their head in either clockwise or counterclockwise direction. Optomotor responses were measured under monitor background illumination of $-4.45 \log \text{cd/m}^2$, which was set by neutral density film filters.

Visual acuity was defined as the threshold for spatial frequency ($F$) of gratings with 100% contrast and measured at the speed ($S$) of 6.0°/s. $F$ was gradually altered by the computer protocol until its combined threshold for both stimuli directions was determined. Temporal frequency ($F_T$) was automatically adjusted by the computer software, based on the following equation: $F_T = S \times F$ (Uminn et al., 2008). Data were analyzed using independent two-tailed Student’s t-test, with an accepted significance level of $p < 0.05$.

**Results**

**Expression of cone PDE6α in rd10 mouse retinas**

An AAV8 Y733F capsid tyrosine mutant vector containing the mouse PDE6α cDNA driven by a ubiquitous smCBA promoter was delivered subretinally to one eye of rd10 mice at P14, whereas the contralateral eyes remained uninjected and served as controls. PDE6α expression in treated retinas was analyzed by immunostaining (Fig. 1A) and Western blot analysis (Fig. 1B) at 5 weeks after injection. PDE6α expression was found in both rods and cones of treated rd10 mice after immunostaining with a cone-specific PDE6α antibody, whereas it was found exclusively in the cones of WT control mouse retinas based on colocalization with a cone OS sheath-specific PNA marker. Photoreceptor cells in untreated retinas were significantly degenerated at this age, and only a weak spotty staining was detected for residual cones (Fig. 1A). Low levels of expression were also observed in the inner retina most likely as a result of nonspecificity of the PDE6α antibody because similar labeling was observed in untreated and treated rd10, as well as in the WT sections (Fig. 1A). Western blot analysis using the same antibody (Fig. 1B) detected abundant PDE6α expression in injected rd10 retinas compared with WT controls in which PDE6α was expressed predominantly in cones. This result provides evidence that PDE6α is robustly expressed in rd10 rods after AAV8 treatment because rods comprise the majority (97%) of photoreceptor cells in the mouse retina. PDE6α expression was reduced to almost undetectable levels in retina from uninjected rd10 animals (Fig. 1B), presumably because of the degeneration of cones caused by the loss of PDE6α-deficient rods.

**Functional and structural retinal preservation in PDE6α+/- treated rd10 eyes**

To determine whether exogenously expressed cone PDE6α can rescue rod function in rd10 mice, full-field scotopic and photopic ERG responses were recorded from uninjected rd10 mice, injected rd10 mice 5 weeks after injection, and age-matched WT controls. Rod-mediated ERG responses were undetectable in rd10 mice at this age (7 weeks old), whereas vector delivery of PDE6α+/- to rd10 rods led to significant restoration of rod-driven ERG responses (Fig. 2A, C). The average rod-mediated b-wave amplitude at a flash intensity of $-1.6 \log \text{cd/m}^2$ in treated eyes was $109 \pm 39 \mu \text{V}$ (mean ± SD), whereas it was undetectable in contralateral untreated eyes ($n = 3, p < 0.01$). The treated eye rod ERG b-wave amplitude was $\sim 35\%$ of the WT level. Cone-mediated ERG amplitudes in injected eyes also showed some improvement compared with uninjected controls (Fig. 2B, D), presumably as a result of better preservation of the cones after restoration of rod function and rod survival (Fig. 3C). The average cone b-wave amplitude was $44 \pm 8 \mu \text{V}$ (mean ± SD) in injected eyes versus $27 \pm 10 \mu \text{V}$ in contralateral untreated eyes at $1.4 \log \text{cd/m}^2$ ($n = 3, p < 0.02$). We also recorded ERG responses...
from some rd10 mice injected with vector expressing PDE6β and observed no significant differences between PDE6β and PDE6α− treatments (Fig. 1E), suggesting that therapy in the rd10 mouse was equivalent whether we used the heterologous rod subunit or the homologous cone subunit. Finally, the scotopic visual acuity of PDE6α−-treated rd10 mice improved significantly (0.349 ± 0.088, n = 8) over that of untreated controls (0.069 ± 0.024, n = 5) (Fig. 2F), although rod visual performance still remained subpar compared with WT mice (0.776 ± 0.072).

Three rd10 mice exhibiting significant ERG rescue were killed 2 d after the recordings, and retinal morphology was analyzed by H&E staining (Fig. 3A). Only one layer of photoreceptor nuclei remained in the outer nuclear layer of untreated rd10 retinas with no evident outer or inner segments. In contrast, retinal structure was partially preserved in injected eyes, with five to seven layers of nuclei remaining compared with 12 layers in WT controls. Additionally, treated retinas retained 20–50% of the normal OS length. Uninjected, injected, and WT retinal sections were also stained with rhodopsin antibody (Fig. 3B) and cone opsin-specific (Fig. 3C) antibody to further confirm the morphological rescue. Expression of both rhodopsin and cone opsin was evident and much more abundant in treated rd10 eyes compared with their spotty staining observed in uninjected controls.

We also recorded ERG responses from rd10 mice at 5 months after treatment (Fig. 4A,B). The average rod-driven ERG b-wave amplitude (Fig. 4A) at a flash intensity of −1.0 log cd/m² was 81 ± 15 μV (mean ± SD), and it was significantly higher than the undetectable ERGs in untreated eyes (n = 3, p < 0.005). The cone-mediated ERG responses (Fig. 4B) were also undetectable in untreated eyes at this age, whereas the average b-wave amplitude in treated eyes at 1.0 log cd/m² was 51 ± 13 μV (mean ± SD) (n = 3, p < 0.03). PDE6α− expression was still evident in both rods and cones in treated eyes at 5 months after injection (Fig. 4C). Transducin was strongly expressed in the treated eyes as determined by immunostaining, whereas it was undetectable in untreated eyes at 5 months after injection (Fig. 4D). Thus, PDE6α−-mediated rescue of rd10 rod structure and function persisted even months after the AAV injection.

Conc PDE6a− binds to rod PDE6g to restore rod function

Restoration of the light-dependent rod response in rd10 animals suggested that PDE6α− expressed by AAV is capable of forming a functional complex with rod PDE6g. Before testing this idea, we investigated the levels of various subunits of rod PDE6 holoenzyme. Uninjected rd10 animals with advanced stage of rod degeneration lacked all three subunits of rod PDE6 (Fig. 5A). Despite preservation of five to eight layers of photoreceptor cells in injected animals, we observed destabilization of both rod
PDE6 catalytic subunits (Fig. 5A). Compared with age-matched WT controls, minor amounts of PDE6α or PDE6β were expressed in total retinal extracts from injected animals. In contrast, there was a dramatic upregulation in PDE6α' expression in these retina. Although lower than in WT controls, we observed robust expression of rod PDE6γ in treated animals likely as a result of complex formation with the viral introduced PDE6α'. To directly test whether the formation of a complex between cone PDE6α' and rod PDE6γ existed, we performed IP with a monoclonal antibody, ROS-1, that exclusively recognizes assembled and functional PDE6 complex from both rods and cones (Kolandaivel et al., 2009, 2011). As expected, we observed assembled rod and cone PDE6 subunits in ROS-1 pull-downs from WT controls (Fig. 5B). Assembled PDE6α' was also observed from surviving cones in un.injected animals. In treated rd10 animals, we detected a complex of PDE6α' and PDE6γ indicating that the restoration of light sensitivity in rd10 rods is attributable to the function of cone PDE6α' assembles with rod PDE6γ (Fig. 5B).

**Single-cell recordings from injected rd10 rods**

To gain additional insight into the light responses generated by rods expressing cone PDE6α', we performed single-cell recordings from injected rd10 rods and WT controls. For comparison, we also obtained responses from rd10 rods treated with vector expressing rod PDE6β. Although all retinas of PDE6α' and PDE6β-treated rd10 mice were still subject to some level of degeneration, we were able to find areas with healthy ROS in portions of the retina in which AAV vectors seemed to have been successfully delivered. We obtained photoresponses from 14 PDE6α'-treated rods (from two animals) and 22 PDE6β-treated rods (from three animals). No significant differences were found between the photoresponses of WT and PDE6β-treated rd10 rods (compare with Fig. 6A,C), indicating that the exogenous expression of PDE6β by AAV infection into rods of rd10 mice successfully rescued rod physiological functions. The dark currents, measured from saturated photoresponses, were comparable among WT, PDE6α'-treated, and PDE6α'-(treated) rods (Table 1). Thus, PDE6α' ectopically expressed in rod photoreceptors could form a functional complex with rod PDE6γ and maintain normal spontaneous activity and dark cGMP levels. However, we also observed several unusual features in the responses of PDE6α'-treated rods. First, PDE6α'-treated rods had higher sensitivity and produced larger single-photon responses than WT rods (Fig. 7A, Table 1). Consistent with this result, intensity–response relationships of dark-adapted rods showed that the flash intensity required for half-saturating response of the PDE6α'-treated rods was approximately twofold lower than that of WT rods (Fig. 6D, inset, Table 1). Second, the time-to-peak and integration time of dim-flash responses were substantially prolonged in PDE6α'-treated rd10 rods (Fig. 7A, Table 1). The rising phase of dim-flash response was similar among WT, PDE6β-treated, and PDE6α'-treated rd10 rods (Fig. 7A), which indicates that light-induced cGMP hydrolysis activated by PDE occurred at comparable rates. However, the response recovery phase was substantially delayed in PDE6α'-treated rods, indicating that the activation of cone PDE6α' was less effective. This slower than normal response shutoff could potentially explain the increased sensitivity and single-photon response amplitude in PDE6α'-treated rd10 rods. Third, unlike in WT and PDE6β-treated rd10 rods, the response kinetics in PDE6α'-treated rods accelerated substantially with increasing flash strength (Fig. 6B), and the resulting intensity–response curves appeared shallower than those of WT and control PDE6β-treated rd10 rods. Both of
Table 1. Rod response parameters of single-cell recordings

<table>
<thead>
<tr>
<th></th>
<th>WT (n = 10)</th>
<th>PDE6α&lt;sup&gt;−/−&lt;/sup&gt; (n = 14)</th>
<th>PDE6β&lt;sup&gt;−/−&lt;/sup&gt; (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark current (pA)</td>
<td>16.0 ± 1.0</td>
<td>14.8 ± 1.1</td>
<td>13.5 ± 0.8</td>
</tr>
<tr>
<td>Sensitivity (photons·μm&lt;sup&gt;−2&lt;/sup&gt;·s&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>35.7 ± 10.4</td>
<td>14.1 ± 2.1</td>
<td>3.5 ± 2.7</td>
</tr>
<tr>
<td>Time to peak (ms)</td>
<td>169 ± 9</td>
<td>471 ± 15</td>
<td>178 ± 7</td>
</tr>
<tr>
<td>Integration time (ms)</td>
<td>448 ± 34</td>
<td>790 ± 35</td>
<td>495 ± 29</td>
</tr>
<tr>
<td>Single-photon response (pA)</td>
<td>0.71 ± 0.08</td>
<td>1.55 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.76 ± 0.09</td>
</tr>
</tbody>
</table>

<sup>a</sup>Significant difference was observed between WT and PDE6β<sup>−/−</sup> parameters. One-way ANOVA with the post hoc Tukey's HSD test determined significant differences (P < 0.05) between WT and PDE6α<sup>−/−</sup>.

Figure 7. A. Single photon responses from WT (black), PDE6α<sup>−/−</sup>-treated (red), and PDE6β<sup>−/−</sup>-treated (blue) rod cells. Single-photon responses were obtained by dividing each dim-flash response (i<sub>max</sub>) by the estimated number of activated rhodopsin per rod, with the collecting area assumed to be 0.5 μm<sup>2</sup>. The traces are averaged from 10 WT, 14 PDE6α<sup>−/−</sup>-treated, and 22 PDE6β<sup>−/−</sup>-treated individual cells. Error bars represent SEM. B. Comparison of dim-flash responses scaled at peak amplitude.

Discussion

In this study, we expressed cone PDE6α<sup>−/−</sup> subunit exogenously in the retina of rd10 mice to investigate its biochemical and light signaling properties in a rod cell environment. Our results demonstrate that cone PDE6α<sup>−/−</sup> can functionally substitute for rod PDE6α to mediate light signaling in rods, as shown by full-field ERG analysis, behavior experiments, and single-cell recordings. Rod PDE6 catalytic subunits are destabilized in PDE6α<sup>−/−</sup>-injected retinas despite the functional and morphological rescue of rods, and restoration of rod light sensitivity is mediated by assembly of cone PDE6α<sup>−/−</sup> with rod PDE6γ. Rods with cone PDE6α<sup>−/−</sup> are approximately two times more sensitive to light than WT cells, and this difference is likely the result of the slower shutoff of their light responses. The slower rate of deactivation indicates that inhibition by rod PDE6γ or the hydrolysis ofρ<sup>−/−</sup>-GTP on PDE6α<sup>−/−</sup>-transducin α complex by regulator of G protein signaling-9 (RGS9) is less efficient than normal.

We demonstrated previously that AAV-mediated subretinal delivery of rod PDE6β<sup>−/−</sup> transgene confers long-term rescue of visual function and morphological preservation of the rd10 retinas (Pang et al., 2011). In the present study, AAV8 Y733E cone PDE6α<sup>−/−</sup>-treated rd10 retinas showed comparable levels of rescue in gross morphology, amplitudes of rod-driven full-field ERG signals, and the maximal amplitude of single-photon responses, clearly demonstrating that cone PDE6α<sup>−/−</sup> can couple effectively to the rod visual signaling pathway in response to light. Our work complements the previous finding of the ability of rod PDE6 to substitute for cone PDE6 to mediate visual signaling in the rd10 mouse model (Kolandaivelu et al., 2011). Although the PDE6α<sup>−/−</sup> transgene was driven by an scCBA ubiquitous promoter, we detected most PDE6α<sup>−/−</sup> in photoreceptor cells in which it is normally expressed. We observed similar phenomenon of endogenous cell-specific expression in the cases of PDE65 (Pang et al., 2006), transducin (Deng et al., 2009), and PDE6β (Pang et al., 2011) proteins when using the ubiquitous CBA promoter. The significant scatter in the sensitivity of the AAV-treated rods (Fig. 6D, inset) most likely reflects the variability of AAV-mediated PDE6 expression.

We also showed that vector-expressed cone PDE6α<sup>−/−</sup> localized properly to OS membranes is essential for rapid activation by transducin (Liebmann et al., 1987). Cone PDE6α<sup>−/−</sup> ectopically expressed in rods of X. laevis was shown to colocalize with endogenous PDE6α on disc rim regions in rod OSs (Muradov et al., 2009). The similar rising phases of dim-flash responses between PDE6α<sup>−/−</sup>-treated rd10 and WT rods as shown with single-cell recordings suggest that the activation rate of the catalytic cone PDE6α subunit in the rod environment is comparable with that of rod PDE6α and that exogenously expressed cone PDE6α<sup>−/−</sup> is appropriately localized to ROS disk membranes.

We further show that the restoration of light sensitivity in rd10 rods is attributable to the assembly of cone PDE6α<sup>−/−</sup> with rod PDE6γ. Robust expression of PDE6γ was observed in injected retinas, most likely as a result of complex formation with the virus-introduced PDE6α<sup>−/−</sup>. The presence of cone PDE6α<sup>−/−</sup> or restoration of rod cells did not help in preserving rod PDE6α, which was degraded without its PDE6β partner. It appears that, regardless of cell type, cone PDE6α<sup>−/−</sup> forms homodimers to be functional in vivo. The same holds true for rod PDE6 in the sense that PDE6α<sup>−/−</sup> and PDE6β are obligated to function as heterodimers (Kolandaivelu et al., 2011). Apparently, the state of association is determined by the properties of the subunits rather than the photoreceptor cell type. All families of vertebrate cyclic nucleotide phosphodiesterases function as homodimers, and, although the reason behind the heterodimerization of rod PDE6 is not known, it presumably exists as a mechanism to control the amount of functional PDE6 enzyme present in rods (Kolandaivelu et al., 2011).

The equivalent rate of activation between WT rods and rd10 rods expressing cone PDE6α<sup>−/−</sup> or PDE6β<sup>−/−</sup> suggests that activated ρ<sup>−/−</sup>-GTP can effectively release the inhibitory constraint of rod PDE6γ from cone PDE6α<sup>−/−</sup> catalytic domain. However, the slower shutoff of PDE6α<sup>−/−</sup>-treated rods indicates that deactivation of cone PDE6α<sup>−/−</sup> by inhibitory rod PDE6γ or the hydrolysis of ρ<sup>−/−</sup>-subunit-bound GTP on PDE6α<sup>−/−</sup>-transducin complex is less efficient. The GAFα domains also bind to the inhibitory γ-subunits and play a role in the dimerization of the PDE6 catalytic subunits (Muradov et al., 2004). The strength of interaction between PDE6γ and GAF domains is modulated by cGMP binding to GAF domain, cGMP binding induces an allosteric GAF conformational change and enhances PDE6γ binding affinity, and, in a reciprocal manner, binding of γ-subunit to PDE6 catalytic dimer increases the binding affinity of cGMP to the GAF domains (Yamazaki et al., 1982; Cote et al., 1994). Accordingly, dissociation of either one weakens the binding of the other. Based on a structural study of PDE6γ (Barren et al., 2009), it has been suggested that the interaction between ρ<sup>−/−</sup>-GTP and PDE6γ induces a hinge-like movement of the last 10 residues away from...
the enzyme active site without the Toe–GTP/PDE6γ complex completely dissociating from the PDE6 holoenzyme. The inactivation of Toe–GTP by its intrinsic GTPase activity is the rate-limiting step to restore the photoreceptor to a dark-adapted state and its regulator RGS9-1 associates with PDE6γ to accelerate the GTPase activity of Toe–GTP (Arashtavan & Burns, 2012). The multiple interactions of PDE6γ with PDE6βα, Toe–GTP, and RGS9-1 complex are likely to occur in a precisely controlled temporal sequence that coordinates the activation and deactivation of PDE6 (Zhang et al., 2012). The major sequence difference between cone and rod PDE6 resides in the GAF domains, with cone PDE6 displaying a lower affinity toward cGMP. The relative affinity of rod PDE6βγ binding to Toe–GTP versus the PDE6 catalytic subunits may be defined by the state of cGMP occupancy on the GAF domains of PDE6αγ. Likewise, the affinity of rod PDE6γ for vector-expressed cone PDE6αγ may be lower than that for the rod PDE6βγ. These differences may contribute to the slower inactivation of the cone PDE6αγ expressed in rods. It would be interesting to study the effects of replacing rod PDE6γ with cone PDE6γ or the entire rod PDE holoenzyme with cone PDE6, because PDE6γ critically regulates phototransduction through on- and off interactions with PDE6βα. Toe–GTP, and RGS9-1. Overall, it is difficult from our results to gain a clear view of the role of PDE in the differences in sensitivity or kinetics between rods and cones. Interestingly, although rods and cones share the same GAP complex, cones express RGS9 at higher levels (Zhang et al., 2003). This observation, together with the slow inactivation of cone PDE6αγ in rods observed by us indicate that, perhaps, the timely Toe–GTP/PDE complex inactivation in cones requires higher GAP activity than in rods.

Finally, our single-cell recordings from cone PDE6αγ-treated rd10 rods demonstrated an unusual response acceleration with increasing flash strength. This, together with a shallower intensity–response curve for these rods indicates a potential acceleration of cone PDE inactivation with increased phototransduction activation. A direct modulation of PDE activity was recently suggested as an additional adaptation mechanism in mouse rods (Chen et al., 2012), although it has not been directly demonstrated. Notably, however, we did not observe substantial response acceleration in wild-type or PDE6βα-treated rd10 rods, suggesting that this is a cone PDE-specific phenomenon. Such a negative feedback modulation of cone PDE6 is an exciting novel concept and represents a potential mechanism for extending the functional range of cones. Future studies should help elucidate the mechanisms that regulate cone PDE6 activity and how this phenomenon affects cone light adaptation.

References
Self-complementary AAV-mediated gene therapy restores cone function and prevents cone degeneration in two models of Rpe65 deficiency. Gene Ther 17:815-826. CrossRef Medline


