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Cone Phosphodiesterase-6α' Restores Rod Function and Confers Distinct Physiological Properties in the Rod Phosphodiesterase-6β-Deficient rd10 Mouse

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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 PDE6α 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 PDE6β subunit. We show that adenovirus-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 PDE6γ. 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., 2001), the signaling properties of rod and cone transducin are not different from 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

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Each of the catalytic subunits of PDE6 consists of two N-terminal regulatory cGMP-binding GAF (for cGMP-specific phosphodiesterases, adenyl cyclases, and FlIA) 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 (Moon and Cote, 2001; Muradov et al., 2010). Among the GAF domains, rod PDE6 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 PDE6y might 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 PDE6y. 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 a 14 hr light period. 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 or PDE6β cDNA was purchased from Invitrogen. The aden-associated virus (AAV) vector containing murine PDE6α or PDE6β cDNA under the control of a small chimeric β-actin (smCBA) promoter was packaged in AAV serotype 8 (AAV8) Y735F 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 AAV8 Y735F-smCBA-PDE6α or PDE6β vector (4.25 × 10^12 vector genomes/ml) was injected subretinally into the left eyes, and the right contralateral eyes served as untreated controls. Subretinal injections were performed as described previously (Pang et al., 2006, 2008). Briefly, a 35-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 blebs. One percent tribromol eye drops and neomycin-polymyxin B/dexamethasone ophthalmic ointment were given after injection.

Electroretinogram analyses. At 5 weeks after injection, in vivo and in vitro electrotetrograms (ERGs) were recorded separately using a UTAS Visual Diagnostic System equipped with Big Shot 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. Photopic cone recording were taken after mice were adapted to a white background light of 30 cd·m²/2 for 5 min. Recordings were performed with four flash intensities at 0, 0.7, 1.0, and 1.4 log cd·m²/2 in the presence of 30 cd·m²/2 background light. Fifty responses were recorded and averaged at each intensity. Scotopic and photopic b-wave amplitudes were analyzed from untreated, treated 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 eyes were killed 4 hours after ERG recordings for morphological and immunohistochemical analysis. The eyes were fixed in a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde for 3 h at room temperature and then paraffin embedded 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 eyeball was rinsed with PBS and then cryoprotected by placing it in 30% sucrose in PBS for 4 h at 4°C. Eyeballs 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 h at room temperature. Anti-PDE6α (5184P) (Kirschman et al., 2010), rhodopsin, or red/green-cone opsin (Millipore Bovine Retina Reagents) antibodies (all 1:1000 dilution) 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 h, and washed with PBS. Sections were mounted with Vectashield Mounting Medium for Fluorescence (H-1000; Vector Laboratories) and covered slipped. Sections were analyzed with a Carl Zeiss CD25 microscope fitted with Axiosvision release 4.6 software.

Western blot analyses. Untreated, AAV8 Y735F-smCBA-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 ex-
Figure 2. ERG responses, retinal morphology, immunohistochemistry, and scotopic visual acuity of rd10 mice after AAV8 Y733F-smCBP-PDE6βα' 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 at 5 weeks after injection. C, Dark-adapted ERG was partially restored in injected rd10 eyes. Statistical analysis demonstrated a significant difference between un.injected and fellow vector-treated eyes for dark-adapted b-waves at t = -2.6, -0.6, and 0.4 log cd/m² (p < 0.01). D, Light-adapted ERG responses were improved in treated rd10 eyes compared with untreated controls as a result of rod function rescue and cell survival (p < 0.02). Error bars are mean ± SEM. E, Comparison of ERG responses between PDE6βα', PDEβ-treated, and PDE6α-treated rd10 eyes at 5 weeks after injection. There were no significant differences in dark-adapted b-wave amplitudes at three light intensities tested between PDE6βα' treated and PDE6α-treated rd10 eyes (all p > 0.1). Error bars represent the mean ± SEM. F, Wave amplitude at flicker intensities was compared by repeated-measures ANOVA, with the Bonferroni’s post-hoc test for ANOVA (p < 0.01) used to compare means at individual flicker intensities. F, Restoration of scotopic visual acuity in PDE6α–treated rd10 mice 5 weeks after injection. Data were derived from mouse optomotor responses to rotating gratings under background monitor luminance of −4.45 log cd/m². Error bars are mean ± SEM.

Immunoprecipitation. Frozen retinal eyecups from untreated, AAV8 Y733F-smCBP-PDE6α'–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 10 mM iodoacetamide using a pestle (VWR) in a 1.5 ml Eppendorf tube on ice (15 s for three times). After homogenization, Triton X-100 was added to a final concentration of 1% (500 μl total volume). The homogenized retinal extracts were preclotted by addition of 10 μl of immunopure immobilized Protein A plus beads (Thermo Fisher Scientific) by incubating at 4°C for 1 h. Supernatants were collected by centrifuging at 16,000 × g (Eppendorf 5414) 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 Lisanlomil sample buffer and separated by 4–20% SDS–polyacrylamide gel (Bio-Rad) and transferred to Immunoblot LF PVDF membrane (Bio-Rad). Immunoblot analyses were performed with individual rod PDE6α, PDE6β, and PDE6γ subunits and cone PDE6βα’ (3184p tail)-specific primary antibodies according to our previously published method (Kolandarew 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 Na₂HPO₄, 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, and 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 (Koenig-Tite), 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 (λ_max = 500 nm) filters (Edmund Optics). Intensity–response data were fit by the Hill equation: R / R_{max} = P / (P + h^n), where P is the transient peak amplitude of response, R_{max} is maximal response amplitude, h is flash intensity, and n 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 (Umino et al., 2007).
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 (Frisky 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 cd/m², which was set by neutral density filters.

Visual acuity was defined as the threshold for spatial frequency (F₀) of gratings with 100% contrast and measured at the speed (S₀) of 6.0°/s. F₀ was gradually altered by the computer protocol until its combined threshold for both stimuli directions was determined. Temporal frequency (F₉) was automatically adjusted by the computer software, based on the following equation: F₀ = S₀ × F₉ (Umino 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 cd/s/m² in treated eyes was 109 ± 39 µV (mean ± SD), whereas it was undetectable in contralateral untreated eyes (n = 3, p < 0.01). The treated eye rod ERG b-wave amplitude was ~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. 1C). The average cone b-wave amplitude was 44 ± 8 µV (mean ± SD) in injected eyes versus 27 ± 10 µV in contralateral untreated eyes at 1.4 log cd/s/m² (n = 3, p < 0.02). We also recorded ERG responses
from some rd 10 mice injected with vector expressing PDE6β and observed no significant differences between PDE6β and PDE6α treatments (Fig 1E), suggesting that therapy in the rd 10 mouse was equivalent whether we used the heterologous rod subunit or the homologous cone subunit. Finally, the scotopic visual acuity of PDE6α-treated rd 10 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 rd 10 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 rd 10 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 rd 10 eyes compared with their spotty staining observed in uninjected controls.

We also recorded ERG responses from rd 10 mice at 5 months after treatment (Fig 4A,B). The average rod-driven ERG b-wave amplitude (Fig 4A) at a flash intensity of −1.6 log cd/m² was 81 ± 15 μV (mean ± SD), and it was significantly higher than the undetectable ERGs in untreated eyes (μ = 3, p < 0.05). 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) (μ = 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 rd 10 rod structure and function persisted even months after the AAV injection.

Cone PDE6α binds to rod PDE6g to restore rod function

Restoration of the light-dependent rod response in rd 10 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 rd 10 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 retinas. 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 (Kolandaivelu 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 uninjected 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' assembled 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 photoreponses from 14 PDE6α'-treated rods (from two animals) and 22 PDE6β-treated rods (from three animals). No significant differences were found between the photoreponses of WT and PDE6β-treated rd10 rods (compare with Fig. 6A,C; Table 1), 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 photoreponses, were comparable among WT, PDE6β-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 occurs at comparable rates. However, the response recovery phase was significantly delayed in PDE6α'-treated rods, indicating that the deactivation 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

|            | WT (n = 10) | PDE6α' (n = 14) | PDE6β (n = 22) |
|------------|-------------|-----------------|----------------|
| Dark current (pA)    | 16.0 ± 1.0  | 14.8 ± 1.1      | 13.5 ± 0.8     |
| Sensitivity (photons/μm²) | 35.7 ± 10.4 | 14.1 ± 2.1      | 35.2 ± 7.7     |
| Time to peak (ms)    | 169 ± 9     | 467 ± 31*       | 178 ± 7        |
| Integration time (ms) | 448 ± 34    | 790 ± 35        | 495 ± 29       |
| Single photon response (pA) | 0.71 ± 0.08  | 1.55 ± 0.22     | 0.76 ± 0.09    |

*Significant difference in the response parameters of PDE6α' compared to WT and PDE6β.

These response features suggest a possible light-dependent feedback modulation directly on cone PDE6α'.

Discussion

In this study, we expressed cone PDE6α' 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' can functionally substitute for rod PDE6αβ to mediate light signaling in rods, as shown by full-field ERG analysis, behavioral experiments, and single-cell recordings. Rod PDE6 catalytic subunits are destabilized in PDE6α' injected retinas despite the functional and morphological rescue of rods, and restoration of rod light sensitivity is mediated by association of cone PDE6α' with rod PDE6γ. Rods with cone PDE6α' are approximately two times more light sensitive than WT cells, and this difference is likely the result of the slower shut off of their light responses. The slower rate of deactivation indicates that inhibition by rod PDE6γ or the hydrolysis of α-GTP on PDE6α'—transducin complex by regulator of G-protein signaling (RGS9) is less efficient than normal.

We demonstrated previously that AAV-mediated subretinal delivery of rod PDE6β transgene conferred long-term rescue of visual function and morphological preservation of the rd10 retinas (Pang et al., 2011). In the present study, AAV8 V733F cone PDE6α' injected 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' 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 Nrl-/- cpfl1 mouse model (Koletti-Dale et al., 2011). Although the PDE6α transgene was driven by an smCBA ubiquitous promoter, we detected most PDE6α' in photoreceptor cells in which it is normally expressed. We observed similar phenomenon of endogenous cell-specific expression in the cases of RPE65 (Pang et al., 2006), transducin (Deng et al., 2009), and PDE6β (Pang et al., 2011) proteins when using the ubiquitous smCBA 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' localized properly in ROS membrane, which is essential for rapid activation by transducin (Liebman et al., 1987). Cone PDE6α' ectopically expressed in rods of Xenopus laevis was shown to colocalize with endogenous PDE6 on disc rim regions in rods (Muradov et al., 2009). The similar rising phases of dim-light responses between PDE6α'-treated rd10 and WT rods as shown in single-cell recordings suggest that the activation rate of the catalytic cone PDE6 subunit in the rod environment is comparable with that of rod PDE6 and that exogenously expressed cone PDE6α' 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' with rod PDE6γ. Robust expression of PDE6γ was observed in injected retina, most likely as a result of complex formation with the virus-introduced PDE6α'. The presence ofcone PDE6α' in rod cells did not help in preserving rod PDE6α, which was degraded without its PDEβ partner. It appears that, regardless of cell type, cone PDE6α' forms homodimers to be functional in vivo. The same holds true for rod PDE6 in the sense that PDE6α and PDE6β are obligated to function as heterodimers (Koletti-Dale 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 (Koletti-Dale et al., 2011).

The equivalent rate of activation between WT rods and rd10 rods expressing cone PDE6α' or PDE6β suggests that activated α-GTP can effectively release the inhibitory constrain of rod PDE6γ from cone PDE6α' catalytic domain. However, the slower shut off of PDE6α'-treated rods indicates that deactivation of cone PDE6α' by inhibitory rod PDE6γ or the hydrolysis of α-subunit-bound GTP on PDE6α'-transducin complex is less efficient. The Gαα 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 Gαα domains is modulated by cGMP binding to Gαα domain. cGMP binding induces an allosteric Gαα 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 Gαα domains (Yamanaka 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 (Barrett et al., 2009), it has been suggested that the interaction between α-GTP and PDE6γ induces a hinge-like movement of the last 10 residues away from
the enzyme active site without the Toc–GTP/PDE6γ complex completely disassociating from the PDE6 holoenzyme. The inactivation of Toc–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 Toc–GTP (Arshavsky and Burns, 2012). The multiple interactions of PDE6γ with PDE6αβ, Toc–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 PDE6β binding to Toc–GTP versus the PDE6 catalytic subunits may be defined by the state of cGMP occupancy on the GAF domains of PDE6αβ. Likewise, the affinity of rod PDE6γ for vector-expressed cone PDE6α may be lower than that for the rod PDE6αβ. 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 PDE6αβ, Toc–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 Toc–GTP/PDE complex inactivation in cones requires higher GAP activity than in rods.

Finally, our single-cell recordings from cone PDE6αβ–treated rd10 rods demonstrated an unusual response activation 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 WT or PDE6αβ-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 mechanism(s) that regulates cone PDE6 activity and how this phenomenon affects cone light adaptation.

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