Cone Phosphodiesterase-6γ’ Subunit Augments Cone PDE6 Holoenzyme Assembly and Stability in a Mouse Model Lacking Both Rod and Cone PDE6 Catalytic Subunits

Wen-Tao Deng
Saravanan Kolandaivelu
Astra Dinculescu
Jie Li
Ping Zhu

See next page for additional authors

Follow this and additional works at: https://researchrepository.wvu.edu/faculty_publications
Part of the Medical Biochemistry Commons, Neurosciences Commons, and the Ophthalmology Commons
Authors
Wen-Tao Deng, Saravanan Kolandaivelu, Astra Dinculescu, Jie Li, Ping Zhu, Vince A. Chiodo, Visvanathan Ramamurthy, and William W. Hauswirth
Cone Phosphodiesterase-6γ’ Subunit Augments Cone PDE6 Holoenzyme Assembly and Stability in a Mouse Model Lacking Both Rod and Cone PDE6 Catalytic Subunits

Wen-Tao Deng1*, Saravanan Kolandaivelu2, Astra Dinculescu1, Jie Li1, Ping Zhu1, Vince A. Chiodo1, Visvanathan Ramamurthy2 and William W. Hauswirth1

1Department of Ophthalmology, University of Florida, Gainesville, FL, United States, 2Departments of Ophthalmology and Biochemistry, Center for Neuroscience, West Virginia University, Morgantown, WV, United States

Received: 27 March 2018
Accepted: 13 June 2018
Published: 09 July 2018

Keywords: phosphodiesterase 6 (PDE6), rd10, Cpf11, adeno-associated viral vector (AAV), achromatopsia, gene therapy, rod, cone
INTRODUCTION

Cyclic nucleotide phosphodiesterases 6 (PDE6) belongs to the highly conserved mammalian PDE family of 11 different families that modulate cellular levels of the second messengers, cAMP and cGMP, by controlling their rates of degradation (Beavo, 1995; Francis et al., 2001). PDE6 is the key regulator of cytoplasmic cGMP concentration in rod and cone photoreceptor cells. Because of its primary role in controlling cGMP, regulation of PDE6 is essential for the speed, sensitivity, recovery and adaptation of visual signals (Burns and Arshavsky, 2005; Fu and Yau, 2007). Rod PDE6 is composed of two catalytic subunits α and β encoded by the PDE6A, PDE6B genes, and two inhibitory subunits γ encoded by PDE6G. In contrast, cone PDE6 is composed of two identical catalytic subunits of α’ encoded by PDE6C and two cone-specific inhibitory subunits γ’ encoded by PDE6H (Gillespie and Beavo, 1988; Hamilton and Hurley, 1990; Li et al., 1990).

Despite the important role it plays in photoreceptor function, the structure and function relationship of PDE6 is not well understood due to failure of its functional expression in various in vitro systems (Qin et al., 1992; Firiev et al., 1993, 2003; Qin and Baehr, 1994). Electron microscopic analysis of purified rod PDE6 catalytic dimers showed that the catalytic domains are located at the C-terminus, and are highly conserved among all known members of PDE family (Beavo, 1995). The N-terminal domains of PDE6 catalytic subunits consist of two regulatory cGMP binding GAF motifs (GAF A and GAF B) termed for their presence in cGMP-regulated PDE, adenylyl cyclases and the E. coli protein E11A (Aravind and Ponting, 1997). It is known that there is a synergistic effect between PDE6γ and cGMP binding to the catalytic subunits because: (1) PDE6γ binding to the catalytic subunits enhances the affinity of cGMP for the noncatalytic sites; and (2) when cGMP binds to the GAF domains, the affinity of PDE6γ for the catalytic subunits increases (Cote et al., 1994; Yamazaki et al., 2002). The region of PDE6γ responsible for this effect has been mapped to the central polycationic domain of PDE6γ known to have high affinity for the catalytic dimer (Artemyev and Hamm, 1992; Mou and Cote, 2001). Functional mapping of PDE6γ with the catalytic subunits showed that the C-terminus of PDE6γ is a key inhibitory domain. It interacts directly with the active site of PDE6 and blocks access of substrate to the catalytic pocket and therefore controls cGMP hydrolysis (Granovsky et al., 1997; Barren et al., 2009; Zhang and Artemyev, 2010). The middle region of PDE6γ stabilizes the overall binding affinity of PDE6γ with the catalytic subunits (Zhang et al., 2012).

One unique feature of rod PDE6 among the PDE family is that its catalytic subunits are composed of heterodimer αβ subunits. Evidence suggests that PDE6αβ may form structurally and functionally distinctive interactions with the α- and β-subunits, and this asymmetric binding of PDE6γ to PDE6αβ may represent an important regulatory mechanism in rod phototransduction (Guo et al., 2005, 2006). The molecular mechanism underlying the distinctive photoreponses of rods vs. cones is poorly understood and is one of the fundamental questions remaining in photoreceptor biology. The potential roles of rod and cone PDE6 in phototransduction is of particular interest because PDE6’s functions in rods and cones as effector enzymes in controlling cGMP levels in the outer segments is key to fully understanding of rod and cone photoreponses. We have shown previously that rods of rd10 mice expressing PDE6α γ-delivered by an adeno-associated virus (AAV) vector are more sensitive to light than wild type rods, most likely due to the slower shutoff of their light responses (Deng et al., 2013). A similar study employing a transgenic approach showed that exchange of cone PDE6α’ for rod PDE6αβ partially mimics the features of light adaptation (Majumder et al., 2015). Here, we study the association affinities among different subunits of PDE6 following AAV-mediated delivery in rd10/cpfl1 mice. The rd10/cpfl1 mice carry mutations in both rod PDE6β and cone PDE6α, and they also lack rod PDE6γ and cone PDE6γ’. We find that cone PDE6α’ has an intrinsically higher affinity for its partner PDE6γ’ than for PDE6γ, and the presence of PDE6γ’ augments cone PDE6 holoenzyme assembly and enhances its stability.

MATERIALS AND METHODS

Animals
Rd10, cpfl1, and wild-type C57BL/6J mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Rd10 and cpfl1 mice were crossed to generate homozygous rd10/cpfl1 mutant mice. Genotyping of rd10 and cpfl1 were performed according to previously published methods (Chang et al., 2007; Kolandaivelu et al., 2011). The mice were 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. Physiological experiments were performed under dim red illumination using a Kodak number 1 Safelight filter (transmittance > 560 nm). All experiments were approved by the local Institutional Animal Care and Use Committees at the University of Florida and West Virginia University and conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and NIH regulations.

Construction and Packaging of AAV Vectors
The murine PDE6γ’ cDNA was synthesized by GenScript (Piscataway, NJ, USA) and subcloned under the ubiquitous, constitutive small chicken β-actin promoter (smCBA; Haire et al., 2006) in self-complimentary AAV vectors to create sc-smCBA-mPDE6γ’. Construction of smCBA-mPDE6β and smCBA-mPDE6α were described previously (Deng et al., 2013). All constructs were packaged in AAV serotype 8-Y733F by transfection of H293 cells according to previously published methods (Zolotukhin et al., 1999).

Subretinal Injections
All vectors were adjusted to the same titer of 5 × 10^{12} vector genomes/ml before injection. One microliter of vector was injected subretinally into the left eyes of rd10/cpfl1 pups at postnatal 14 (P14) while the right eyes served as untreated controls. For co-injection, smCBA-mPDE6α’...
and sc-smCBA-mPDE6γ’ vectors were mixed at a 2:1 ratio and 1 µl injected. Subretinal injections were performed as previously described (Pang et al., 2006, 2008). Briefly, eyes were dilated with 1% atropine (Akorn, Inc. Lake Forest, IL, USA) and 2.5% phenylephrine hydrochloride solution (Paragon Biotek, Portland, OR, USA). An aperture within the pupil area was made through the cornea with a 30 gauge disposable needle. A 33-gauge blunt needle mounted on a 5-µl Hamilton syringe (Hamilton Co., Reno, NV, USA) was then introduced through the corneal opening, avoiding the lens and reaching the subretinal space. Injections were visualized by fluorescein-positive subretinal bleb. 1% atropine eye drops and neomycin/polymyxin B/dexamethasone ophthalmic ointment (Bausch and Lomb Inc. Tampa, FL, USA) were given after injection.

**ERG Analysis**

At 5 weeks post-injection, dark-adapted and light-adapted electroretinograms (ERGs) were recorded separately using a UTAS Visual Diagnostic System equipped with Big Shot Ganzfeld (LKC Technologies, Gaithersburg, MD, USA) according to protocols previously describe (Deng et al., 2013). Scotopic rod recordings were performed with three increasing light intensities at −1.6, −0.6, and 0.4 log cd/m² after overnight dark adaptation. 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 for 10 min. Recordings were performed with four flash intensities at 0.1, 0.7, 1.0 and 1.4 log cd/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, treated rd10/cpf1 and wild-type controls at each intensity were averaged and used to generate a standard deviation.

**Immunohistochemistry and Morphology**

Three days after ERG recordings, mice were sacrificed and eyes were enucleated for immunohistochemical analysis. Eyes were fixed in 4% paraformaldehyde at room temperature for 3 h. Cornea, lens and vitreous were removed from eyes without disturbing the retina. The remaining eyecup was rinsed with PBS and then cryoprotected by immersion in 30% sucrose in PBS at −80°C. Eyecups were then embedded in cryostat compound (Tissue Tek OCT, Sakura Finetek USA, Inc., Torrance, CA, USA) and frozen at −80°C. Embedded eyecups were sectioned at 12 µm thickness, rinsed in PBS, and blocked in 3% BSA, 0.3% Triton X-100 in PBS for 1 h at room temperature. Primary antibodies were: PDE6α’ (3184P, A polyclonal antibody raised by one-way ANOVA with Bonferroni test to compare means at indicated flash intensities. Protein quantifications were analyzed by one-way ANOVA with Bonferroni post hoc test to compare means of each treatment. Data were expressed as mean ± SEM.

**RESULTS**

**Histological Analysis of rd10/cpf1 Mouse Retinas**

We first compared rod photoreceptor cell degeneration in rd10/cpf1 mice to rd10 mice reared in the dark. Histological
We first characterized the expression of cone PDE6
Retinas
rd10/cpfl1
PDE6
Rod and Cone Function in PDE6
3 weeks of age (data not shown).

mice exhibited both flat scotopic and photopic ERG responses at
age (Chang et al., 2009).

Rd10/cpfl1
cone degeneration and complete cone loss by 10 weeks of
is vacuolization of a small subset of cells in the photoreceptor
retinas display grossly normal morphology and layering, there
involuciolation of a small subset of cells in the photoreceptor
layer indicating cone loss as early as 3 weeks, with continued
cone degeneration and complete cone loss by 10 weeks of
age (Chang et al., 2009). Rd10/cpfl1 mice show a cone opsin
staining pattern similar to cpfl1 at 2 weeks of age, followed by
rapid cone photoreceptor cell degeneration with a majority
of cones absent by 4 weeks of age. By 8 weeks, cone opsin
staining was undetectable (Figure 1B). Cones of rd10/cpfl1 mice
degenerated substantially faster than those in cpfl1 mice most likely
due to concomitant rod degeneration. Furthermore, rd10/cpfl1
mice exhibited both flat scotopic and photopic ERG responses
at 3 weeks of age (data not shown).

Rod and Cone Function in PDE6α' and
PDE6α'+PDE6γ'-treated rd10/ cpfl1
Retinas
We first characterized the expression of cone PDE6α' in
rd10/cpfl1 mice treated with AAV8 Y733F-smCBA-PDE6α'. The
vector was subretinally delivered into one eye of postnatal 14
(P14) rd10/cpfl1 mice, while the contralateral eyes remained
untreated and served as controls. PDE6α' expression
was analyzed by immunostaining at 5 weeks post-injection.
In C57BL/6 wild-type eyes, PDE6α' is expressed specifically in
cones (Figure 2). Untreated rd10/cpfl1 eyes showed no PDE6α'
expression, whereas injected eyes showed robust PDE6α'
expression in both rods and cones.

We next characterized the rod function in PDE6α'-
and PDE6α'+PDE6γ'-treated rd10/cpfl1 eyes by full-field ERG
analysis. Expression of PDE6α' or PDE6α'+PDE6γ' driven by
smCBA promoter led to significant restoration of scotopic
ERG responses at 5 weeks post-injection (Figures 3A–C). Both
average b-wave and a-wave amplitudes in treated eyes are
significantly higher than their undetectable ERG responses in
contralateral untreated eyes at three light intensities tested. The
average rod-mediated b-wave amplitude at a flash intensity of
−1.6 log cd s m−2 was 166 ± 14 µV (mean ± SEM) in PDE6α'-
treated eyes, significantly higher than untreated controls (n = 6,
P < 0.001, F = 253.0), and approximately 50% of those in the
wild-type controls. The average rod-mediated a-wave amplitude
at a flash intensity of −1.6 log cd s m−2 was 21 ± 4.9 µV in
PDE6α'-treated eyes, significantly higher than untreated controls
(n = 6, P < 0.01, F = 28.34). Measured rod ERG responses were
similar in eyes receiving PDE6α' alone or PDE6α' and PDE6γ'
mixture (n = 6, P > 0.05).

Rescue of rod photoreceptor cells in PDE6α'-treated eyes
was also confirmed by examination of retinal morphology and
immunostaining for rhodopsin. Hematoxylin and eosin
staining (H&E) showed partial retinal structure preservation in
treated eyes with 7–8 layers of photoreceptor nuclei retained
in the ONL, while only one layer remained in contralateral
untreated eyes. Retinal outer segments were also preserved in
treated eyes (Supplementary Figure S1A). Consistent with this
result, immunostaining with a rhodopsin antibody showed much
stronger rhodopsin expression in treated eyes compared to
almost undetectable staining in untreated eyes (Supplementary
Figure S1B).

In contrast to robust rod rescue in rd10/cpfl1 mice by
PDE6α’, rescue of cone function was significantly muted in
PDE6α'-treated eyes (Figures 3D,E). To test the hypothesis
that the presence of PDE6γ' might assist proper folding
of cone PDE6 holoenzyme and improve cone ERG rescue,
PDE6γ' driven by a smCBA promoter packaged in a fast-acting
self-complimentary AAV vector was co-injected with PDE6α'.
Improved photopic ERG responses were obtained with an
average cone b-wave amplitude of 38 ± 1.5 µV (n = 6) at
1.4 log cd s m−2, which was significantly higher than amplitudes
from eyes treated with PDE6α' alone (12 ± 1.9 µV, P < 0.001,
F = 580.4). Immunostaining with M/S-opsin antibodies and
PNA in co-injected eyes showed cone morphology rescue
(Supplementary Figure S1C). Wild cone morphology rescue was
also observed in PDE6α'-treated eyes (data not shown), however,
these cones mostly existed because of slower degeneration due to
rescue of rods. Our results suggest that the presence of PDE6γ'
from an earlier onset expression vector may help the proper
folding, assembly, and stabilization of PDE6α' more efficiently.
The stability of the PDE6 α’γ’ complex could be vital for
enhancing the survival and function of cones.

Presence of PDE6γ' Augments Cone
PDE6 Assembly and Stability
Rd10/cpfl1 mice carry mutations in both rod PDE6β and cone
PDE6α'. Next we investigated the levels of PDE6α’, PDE6γ'
and PDE6γ subunits by Western blot analysis from retinal
extracts of C57Bl/6 wild-type control, uninjected rd10/cpfl1, and
rd10/cpfl1 injected with PDE6β, PDE6α’, or PDE6α' + PDE6γ'.
We took advantage of the fact that cone specific PDE6γ' has
a slightly higher molecular weight than rod PDE6γ (Gillespie and
Beavo, 1988) and used the same antibody to distinguish them.
Western blot analysis showed that untreated rd10/cpfl1 eyes
lacked all three rod PDE6 subunits (Figure 4A; Supplementary
Figure S2), and PDE6α’ treatment restored expression of rod
PDE6γ subunits, similar to eyes treated with rod PDE6β
(Figure 4A). However, cone PDE6γ' expression was not detected
in PDE6α'-treated eyes. In contrast, rd10/cpfl1 eyes co-injected
with PDE6α' + PDE6γ' showed significantly higher levels of
expression of both PDE6α' and PDE6γ' (Figure 4A). Qualitative analysis of the amount of PDE6α', PDE6γ and PDE6γ' from these samples showed that PDE6α' levels were approximately double in co-injected eyes compared to eyes receiving only PDE6α' (Figure 4B, top panel, N = 3, P < 0.005). The levels of PDE6γ in PDEβ- and PDE6α'-treated eyes were similar (N = 3, P > 0.05) and were about half of that in the wild-type control (N = 3, P < 0.001). However, the levels of PDE6γ were reduced (N = 3, P < 0.05) while the amount of PDE6γ' was about twice as much as PDE6γ in co-injected eyes (Figure 4B, bottom panel). PDE6γ' is delivered in half of the vector genomes of PDE6α' (vector genome ratio 1:2). PDE6γ' expression is expected to occur in both
rods and cones following AAV vector delivery because we are using the nonspecific, ubiquitous smCBA promoter. However, we do not have a PDE6γ'-specific antibody to demonstrate this by immunohistochemistry. The higher PDE6α' levels in co-injected eyes is mostly likely due to the presence of PDE6γ'.

Finally, we investigated the assembly status of PDE6 from the above samples by IP using ROS-I, a mouse monoclonal antibody that exclusively recognizes assembled and functional PDE6 complexes in both rods and cones in rod PDE6α' and cone PDE6γ' mice. We have shown previously that restoration of light sensitivity by cone PDE6α' in rods of rd10 mice was attributed to functional complex formation between cone PDE6α' and rod PDE6γ' (Deng et al., 2013). Here we confirm that rescue of rod function by PDE6α' in rd10/cpfl1 mice is also due to stabilization of endogenous PDE6γ and the resultant functional complex formation of PDE6α'γ' (Figure 4C, Supplementary Figure S2). Interestingly, in PDE6α'+PDE6γ' co-injected eyes, the majority of PDE6α' coupled with PDE6γ', with only a small amount of PDE6α' associated with rod PDE6γ' (Figure 4C), although PDE6γ was restored due to presence of PDE6α'. These results suggest that the reduced level of PDE6γ in co-injected eyes might be the result of degradation due to its reduced coupling to PDE6α'. Overall, these results clearly demonstrate that cone PDE6γ' delivered with PDE6α' specifically enhances cone PDE6 assembly and its stability, while assembly of endogenous rod PDE6γ with PDE6α' is severely reduced in presence of PDE6γ'.

**DISCUSSION**

We studied the association among PDE6 subunits in vivo using rd10/cpfl1 mice. The rd10/cpfl1 mice have no detectable rod and cone ERG function, and carry mutations in both rod PDE6β' and cone PDE6α'. Interestingly, these mice also lack rod PDE6αγ and cone PDE6γ'. The loss of both rod and cone PDE6 in these mice lead to early onset and rapid rod and cone photoreceptor cell degeneration. We show here that AAV vector-mediated expression of cone PDE6α' driven by a constitutive promoter can rescue rod-mediated light responses and this functional rescue is achieved through stabilization of endogenous rod PDE6γ and complex formation of PDE6α'γ'. However, improved photopic ERG responses were attained only in eyes co-injected with vectors expressing both PDE6α' and PDE6γ', but not in the eyes injected with the PDE6α' vector alone. The levels of PDE6α' are significantly higher in co-injected eyes and the majority of PDE6α' complexed with PDE6γ', although PDE6γ is also detected. These results demonstrate that PDE6α' has higher intrinsic affinity for PDE6γ' and its presence facilitates cone holoenzyme assembly and augmented its stability.

One of the biggest challenges hampering PDE6 research is failure of functional expression of this enzyme in various in vitro systems. It was recently shown that AIPL1 is an obligate chaperone required for heterologous expression of active PDE6α' in cultured cells, and that the presence of PDE6γ dramatically increases the proportion of correctly folded, functional PDE6 produced in the presence of AIPL1 (Gopalakrishna et al., 2016). Our results showing that the presence of PDE6γ' helps to stabilize cone PDE6α holoenzyme is consistent with this observation. In our experiment, we also find that PDE6γ' increases the stability of PDE6α' and facilitates cone PDE6 assembly demonstrated by the significant elevated amount of PDE6α' as well as assembled and functional cone PDE6. We also found that levels of PDE6γ is reduced in co-injected eyes compared to eyes received PDE6α' alone. We speculate that this reduction is the result of degradation due to its reduced coupling to PDE6α' when PDE6γ' is present. This is supported by previous studies suggesting that stability of PDE6 complex is dependent on the association of its interacting partners. First, in rd10 mice carrying a mutation of PDE6β, PDE6α and PDE6γ were not detected (Deng et al., 2013). Second, AIPL1 interacts with PDE6α and the interaction is essential for PDE6 assembly. In the absence
of AIPL1, all PDE6 subunits are degraded regardless of their normal synthesis (Kolandaivelu et al., 2009). Therefore, our data support the same conclusion that PDE6γ potentiates the proper folding of PDE6α and plays a critical role in the maturation of cone PDE6 holoenzyme.

An interesting question raised by this study is why PDE6γ enhances cone PDE6 assembly and stability compared with rod PDE6γ? A previous study demonstrated that the rod PDE6γ naturally exists in mouse cones and can couple with cone PDE6α to protect cone function and stability (Brennenstuhl et al., 2015). Our data show that the PDE6α can couple with PDE6γ when PDE6γ is absent. However, when PDE6γ is present along with the endogenous PDE6α, PDE6α has a higher association affinity for its natural partner. Such a higher affinity might play a role in the faster light response kinetics of cone photoreceptor cells. Although the overall three-dimensional structure of the rod PDE6 holoenzyme was defined by using negative-stain electron microscopy, the quaternary structure of PDE6αβ as well as the

FIGURE 3 | Scotopic and photopic electroretinogram (ERG) responses in rd10/cpfl1 mice treated with PDE6α alone or PDE6α and PDE6γ together. (A) Representative examples of dark-adapted ERG traces from rd10/cpfl1 mice at 5 weeks following delivery of AAV-smCBA-PDE6α. (B) Dark-adapted b-wave is partially restored in injected rd10/cpfl1 eyes. One-way ANOVA with the post hoc Bonferroni test demonstrated a significant difference between uninjected and contralateral vector-treated eyes at light intensities of −1.6, −0.6 and 0.4 log cds/m² (data shown are mean ± SEM, N = 6, *p < 0.001 at all three light intensities, \( F = 253.0, 134.6 \) and 212.9 at −1.6, −0.6 and 0.4 log cds/m², respectively). (C) Dark-adapted a-wave is partially restored in injected rd10/cpfl1 eyes. One-way ANOVA with the post hoc Bonferroni test demonstrated a significant difference between uninjected and contralateral vector-treated eyes (mean ± SEM, N = 6, *p < 0.01 at all three light intensities, \( F = 28.34, 20.98 \) and 63.20 at −1.6, −0.6 and 0.4 log cds/m², respectively). (D) Representative examples of full-field light-adapted ERG traces from rd10/cpfl1 mice at 5 weeks following delivery of AAV-smCBA-PDE6α alone or co-injection of AAV-smCBA-PDE6α and scAAV-smCBA-PDE6γ. (E) Photopic b-wave amplitudes are significantly higher in PDE6α+γ co-injected eyes than eyes receiving PDE6α alone. The bar graph shows averaged ERG responses from 6 mice at a light intensity of 1.4 log cds/m² (data shown are mean ± SEM, N = 6, *p < 0.01, \( F = 580.4 \)).
Deng et al. PDE6γ Augments Cone PDE6 Assembly

FIGURE 4 | Cone PDE6 assembly and stability is enhanced when PDE6α' and PDE6γ' are co-expressed. (A) Immunoblotting of retinal extracts from wild type control, uninjected rd10/cpfl1, rd10/cpfl1 mice injected with PDE6β, PDE6α' and PDE6α'+PDE6γ', and analyzed with the indicated antibodies. (B) Quantification of cone PDE6α', rod PDE6γ and cone PDE6γ' levels in retinal extracts from samples in (A). Band densities were measured with a Li-COR Odyssey image analyzer. Statistical analysis shows that PDE6α' is significantly higher in PDE6α'+PDE6γ'-treated eyes than eyes treated with PDE6α' alone (top panel, mean ± SEM, N = 3, *p < 0.005), whereas PDE6γ is reduced in PDE6α'+PDE6γ'-treated compared to eyes treated with PDE6α' alone (bottom panel, mean ± SEM, N = 3, *p < 0.05, F = 53.44). There is no significant difference of PDE6γ levels between PDE6β- and PDE6α'-treated eyes (N = 3, p > 0.05). (C) Immunoblots show the assembled rod PDE6 and cone PDE6 when probed with ROS mouse monoclonal antibody from retinal extracts of wild type controls, uninjected rd10/cpfl1, and rd10/cpfl1 mice injected with PDE6β, PDE6α', and PDE6α'+PDE6γ'. Immunoblots were probed with indicated antibodies. Immunoprecipitation (IP) samples are 10 times more concentrated than input fractions. Protein molecular weight markers are shown on the left. T, Total; U, Unbound and E, Elution.

interface between PDE6αβ and their inhibitory subunits PDE6γ remains elusive (Kameni Tcheudji et al., 2001; Goc et al., 2010). In addition, the heterogeneity of the rod PDE6α and β catalytic subunits in terms of their interactions with PDE6γ is poorly understood. There is evidence that PDE6γ may form distinctive structural and functional interactions with the PDE6α and PDEβ subunits (Guo et al., 2005). Despite the fact that rod and cone PDE6 subunits share similar domain organizations, the detailed structural relationships between PDE6α' and γ' is unknown and cone PDE6 may have a different mode of regulation and therefore display different enzymatic kinetics. Single cell recordings from rods expressing PDE6α'γ and PDE6α'γ' would give detailed
information regarding light signaling properties between the two types of rods and might reveal if PDE6γ′ plays a role in the faster light response of cones. The ideal model to perform this experiment would be a knock-in mouse line with endogenous PDE6γ replaced with cone PDE6γ′. In these mice, PDE6γ′ is expressed at the same level as PDE6γ in wild type mice, therefore detailed light response kinetics can be compared. Single cell recordings were not performed here due to the stochastic nature of AAV vector expression.

In our previous study, we showed that rod visual performance measured by optomotor responses was significantly improved in rd10 mice treated with PDE6α′ but remained subpar compared to wild-type controls (Deng et al., 2013). We would expect similar improvement of scotopic visual acuity in rd10/cpfl1 mice treated with PDE6α′ alone or a PDE6α′γ′ mixture. We also note that although improved photopic ERG responses were obtained with co-injection of vectors expressing both PDE6α′ and PDE6γ′, there is a possibility that these photopic recordings were partially derived from rods expressing cone PDE6α′γ′. These rods might be desensitized and could not be saturated by standard scotopic conditions, although our photopic ERG recordings were performed after longer than standard light exposure time for saturating normal rods. One approach to address this issue would be to express PDE6γ′ under a cone-specific promoter instead of a constitutive promoter such as CBA. However, our best cone-preferred promoter still confers some level of expression in the mouse rods (Dyka et al., 2014).

In summary, we show that cone PDE6α′ has an intrinsically higher affinity for its partner PDE6γ′ than for rod PDE6γ and that the presence of PDE6γ′ helps to stabilize cone PDE6 holo-enzyme and augment its assembly. This finding might be important for designing future gene therapy studies for treating patients with PDE6α′-associated achromatopsia.

**AUTHOR CONTRIBUTIONS**
W-TD and SK designed and performed the experiments, and wrote the manuscript. JL, PZ and VC conducted experiments. AD contributed to the data analysis. VR and WH contributed to study supervision. W-TD, SK, AD, VR and WH contributed to the critical revision of the manuscript.

**FUNDING**
This work was supported by National Institutes of Health (NIH) grant T32EY021721 (to WH), unrestricted grants to the Departments of Ophthalmology at the University of Florida from Research to Prevent Blindness (RPB; New York), Macula Vision Research Foundation, and AGTC Inc. This work was also supported by the SK startup fund (VVU) and NIH grant RO1EY017035 (to VR).

**SUPPLEMENTARY MATERIAL**
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnmol.2018.00233/full#supplementary-material

**REFERENCES**

Frontiers in Molecular Neuroscience | www.frontiersin.org 9 July 2018 | Volume 11 | Article 233


Conflict of Interest Statement: WH and the University of Florida have a financial interest in the use of AAV therapies, and WH owns equity in a company (AGTC Inc.) that might, in the future, commercialize some aspects of this work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Deng, Kolandaivelu, Dinculescu, Li, Zhu, Chiodo, Ramamurthy and Hauswirth. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.