Published in final edited form as:

AFCS Nat Mol Pages. 2011 ; 2011: . doi:10.1038/mp.a001758.01.

Phosphodiesterase 6H, cone-specific inhibitor:

Basis Sequence: Mouse

Xiu-Jun Zhang¹, Rick H Cote¹

¹Molecular, Cellular & Biomedical Sciences, University of New Hampshire, NH 03824, US.

Protein Function

PDE6H is the inhibitory γ -subunit of the cone-specific cGMP phosphodiesterase (cone PDE6), which has a central role in the visual transduction pathway in cone photoreceptor cells of the vertebrate retina. PDE6H is more commonly referred to as cone P γ or γ' and is composed of 83 amino acids whose sequence is highly homologous to the 87-amino-acid rod PDE6 γ -subunit (gene name: *PDE6G*).

In the dark-adapted state, cone P γ tightly binds to cone PDE6 catalytic dimer and suppresses cGMP hydrolysis to a low (basal) level of activity. Following activation of the cone signaling pathway by light, activated cone transducin binds to cone P γ and relieves inhibition of catalysis, thereby accelerating cGMP hydrolysis by cone PDE6, lowering the intracellular concentration of cGMP, and ultimately causing closure of cGMP-gated ion channels that hyperpolarize the cone cell membrane (reviewed in Kawamura and Tachibanaki 2008).

The characterization of PDE6H as a regulatory subunit of cone PDE6 was first reported by Gillespie and Beavo (1988), and its sequence and localization to cone photoreceptors were determined by Hamilton and Hurley (1990).

Given the high degree of sequence conservation between PDE6G and PDE6H throughout vertebrate evolution (Muradov *et al.* 2007), it is reasonable to expect that the structure/function relationships for the two inhibitory subunits are very similar. Thus, the wealth of knowledge about the rod γ -subunit is probably applicable to the PDE6H subunit, but direct examination is required. These rod γ -subunit functions include (reviewed in Guo and Ruoho 2008): (1) binding of the carboxy-terminal region of the molecule to the active site of the PDE6 catalytic subunit; (2) stimulation of cGMP binding to the GAFa domain (named after cGMP-specific phosphodiesterase, adenylyl cyclase and FhlA) of the PDE6 catalytic subunits by interaction with the polycationic region of the γ -subunit; (3) interaction of several regions of the γ -subunit with activated transducin α -subunit during the PDE6 activation mechanism; and (4) interaction of the γ -subunit with a GTPase accelerating protein (RGS9-1) to facilitate GTPase acceleration of transducin deactivation.

Regulation of Activity

PDE6H has no intrinsic catalytic activity of its own, but rather is a regulatory subunit of cone PDE6C that modulates its catalytic activity in a transducin-dependent manner (Gillespie and Beavo 1988; Zhang *et al.* 2003).

The regulation of the rod γ -subunit (PDE6G) by post-translational modifications and by binding to signaling proteins has been extensively studied (reviewed in Guo and Ruoho 2008; Janisch *et al.* 2009), but the functional significance of this remains unclear. Janisch *et al.* (2009) reported phosphorylation of residues Thr 20 and Thr 33 in chicken PDE6H (corresponding to the Thr 22 and Thr 35 residues in PDE6G), but no light-dependent changes in phosphorylation of PDE6H were detected at either position. The authors concluded that reversible phosphorylation of PDE6H was unlikely to contribute to cone PDE6C regulation of the cone phototransduction pathway.

Interactions with Ligands and Other Proteins

The ability of PDE6H to regulate cone PDE6 catalytic activity fundamentally depends on the coordination of its interactions with the PDE6C catalytic subunits (to inhibit catalysis) and with activated cone transducin α -subunit (GNAT2) to relieve this inhibitory constraint and to accelerate cGMP hydrolysis.

Early studies reported that PDE6H binds to and inhibits cone PDE6 catalytic activity with high affinity (K_i ~100–200 pM; Gillespie 1990; Hamilton *et al.* 1993), but that it was even more potent at inhibiting the rod PDE6 catalytic dimer (K_i ~50–80 pM; Gillespie 1990; Hamilton *et al.* 1993). More recent evidence (Muradov *et al.* 2010) fails to observe significant differences in affinity of PDE6H for the rod or cone catalytic domains (K_i ~30–40 pM), nor do PDE6G and PDE6H show much difference in inhibitory potency (Muradov *et al.* 2010).

Direct assays of the interaction of GNAT2 with PDE6H have not been reported, in contrast to the well-studied interactions of the homologous rod proteins. Nonetheless, the ability of activated transducin to bind to and activate cone PDE6 implies a direct binding of GNAT2 to PDE6H. In partially purified preparations of cone outer segments from fish, Tachibanaki *et al.* (2001) observed that transducin activation of cone PDE6 was ~100-fold less light-sensitive than in the rod phototransduction pathway, although the maximum extent of activation with bright lights was similar. Likewise, Zhang *et al.* (2003) used crude extracts from conedominated chipmunk retina to document light-induced cone Pde6 activation by cone transducin, presumably resulting from Gnat2 displacing Pde6h from its site of inhibition within the cone PDE6 catalytic domain.

Biochemical studies of reconstituted transducin and PDE6 have revealed marked differences in the ability of different PDE6 isoforms to be activated by transducin. For example, a 50-fold lower concentration of rod transducin α -subunit is needed to activate cone PDE6 compared with that needed to activate rod PDE6 (Gillespie and Beavo 1988). More recently, Muradov *et al.* (2010) confirmed this finding and also demonstrated that rod and cone transducin have similar abilities to activate rod and cone PDE6. Using chimeric catalytic

subunits, they demonstrated that the amino-terminal GAF domain of cone PDE6 is responsible for the increased efficacy with which activated transducin can activate PDE6 catalysis (Muradov *et al.* 2010).

Based on the well-established ability of PDE6G to bind to RGS9–1 to modulate its catalytic activity (He *et al.* 1998), it is reasonable to expect that PDE6H will serve a similar role in cone phototransduction. In this regard, note that cones contain significantly greater levels of RGS9–1 than do rods (Cowan *et al.* 1998; Zhang *et al.* 2003; Martemyanov *et al.* 2008; Lobanova *et al.* 2010).

As has been proposed for the rod PDE6 γ -subunit, PDE6H may also have additional binding partners in cone photoreceptors or other cell types that are not currently elucidated, based on the ability of PDE6H transfected into human embryonic kidney 293 cells to stimulate the activation of mitogen-activated protein kinase (Wan *et al.* 2001).

Additional citation:

Gillespie, P. G. Phosphodiesterases in visual transduction by rods and cones. In *Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action*. Beavo, J. and Houslay, M. D., editors. New York: John Wiley & Sons, 1990.

Regulation of Concentration

The only direct measurement of Pde6h concentration has been performed with retinal membrane preparations from cone-dominant chipmunk retina, and the molar ratio of Pde6h to cone opsin was determined to be 1:68 (Zhang *et al.* 2003).

Subcellular Localization

The PDE6H gene product is synthesized in the cone inner segment and then presumably transported to the outer segment in a complex with PDE6C catalytic subunits (cone PDE6 holoenzyme). Because PDE6C is isoprenylated, it is presumed that PDE6H is also membrane-associated.

When photoreceptor proteins were isolated from retinal extracts, the majority of bovine cone PDE6 was found in the soluble fraction in a complex with the prenyl binding protein δ (PDE6D; Gillespie and Beavo 1988; Pentia *et al.* 2005). There is reason to think that the occurrence of cone PDE6 as a soluble protein is an artifact of the protein isolation process, and that *in vivo* the cone PDE6 holoenzyme exists in a membrane-associated state.

Major Sites of Expression

The primary site of expression of PDE6H is in retinal cone photoreceptor cells, where it is synthesized in the inner segment and then transported to the cone outer segment (Hamilton and Hurley 1990; Shimizu-Matsumoto *et al.* 1996).

In addition, there are reports of PDE6H expression in other light-sensing tissues that also express the PDE6C subunit, including chicken pineal gland (Morin *et al.* 2001) and lizard

parietal eye (Su *et al.* 2006). Not unexpectedly, transcripts for PDE6H were identified in two different retinoblastoma cell lines, Y79 and WERI, although functional protein was not detected (White *et al.* 2004).

Tate *et al.* (2002) reported that mouse lung contains transcripts and protein immunoreactivity for PDE6H, but not PDE6 catalytic subunits. Nikolova *et al.* (2010) also identified expression of *PDE6H* transcripts in human lung cells, as well as expression of cone PDE6 catalytic subunit (PDE6C), rod PDE6 subunits (PDE6A, PDE6B and PDE6G) and the prenyl binding protein/δ (PDE6D). This study reported downregulation of PDE6H expression in idiopathic pulmonary fibrosis, but the relationship of PDE6H expression to the regulation of cyclic nucleotide metabolism in normal and diseased lung tissue is not understood.

Phenotypes

Although transgenic mice studies showed that knockout of the rod P γ gene (*Pde6g*) leads to retinal degeneration (Tsang *et al.* 1996), no similar test of *Pde6h* has been conducted in mice. In humans, an uncommon G-to-C substitution in the 5'-untranslated region of the *PDE6H* gene was found in a patient and family members with cone dystrophy (Piri *et al.* 2005). However, Wu *et al.* (2006) failed to confirm *PDE6H* as the disease-causing gene and identified mutations in a voltage-gated potassium channel subunit gene (*KCNV2*) to be responsible for the cone dystrophy.

Splice Variants

Tate *et al.* (2002) reported that mouse lung contains a short form of the *Pde6h* gene consisting of the same N-terminal half as the long form, but which is interrupted with a frameshift mutation resulting in a shortened, different C-terminal sequence. Protein expression of this transcript was not detected in lung, but expression at the transcriptional level was also detected in other non-retinal mouse and rat tissues.

Antibodies

Because of the high sequence similarity to PDE6G, PDE6H-specific antibodies are difficult to generate. Hamilton and Hurley reported rod-and cone-specific antibodies to the N-terminal region of P γ . Cone specific polyclonal antibody can specifically bind to cones (Hamilton and Hurley 1990).

Tate *et al.* (2002) produced cone-specific antibody using the N-terminal peptide MSDSPCLSPP. The antibody did not show cross-reactivity with rod P γ . Janisch *et al.* (2009) generated and characterized a PDE6H anti-peptide antibody containing a phosphorylated T20 residue; this antibody failed to react with samples containing mouse PDE6G.

References

- Cowan CW, Fariss RN, Sokal I, Palczewski K, Wensel TG High expression levels in cones of RGS9, the predominant GTPase accelerating protein of rods. *Proc Natl Acad Sci U S A*, 95, 9 28 4 1998

- Gillespie PG, Beavo JA Characterization of a bovine cone photoreceptor phosphodiesterase purified by cyclic GMP-sepharose chromatography. *J Biol Chem*, 263, 17 15 6 1988
- Guo LW, Ruoho AE The retinal cGMP phosphodiesterase gamma-subunit - a chameleon. *Curr Protein Pept Sci*, 9, 6 12 2008
- Hamilton SE, Hurley JB A phosphodiesterase inhibitor specific to a subset of bovine retinal cones. *J Biol Chem*, 265, 19 5 7 1990
- Hamilton SE, Prusti RK, Bentley JK, Beavo JA, Hurley JB Affinities of bovine photoreceptor cGMP phosphodiesterases for rod and cone inhibitory subunits. *FEBS Lett*, 318, 2 1 3 1993
- Janisch KM, Kasanuki JM, Naumann MC, Davis RJ, Lin CS, Semple-Rowland S, Tsang SH Light-dependent phosphorylation of the gamma subunit of cGMP-phosphodiesterase (PDE6gamma) at residue threonine 22 in intact photoreceptor neurons. *Biochem Biophys Res Commun*, 390, 4 25 12 2009
- Kawamura S, Tachibanaki S Rod and cone photoreceptors: molecular basis of the difference in their physiology. *Comp Biochem Physiol A Mol Integr Physiol*, 150, 4 8 2008
- Lobanova ES, Herrmann R, Finkelstein S, Reidel B, Skiba NP, Deng WT, Jo R, Weiss ER, Hauswirth WW, Arshavsky VY Mechanistic basis for the failure of cone transducin to translocate: why cones are never blinded by light. *J Neurosci*, 30, 20 19 5 2010
- Martemyanov KA, Krispel CM, Lishko PV, Burns ME, Arshavsky VY Functional comparison of RGS9 splice isoforms in a living cell. *Proc Natl Acad Sci U S A*, 105, 52 30 12 2008
- Morin F, Lugnier C, Kameni J, Voisin P Expression and role of phosphodiesterase 6 in the chicken pineal gland. *J Neurochem*, 78, 1 7 2001
- Muradov H, Boyd KK, Artemyev NO Rod phosphodiesterase-6 PDE6A and PDE6B subunits are enzymatically equivalent. *J Biol Chem*, 285, 51 17 12 2010
- Muradov H, Boyd KK, Kerov V, Artemyev NO PDE6 in lamprey *Petromyzon marinus*: implications for the evolution of the visual effector in vertebrates. *Biochemistry*, 46, 35 4 9 2007
- Nikolova S, Guenther A, Savai R, Weissmann N, Ghofrani HA, Konigshoff M, Eickelberg O, Klepetko W, Voswinkel R, Seeger W, Grimminger F, Schermuly RT, Pullamsetti SS Phosphodiesterase 6 subunits are expressed and altered in idiopathic pulmonary fibrosis. *Respir Res*, 11 2010
- Pentia DC, Hosier S, Collupy RA, Valeriani BA, Cote RH Purification of PDE6 isozymes from mammalian retina. *Methods Mol Biol*, 307 2005
- Piri N, Gao YQ, Danciger M, Mendoza E, Fishman GA, Farber DB A substitution of G to C in the cone cGMP-phosphodiesterase gamma subunit gene found in a distinctive form of cone dystrophy. *Ophthalmology*, 112, 1 1 2005
- Shimizu-Matsumoto A, Itoh K, Inazawa J, Nishida K, Matsumoto Y, Kinoshita S, Matsubara K, Okubo K Isolation and chromosomal localization of the human cone cGMP phosphodiesterase gamma cDNA (PDE6H). *Genomics*, 32, 1 15 2 1996
- Su CY, Luo DG, Terakita A, Shichida Y, Liao HW, Kazmi MA, Sakmar TP, Yau KW Parietal-eye phototransduction components and their potential evolutionary implications. *Science*, 311, 5767 17 3 2006
- Tachibanaki S, Tsushima S, Kawamura S Low amplification and fast visual pigment phosphorylation as mechanisms characterizing cone photoresponses. *Proc Natl Acad Sci U S A*, 98, 24 20 11 2001
- Tate RJ, Arshavsky VY, Pyne NJ The identification of the inhibitory gamma- subunits of the type 6 retinal cyclic guanosine monophosphate phosphodiesterase in non-retinal tissues: differential processing of mRNA transcripts. *Genomics*, 79, 4 4 2002
- Tsang SH, Gouras P, Yamashita CK, Kjeldbye H, Fisher J, Farber DB, Goff SP Retinal degeneration in mice lacking the gamma subunit of the rod cGMP phosphodiesterase. *Science*, 272, 5264 17 5 1996
- Wan KF, Sami BS, Frame M, Tate R, Pyne NJ The inhibitory gamma subunit of the type 6 retinal cyclic guanosine monophosphate phosphodiesterase is a novel intermediate regulating p42/p44 mitogen-activated protein kinase signaling in human embryonic kidney 293 cells. *J Biol Chem*, 276, 41 12 10 2001
- White JB, Thompson WJ, Pittler SJ Characterization of 3',5' cyclic nucleotide phosphodiesterase activity in Y79 retinoblastoma cells: absence of functional PDE6. *Mol Vis*, 10 6 10 2004

- Wu H, Cowing JA, Michaelides M, Wilkie SE, Jeffery G, Jenkins SA, Mester V, Bird AC, Robson AG, Holder GE, Moore AT, Hunt DM, Webster AR Mutations in the gene KCNV2 encoding a voltage-gated potassium channel subunit cause “cone dystrophy with supernormal rod electroretinogram” in humans. *Am J Hum Genet*, 79, 3 9 2006
- Zhang X, Wensel TG, Kraft TW GTPase regulators and photoresponses in cones of the eastern chipmunk. *J Neurosci*, 23, 4 15 2 2003

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript