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Unc119 gene deletion partially rescues the GRK1 transport defect of *Pde6d*$^{-/-}$ cones

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Abstract

PrBP/δ, encoded by the *Pde6d* gene, is an isoprenyl-binding protein that regulates trafficking of isoprenylated proteins, such as PDE6 and GRK1, from photoreceptor inner segments to outer segments. Trafficking of PDE6 and GRK1 to photoreceptor outer segments is impeded in *Pde6d* knockout mice. In *Pde6d*$^{-/-}$cones, PDE6 and GRK1 are nearly undetectable and the b-wave amplitudes of photopic ERGs in *Pde6d*$^{-/-}$ mice are reduced by over 50%. We reported recently that UNC119, a homolog of PrBP/δ highly expressed in photoreceptors, functions as an acyl-binding protein and regulates transport of G-proteins in sensory neurons. Since both PrBP/δ and UNC119 regulate peripheral protein trafficking in photoreceptors, we generated *Pde6d;Unc119* double knockout mice in order to study how PrBP/δ and UNC119 may interact. Surprisingly, knockout of Unc119 partially reversed the transport defect of GRK1 in cone photoreceptors caused by deletion of *Pde6d*, and the b-wave amplitudes of photopic ERGs in the double knockout mice were significantly higher than those in the *Pde6d*$^{-/-}$ mice. These results suggest that cone transport of isoprenylated and acylated proteins is interdependent.

Keywords

PrBP/δ; Pde6d; UNC119; photoreceptors; GRK1; PDE6; ERG

62.1 Introduction

PrBP/δ, encoded by the *Pde6d* gene, is a small polypeptide consisting of 150 amino acids in mammals with an apparent molecular weight of ~17kD [1]. As PrBP/δ co-purified with photoreceptor PDE6, it was thought initially to be a fourth subunit of rod PDE6 [2]. Many more PrBP/δ-interacting proteins, identified by yeast-two hybrid screening (e.g., GRK1, Ras, and other small GTPases in the Ras family [3-5]), suggested a more general function of PrBP/δ. Close examination of PrBP/δ-interacting proteins revealed that most share a common feature, a C-terminal isoprenyl group. The FRET assay shows that PrBP/δ has

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distinct affinities for farnesyl and geranylgeranyl moieties, binding farnesyl (k_d = 0.7 μM) more tightly than geranylgeranyl (k_d = 19 μM) [5]. Transport of isoprenylated proteins (GRK1, rod PDE6, and cone PDE6) to photoreceptor outer segments was impeded in *Pde6d* knockout photoreceptors, resulting in a slow degeneration of cones followed by degeneration of rods, a phenotype similar to human patients with cone-rod dystrophy [6].

UNC119, first discovered in *C.elegans*, shares 30-40% sequence similarity with PrBP/δ [7]. UNC119 is highly expressed in the synaptic termini of photoreceptors, and to a less extent in the inner segments [8]. Knockout of the *Unc119* gene in mouse led to slow retinal degeneration starting at 6 months postnatally, a phenotype similar to human *RP* (*Retinitis Pigmentosa*) [8], although the mechanism of degeneration is unclear. We recently proposed that UNC119 is an acyl-binding protein. UNC119 binds the acylated N-terminal peptide of transducin α with a k_d of 0.5 μM [9]. UNC119 can extract transducin from retina membranes in the presence of GTP, and is required for efficient return of transducin to photoreceptor outer segments after light-induced translocation of transducin from outer segments to inner segments during light adaptation, suggesting that UNC119 regulates trafficking of transducin in photoreceptors [9].

In this study, we generated PrBP/δ and UNC119 double knockout mice and observed that GRK1 is up-regulated in the mutant cone photoreceptors. With more GRK1 present in the cone photoreceptor cells, photopic ERGs reflecting cone function exhibit higher responses in the double knockout mice compared with those in the *Pde6d* single knockout mice.

62.2 Materials and methods

62.2.1. Mouse breeding genotyping

Procedures for the animal experiments were approved by the University of Utah IACUC and conformed to recommendations of the Association of Research for Vision and Ophthalmology. Animals were maintained in cyclic light (12 h light/12 h dark) conditions. *Unc119*$^{-/-}$ mice were provided by Dr. George Inana at the University of Miami. *Pde6d;Unc119* double knockout mice were generated by mating *Pde6d*$^{-/-}$ mice to *Unc119*$^{-/-}$ mice. Genotyping of *Pde6d*$^{-/-}$ and *Unc119*$^{-/-}$ mice was described previously [6, 8].

62.2.2. Immunohistochemistry

Immunostaining of mouse retina sections was performed as described [10]. Retina cryosections were cut and immunolabeled using mouse monoclonal GRK1 antibody (G8, 1:1000, from Dr. Kris Palczewski, Case Western Reserve University) or rabbit polyclonal cone PDE6 antibody (!:500, from Dr. Tiansen Li, NEI/NIH).

62.2.3. Photopic Electroretinogram (ERG)

ERGs were recorded with a UTAS E-3000 (LKC Technologies, Inc.) as described [6, 11]. For photopic ERGs to record cone function, the mice were light-adapted under background light of 10 db (1.48 logcds·m^{-2}) for 15 minutes. Single-flash responses were usually recorded at stimulus intensities of −4 db (−0.6 logcds·m^{-2}) to 15 db (1.86 logcds·m^{-2}).

62.3 Results

62.3.1. Knockout of the *Unc119* gene in *Pde6d*$^{-/-}$ mice increases expression of GRK1 in photoreceptor outer segments

PrBP/δ and UNC119 each regulate transport of lipid-conjugated peripheral proteins to photoreceptor outer segments. Both, in turn, are regulated by the small GTPase ARL3, which controls cargo release from PrBP/δ and UNC119 [12, 13]. It remains unclear whether PrBP/δ and UNC119 coordinately regulate the transport of isoprenylated proteins and acylated protein to photoreceptor outer segments. We were interested to define the functional relationship of these two proteins by generating *Pde6d* and *Unc119* double knockout mice. In the *Pde6d*$^{-/-}$*Unc119*$^{-/-}$ mice, cone PDE6 is nearly undetectable, which is similar in the *Pde6d* single knockout (Fig. 1B). Expression of GRK1 in the *Pde6d*$^{-/-}$ and *Pde6d*$^{-/-}$*Unc119*$^{-/-}$ mice is almost absent throughout the rods (Fig. 1A). However, expression of GRK1 is surprisingly increased in the cone outer segments, as compared to the *Pde6d*$^{-/-}$ cones with little GRK1 in COS (Fig. 1A).

62.3.2. Knockout of the *Unc119* gene in *Pde6d*$^{-/-}$ background increases cone photoresponses

GRK1 is a key enzyme to shut down the phototransduction cascade. GRK1 phosphorylates rhodopsin and cone opsins, thereby desensitizing rhodopsin and cone opsins following activation. In GRK1 gene knockout mice, both rod and cone photoreceptors showed slower recovery from photoactivation [14]. In *Pde6d* knockout mice, expression of GRK1 is significantly down-regulated and photoreceptors exhibit a phenotype of slower dark-adaption [6]. Lowered GRK1 in *Pde6d*$^{-/-}$ mice essentially delays desensitization of activated cone opsins and increase basal level of photoresponses under constant light, resulting in lower amplitude of photopic ERGs. In the *Pde6d*$^{-/-}$*Unc119*$^{-/-}$ mice, the expression of GRK1 increases relative to that observed in *Pde6d* knockout mice. As expected, the amplitude of photopic ERGs in the *Pde6d*$^{-/-}$*Unc119*$^{-/-}$ is higher than that in the *Pde6d*$^{-/-}$ mice; however, it is still lower than that in wild type mice (Fig. 2), which may be due to partial up-regulation of GRK1 and lower cone PDE6 expression in the double knockout.

62.4 Discussion

We show that deletion of the Unc119 gene in the *Pde6d*-/- background produced up-regulated expression of GRK1 in cone photoreceptors and consequently, cone b-wave photoresponses increased relative to that observed in *Pde6d* single knockout mice (Fig. 2). GRK1 is a peripheral membrane protein, and undergoes posttranslational isoprenylation at its C-terminus. PrBP/δ solubilizes GRK1 from membranes by binding the isoprenyl tail of GRK1, and forming a soluble complex to facilitate transport of GRK1. PrBP/δ also interacts with a small GTPase ARL3 [15], and GTP-bound ARL3 likely promotes release of GRK1 from PrBP/δ at the target membrane, analogous to release of farnesylated Rheb from PrBP/δ [12]. In contrast, UNC119 is an acyl-binding protein. Deletion of the UNC119 gene does not interfere with transport of GRK1 and other isoprenylated proteins [9]. However, UNC119 also interacts with ARL3, and GTP-bound ARL3 facilitates the release of acylated protein cargo from UNC119 [13]. Our result that the *UNC119;Pde6d* double knockout improves

transport of GRK1 in cones is counter-intuitive, because ARL3 is not involved in regulating PrBP/δ cargo release when PrBP/δ is absent. However, in *Pde6d* knockout cones, residual GRK1 is present in cone outer segments, suggesting that GRK1 transport may be facilitated via alternative mechanisms. One possible mechanism is that other isoprenyl binding proteins may play a minor role in transport of GRK1 to outer segments, and one 'candidate isoprenyl binding protein' is RhoGDI that binds geranylgeranylated CDC42. Although RhoGDI is a possible candidate that may enhance transport of GRK1 to cone outer segments, we cannot exclude other isoprenyl-binding proteins as possible candidates, such as RabGDI; nor could we exclude possible common factors that play a role in transport pathways of both isoprenylated proteins and acylated proteins.

PrBP/δ and RhoGDI each contain a hydrophobic pocket sandwiched by two β-sheets and each can bind isoprenyl groups. It is possible that RhoGDI can accommodate isoprenyl tail of GRK1. Expression of Rho GTPase proteins in photoreceptors have been documented [16], suggesting that RhoGDI, an essential functional partner for Rho GTPase, is also expressed in photoreceptors. When PrBP/δ/GRK1 complex reaches its destination membrane, release of GRK1 requires interaction with GTP-bound ARL3. Similarly, RhoGDI/GRK1 complex may require ARL3 to discharge GRK1 from RhoGDI upon their arrival to the targeted membrane. ARL3 not only traffics isoprenylated proteins but also traffics acylated proteins, a process which is modulated by UNC119. Other factors besides ARL3 may share transport of isoprenylated and acylated proteins. Therefore, knockout of Unc119 gene will free at least some ARL3 and other unknown factors from the pathway of trafficking acylated proteins and enhance transport of GRK1 by the alternative transport pathway.

Future investigations will determine if rescue of GRK1 transport in the *Pde6d;Unc119* double knockout mice would slow down the degeneration of cone photoreceptors. We will also investigate the expression profile of photoreceptor RhoGDI and test the binding affinity of RhoGDI to GRK1 and PDE6. This will address the role of RhoGDI in the observed rescue of GRK1 transport in *Unc119;Pde6d* double knockout mice.

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Abbreviations

RhoGDI	Rho GTPase Guanine nucleotide Dissociation Inhibitor
ERG	electroretinogram
COS	cone outer segment

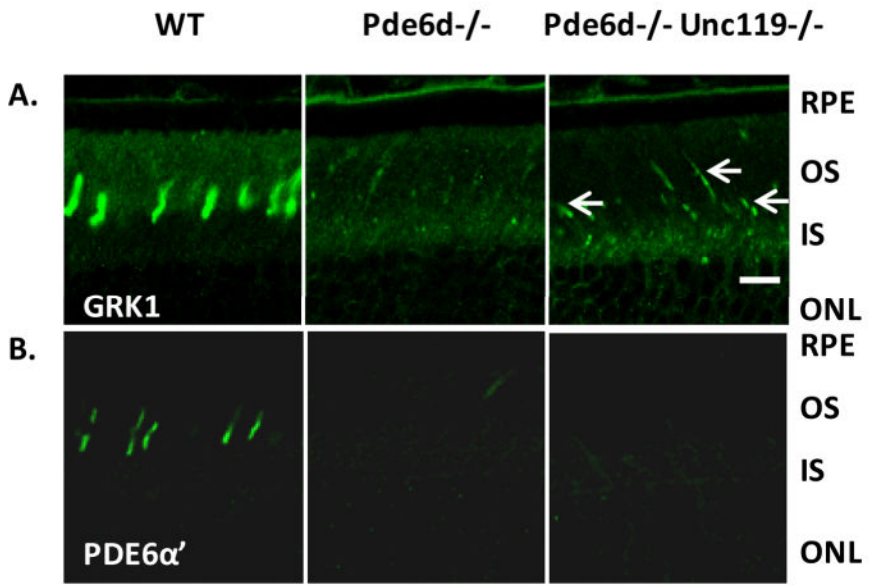

Fig. 62.1.

Immunolocalization of GRK1 and PDE6α′ in WT, *Pde6d*$^{-/-}$, and *Pde6d*$^{-/-}$*Unc119*$^{-/-}$ retinas. The eyes were co-embedded, and central retina sections were probed simultaneously using anti-GRK1 (A) and PDE6α′ (B). RPE, Retinal pigment epithelium; OS, outer segments; IS, inner segments; ONL, outer nuclear layer. Arrows points cone outer segments. Scale bar, 10 μm.

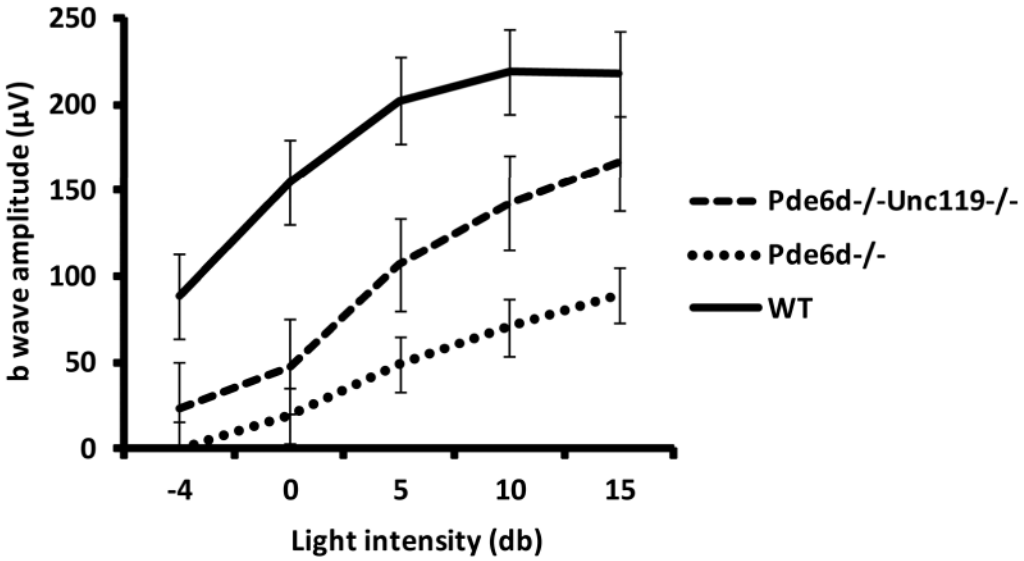

Fig. 62.2.

Photopic ERGs of WT, *Pde6d*$^{-/-}$, and *Pde6d*$^{-/-}$*Unc119*$^{-/-}$ mice. Photopic b-wave amplitudes are plotted as a function of light intensities. Error bars represent mean ± SD (n = 3).