Vision Research 75 (2012) 19–25

Contents lists available at SciVerse ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

The prenyl-binding protein PrBP/δ: A chaperone participating in intracellular trafficking

Houbin Zhang [a], Ryan Constantine [a,b], Jeanne M. Frederick [a], Wolfgang Baehr [a,c,d,*]

[a] Department of Ophthalmology, John A. Moran Eye Center, University of Utah Health Science Center, 65 Mario Capecchi Dr., Salt Lake City, UT 84132, USA
[b] Graduate Program in Neuroscience, University of Utah Health Science Center, Salt Lake City, UT 84132, USA
[c] Department of Neurobiology and Anatomy, University of Utah Health Science Center, Salt Lake City, UT 84132, USA
[d] Department of Biology, University of Utah, 257 South 1400 East, Salt Lake City, UT 84112, USA

ARTICLE INFO

Article history:
Received 6 July 2012
Received in revised form 17 August 2012
Available online 29 August 2012

Keywords:
cGMP phosphodiesterase 6 (PDE6)
Prenyl binding protein PrBP/δ
Prenylation
PrBP/δ crystal structure
Small GTPases
Rod photoreceptors
G-protein coupled receptor kinase 1 (GRK1)

ABSTRACT

Expressed ubiquitously, PrBP/δ functions as chaperone/co-factor in the transport of a subset of prenylated proteins. PrBP/δ features an immunoglobulin-like β-sandwich fold for lipid binding, and interacts with diverse partners. PrBP/δ binds both C-terminal C15 and C20 prenyl side chains of phototransduction polypeptides and small GTP-binding (G) proteins of the Ras superfamily. PrBP/δ also interacts with the small GTPases, ARL2 and ARL3, which act as release factors (GDFs) for prenylated cargo. Targeted deletion of the mouse *Pde6d* gene encoding PrBP/δ resulted in impeded trafficking to the outer segments of GRK1 and cone PDE6 which are predicted to be farnesylated and geranylgeranylated, respectively. Rod and cone transducin trafficking was largely unaffected. These trafficking defects produce progressive cone-rod dystrophy in the *Pde6d*$^{-/-}$ mouse.

1. Introduction

The mouse *Pde6d* gene encodes PrBP/δ, a 17 kDa protein that functions as a prenyl binding protein (Cook et al., 2000; Gillespie et al., 1989; Ismail et al., 2011; Norton et al., 2005; Zhang et al., 2004). By NCBI homology search, PrBP/δ orthologs were identified in essentially all animals (Fig. 1), e.g., fruit fly, the eyeless *Caenorhabditis elegans* (Li & Baehr, 1998), and the unicellular protozoan *Paramecium* (Zhang et al., 2007). PrBP/δ protein sequence is highly conserved throughout the animal kingdom, with at least 70% sequence identity within vertebrates, and ~50% sequence identity among invertebrates. The closest relatives of PrBP/δ are the two UNC119 paralogs, UNC119A and UNC119B (see accompanying paper (Constantine et al., in press)). *PDE6D* and UNC119 paralogs constitute a new class of neural genes whose common function as lipid-binding proteins has been maintained through metazoan evolution. This review focuses on structure/function relationships of PrBP/δ with some of its interaction proteins.

2. Protein prenylation

Protein prenylation is a common posttranslational modification of eukaryotic cells affecting up to 2% of all proteins expressed in mammalian cells (referred to as the "prenylome") (Nguyen et al., 2010, chap. 14). Prenyl side chains are synthesised in all living organisms via the mevalonate pathway and attached to newly synthesised cytosolic proteins carrying a C-terminal CAAX box motif (C = cysteine, A = aliphatic amino acid, X = any amino acid) (Magee & Seabra, 2005; McTaggart, 2006). The C-terminal X determines the nature of the lipid chain as leucine specifies geranylgeranylation and all other residues result in farnesylation. The prenyl chain is attached to the CAAX box cysteine via a thioether bond by cytosolic prenyl transferases (Zhang & Casey, 1996). Prenylated proteins dock to the endoplasmic reticulum (ER) and are further processed by the ER-associated enzymes RCE1 protease (ras-converting enzyme 1), which cleaves AAX of the CAAX box and an isoprenyl cysteine carboxymethyl transferase (ICMT), which carboxymethylates the cysteine COOH (Winter-Vann & Casey, 2005) (Fig. 2). Both enzymes are essential for mouse development as deletion of either RCE1 or ICMT are embryonic lethal (Bergo et al., 2001, 2002). Deletion of RCE1 in retina prevented transport of rod PDE6 to the outer segments, but had no effect on GRK1 (Christiansen et al., 2011). The number of CAAX box-containing proteins in the human and mouse genome, as defined by ORFs followed by CAAX box and a stop codon, has been estimated as

* Corresponding author at: Department of Ophthalmology, John A. Moran Eye Center, University of Utah Health Science Center, 65 Mario Capecchi Dr., Salt Lake City, UT 84132, USA.
E-mail address: wbaehr@hsc.utah.edu (W. Baehr).

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http://dx.doi.org/10.1016/j.visres.2012.08.013

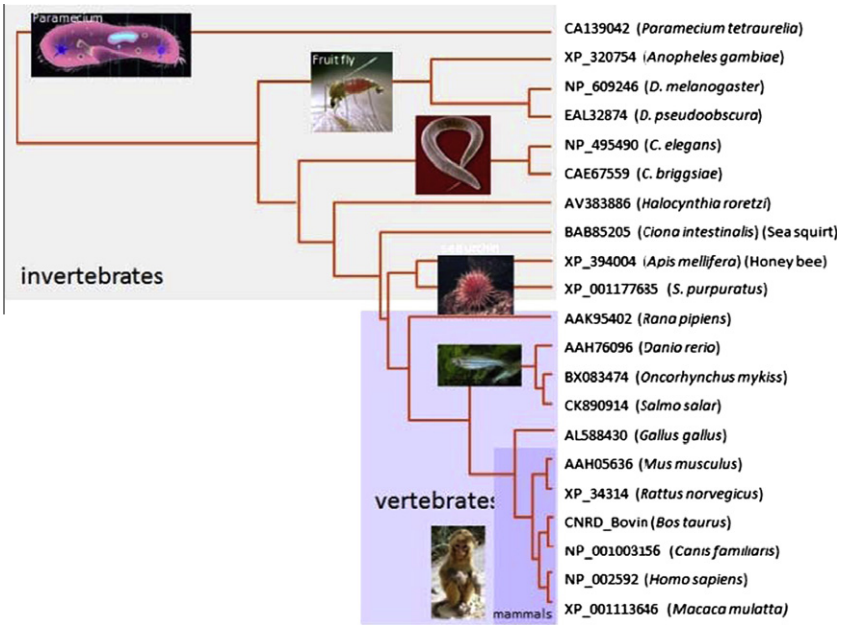

Fig. 1. Dendrogram of 21 PrBP/δ orthologs. Amino acid sequences were retrieved using the accession numbers shown, and aligned using ClustalW. The dendrogram was generated from the alignment. Sequences among vertebrates are highly conserved. PrBP/δ sequences of *C. elegans* and human are 65% similar suggesting conserved function through evolution.

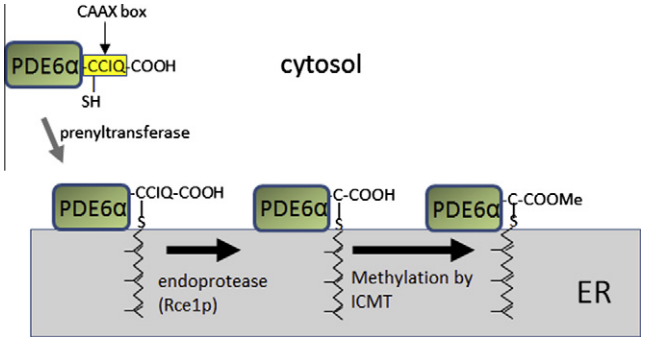

Fig. 2. CAAX-protein prenylation and processing (example: PDE6α). After prenylation in the cytosol, PDE6α protein docks at the ER and undergoes enzymatic processing by RCE1P protease that removes AAX, and an S-adenosylmethionine-dependent methyltransferase (ICMT) that carboxymethylates the C-terminal cysteine.

~280 candidates (Winter-Vann & Casey, 2005). Known prenylated CAAX proteins include visual cascade components, members of the Ras superfamily and G protein γ-subunits, among others (Nguyen, Goody, & Alexandrov, 2010; Winter-Vann & Casey, 2005) (Table 1).

3. Photoreceptor PDE6 and PrBP/δ

cGMP-specific phosphodiesterase 6 (PDE6) belongs to a large PDE superfamily (PDE1–11) whose members regulate cellular concentrations of cAMP and cGMP (Conti & Beavo, 2007). PDE6 is expressed in rods and cones and consists of two catalytic subunits (rod Pde6αβ and cone Pde6α', respectively) and two inhibitory PDE6γ subunits (Baehr, Devlin, & Applebury, 1979; Miki et al., 1975). PDE6α (~99 kDa), PDE6β (~99 kDa) and two PDE6γ (~10 kDa) form a heterotetramer PDE6αβγγ (Fung et al., 1990). Each catalytic subunit carries a C-terminal cysteine, part of a CAAX box motif for post-translational prenylation (Anant et al., 1992). Mammalian PDE6α is farnesylated while PDE6β is geranylgeranylated, resulting in modifications that facilitate membrane attachment. PrBP/δ was originally identified as a protein co-purifying with PDE6αβγ_2 and named PDE6δ (Gillespie et al., 1989). Under isotonic conditions, most PDE6 is peripherally membrane-associated (Baehr, Devlin, & Applebury, 1979), but a fraction (20–30%) remains soluble (Gillespie et al., 1989). Affinity purification using a monoclonal antibody column to purify soluble PDE6, yielded a novel 15 kDa polypeptide (Gillespie et al., 1989). Cloning this peptide's cDNA and northern blotting revealed that PrBP/δ was present in several different bovine tissue mRNA preparations, the strongest of which was present in the retina (Florio, Prusti, & Beavo, 1996).

Addition of a GST-PrBP/δ fusion protein to permeabilized rod outer segment preparations resulted in a reduction of the maximal rate of cGMP hydrolysis in response to light (Cook et al., 2001) suggesting that GST-PrBP/δ may modify the activity of the phototransduction cascade by uncoupling transducin's normal activation of PDE6. However, it was later demonstrated that very little PrBP/δ is present in the rod outer segment (ROS) rendering this *in-vitro* uncoupling mechanism physiologically insignificant (Norton et al., 2005) has purified PrBP/δ has no effect on PDE activity *in vitro*.

Micromolar concentrations of prenylated and carboxymethylated PrBP/δ C-terminal peptides block the Pde6- PrBP/δ interaction. Soluble PDE6 from ROS was five-fold more highly methylated than membrane-bound PDE6 suggesting that PrBP/δ preferentially binds to carboxymethylated PDE6 (Cook et al., 2000). The PDE6-PrBP/δ complex is relatively stable with a half-life of about 3.5 h. Exploiting the intrinsic tryptophan fluorescence of PrBP/δ and using dansylated prenyl cysteines as fluorescent ligands in a fluorescence resonance energy transfer (FRET) experiment, recombinant PrBP/δ was shown to specifically bind geranylgeranyl and farnesyl moieties lacking bound amino acids with K_ds of ~20 and ~1 μM, respectively, establishing unambiguously that PrBP/δ functions as a prenyl-binding protein (Zhang et al., 2004). In photoreceptors, PrBP/δ was shown to interact with PDE6 subunits, farnesylated rhodopsin kinase (GRK1) and geranylgeranylated GRK7 (Zhang et al., 2004). A cryo-EM reconstruction of the PDE6/PrBP/δ complex at 18 Å (Goc