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Phosphodiesterase 6D, cGMP-specific rod delta

Hannah J Gitschier¹ and Rick H Cote¹

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

Abstract

Network Map States Transitions Functions Protein Classes

Sequence Interactions Pathways Domains & Motifs Protein Structure Orthologs

Sequence Interactions Pathways Domains & Motifs Protein Structure Orthologs Blast Data

Protein Function

In general, the physiological function of prenyl-binding protein (PrBP δ) is poorly understood. Essentially the entire protein comprises a single domain that serves to bind prenyl groups covalently attached to proteins, specifically farnesyl- and geranylgeranyl-containing proteins. Binding of PrBP δ to a prenylated protein serves to solubilize the protein and/or prevent membrane attachment of the prenyl moieties. Thus, the prevailing view is that PrBP δ aids in the transport of prenylated proteins from their site of synthesis to their final destination by sequestering the lipophilic prenyl group during protein transport. The functional significance of PrBP δ binding to non-prenylated proteins is unclear at present.

In mammalian photoreceptor cells (where PrBP δ has been studied most extensively), the current prevailing view is that PrBP δ plays a role in the transport of prenylated proteins from the inner segment to the outer segment where they may function in the photoresponse (Karan *et al.* 2008). In the retina, PrBP δ has been shown to interact *in vitro* with the α and β subunits of cyclic nucleotide phosphodiesterase (PDE6), as well as with opsin kinases (G-protein receptor kinase-1 (GRK1) and G-protein receptor kinase-7 (GRK7)) and the retinitis pigmentosa G protein regulator (RPGR) (Florio *et al.* 1996; Gillespie *et al.* 1989; Zhang *et al.* 2005). Further, transport defects are apparent in PrBP δ -deficient mice (*Pde6d*^{-/-}) as farnesylated GRK1 and geranylgeranylated cone PDE6 α subunits are absent from cone outer segment (Zhang *et al.* 2007).

There is *in vitro* evidence that PrBP δ can solubilize PDE6 from rod outer segment (ROS) disk membranes and reduce the ability of activated transducin to activate PDE6 (Norton *et al.* 2005). However, there is currently no support for this regulatory mechanism operating *in vivo*.

Regulation of Activity

PrBP8 lacks intrinsic enzymatic activity, and is designed to bind prenyl groups within a highly hydrophobic pocket (Hanzal-Bayer *et al.* 2002). No known regulatory mechanisms have been reported to modulate prenyl binding to PrBP8.

Binding of PrBP8 to PDE6 does not alter catalytic activity of PDE6, but does enhance nucleotide exchange at the non-catalytic cGMP binding sites of PDE6 (Mou *et al.* 1999; Gillespie *et al.* 1989).

Interactions with Ligands and Other Proteins

Ligands that bind to PrBP8 are known as prenyl groups (also referred to as isoprenoids or isoprenyl groups). PrBP8 can interact with farnesyl and geranylgeranyl side chains in the absence of polypeptides but interacts most favorably with prenylated peptides containing methylated C-termini when compared with non-methylated prenylated peptides (Zhang *et al.* 2004; Zhang *et al.* 2005; Zhang *et al.* 2007; Cook *et al.* 2000).

Numerous prenylated proteins have been shown to interact with PrBP8 *in vitro*, including PDE6, GRK1, GRK7, Rab8 and Rab13 (Norton *et al.* 2005; Wilson *et al.* 1998; Karan *et al.* 2008; Marzesco *et al.* 1998; Florio *et al.* 1996; Gillespie *et al.* 1989; Zhang *et al.* 2004; see Zhang *et al.* 2005 for review).

For the case of PDE6, binding of PrBP8 has been localized by transmission electron microscopy to the catalytic domain of the PDE6 holoenzyme (Goc *et al.* 2010). Recent evidence suggests that each catalytic subunit of the PDE6 dimer is capable of binding PrBP8 (Florio *et al.* 1996; Gillespie *et al.* 1989; Goc *et al.* 2010).

In addition, PrBP8 interacts *in vitro* with non-prenylated proteins of the Ras and Rho GTPase family, such as retinitis pigmentosa GTPase regulator (RPGR) (Linari *et al.* 1999a) and Arl2/Arl3 (Hanzal-Bayer *et al.* 2002; Linari *et al.* 1999b), suggesting a possible role for PrBP8 in linking prenylated and non-prenylated proteins (Zhang *et al.* 2005).

Low-affinity binding of PrBP8 to a PDE5 inhibitor PF-4540124 has been reported as well (Dadvar *et al.* 2009).

Regulation of Concentration

Quantitative immunoblot assays suggest the stoichiometry of PrBP8 bound to purified, soluble bovine rod PDE6 isolated from bovine retinal extracts is 1.0 $\pm$ 0.1 (Norton *et al.* 2005). However, the detectable content of PrBP8 in bovine retinal extracts is 0.6 $\pm$ 0.1 mol per mol of PDE6, and in bovine purified ROS is even less, at 0.09 $\pm$ 0.01 mol PrBP8 per mole of PDE6. These data suggest there are sub-stoichiometric concentrations of PrBP8 relative to PDE6 in bovine retina or in photoreceptor cells.

Subcellular Localization

Localization within photoreceptor cells

The subcellular localization of PrBP δ within photoreceptor cells is not entirely clear. In some immunocytochemistry studies in both frog and bovine retina, the majority of PrBP δ immunoreactivity was localized to the inner segment, or the connecting cilium that joins the inner segment and outer segment (Norton *et al.* 2005). In immunoelectron microscopy studies, PrBP δ was found associated with ciliary axonemes, extending into the rod and cone outer segments. This finding may be indicative of PrBP δ playing a role in prenylated protein transport from the inner segment to the outer segment. However, other immunocytochemistry studies have shown primary localization of PrBP δ to rod outer segment (Zhang *et al.* 2004; Zhang *et al.* 2005).

Localization within other cell types

While the subcellular location of endogenous PrBP δ has not been thoroughly studied, a few immunofluorescence experiments indicate that the majority of transfected epitope-tagged PrBP δ is found in the cytosol of endothelial cells with a small portion (~12%) detected in peripheral vesicular compartments along the plasma membrane (Marzesco *et al.* 1998). Interestingly, deletion of the RVGLFYV C-terminus amino acid sequence in this tagged PrBP δ results in diffusely distributed PrBP δ in the cytoplasm without peripheral staining.

Major Sites of Expression

PrBP δ is ubiquitously expressed in many cell types and tissues, including non-retinal tissues that do not express PDE6 (Florio *et al.* 1996). PrBP δ mRNA has been detected in many mammalian tissues, including those of brain, heart, adrenal gland, spleen, skeletal muscle, prostate, testis, ovary, small intestine, colon, and peripheral blood, but remains present at the highest level in retinal tissue (Florio *et al.* 1996). Further, PrBP δ is a highly conserved protein with 99% amino acid sequence identity between human and cow, and 98% amino acid sequence identity between human and mouse. Other homologues have been detected in vertebrates (e.g. chicken, pig, gorilla), invertebrates (e.g. *Caenorhabditis elegans* with 69% amino acid sequence identity), and even single-celled eukaryotes (e.g. *Paramecium*) (Lorenz *et al.* 1998).

Phenotypes

PrBP δ -deficient mice (*Pde6d*^{-/-}) are fertile and develop normally, but have 20–30% reduced body weight compared with wild-type or heterozygous littermates (Zhang *et al.* 2007). Additionally, prenylated phototransduction proteins such as rod PDE6, cone PDE6, and GRK1 are downregulated in knockout retinas and mislocalize to the inner segment. Rod and cone physiology is compromised because a slow degeneration of the retina with the shortening of ROS and thinning of the outer nuclear layer is also apparent in PrBP δ -deficient mice (Zhang *et al.* 2007). Due to the depletion of GRK1 in ROS there is also a delay in the return to the dark-adapted state in rods.

Splice Variants

No splice variants of PrBP δ have been detected to date.

Antibodies

Commercial antibodies are available: mouse monoclonal and rabbit polyclonal (Santa Cruz Biotechnology, several antibodies offered; Affinity BioReagents, #PAI-726).

Full-length (FL) antibody: Rabbit polyclonal antiserum to the PrBP δ protein was raised by immunization with the FL recombinant bovine protein (Norton *et al.* 2005).

17K-I and 17K-II: synthesized polyclonal antibodies. Both antibodies recognize PrBP δ in immunocytochemistry experiments and immunosorbent assays, but only 17K-II identifies a 17-kDa band in western analysis (Florio *et al.* 1996).

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