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ScienceDirect
Vision Research 48 (2008) 442–452
Vision Research
www.elsevier.com/locate/visres

A model for transport of membrane-associated phototransduction polypeptides in rod and cone photoreceptor inner segments

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Received 27 July 2007; received in revised form 21 August 2007

Abstract

We discuss putative mechanisms of membrane protein transport in photoreceptors based on *Pde6d* and *Gucy2e*/*Gucy2f* knockout mice. Knockout of the *Pde6d* gene encoding PrBP/δ, a prenyl binding protein present in the retina at relatively high levels, was shown to impair transport of G-protein coupled receptor kinase 1 (GRK1) and cone phosphodiesterase α′ subunit (PDE6α′) to the rod and cone outer segments. Other prenylated proteins are minimally affected, suggesting some specificity of interaction. Knockout of the *Gucy2e* gene encoding guanylate cyclase 1 (GC1) disrupted transport of G-protein coupled receptor kinase 1 (GRK1), cone PDE6α′, cone transducin α and γ subunits (cTα and cTγ) to the cone outer segments, while a GC1/GC2 double knockout prevented transport of rod PDE6, but not transducin, GRK1, or rhodopsin, to the rod outer segments. These knockout phenotypes suggest that PrBP/δ functions in extracting prenylated proteins from the endoplasmic reticulum (ER) where they dock after prenylation, and that GC-bearing membranes may co-transport peripheral membrane proteins in vesicles. We conclude that distinct pathways have evolved in rods and cones for transport of integral and peripherally membrane-associated proteins.

Keywords: Phototransduction; Rod and cone photoreceptors; Vesicular transport; Guanylate cyclase knockout; Pde6d knockout

1. Introduction

Rod and cone photoreceptors cells receive light and convert it into an electrical signal. To perform this task efficiently, photoreceptors have evolved into highly polarized structures consisting of three distinct areas: the outer segment containing compacted membrane disks housing the phototransduction machinery, the inner segment where biosynthesis occurs, and the synaptic region that transmits excitation by light to a downstream neuron. The inner segment is connected to the outer segment through a fragile cilium, and to the synaptic region by an elongated soma (for review, see http://webvision.med.utah.edu/). In the past, bovine retinas served as a tissue source to isolate biochemically the components comprising the rod phototransduction cascade and to elucidate many of the basic mechanisms (Burns & Arshavsky, 2005; Ridge, Abdulaev, Sousa, & Palczewski, 2003; Lamb & Pugh, 2006). In the last 15 years however, an abundance of naturally occurring, transgenic and knockout/knockin mouse models (Chang et al., 2002; Dalke & Graw, 2005; Hafezi, Grimm, Simmen, Wenzel, & Reme, 2000) permitted analyses of mechanistic details of rod and cone phototransduction *in vivo*. The mouse retina is rod-rich, and only about 3% of photoreceptors are cones distributed more or less evenly throughout the retina. Despite this low abundance, cone

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doi:10.1016/j.visres.2007.08.020

photoreceptor physiology can be explored readily by manipulation of the mouse genome, by electrophysiology, and sophisticated imaging techniques (Fu & Yau, 2007).

Mouse photoreceptors are postmitotic (withdrawn from the cell cycle) after postnatal day 7. However, the light-sensitive outer segments of rods and cones are renewed roughly every 10 days (Besharse & Hollyfield, 1979; LaVail, 1976; Young, 1976). Renewal is achieved by disk assembly/morphogenesis at the proximal end of the outer segment with concomitant disk shedding at the distal end, and phagocytosis of shed disk membrane by the adjacent RPE (Ritter et al., 2004). Daily renewal of ~10% (about 100 disks) of the outer segment membrane requires very active metabolism, i.e., a continuous high rate of biosynthesis to replace OS proteins, and reliable transport and targeting pathways.

Due to their characteristic shape and enormous biosynthetic requirement, photoreceptors are regarded model cells to study protein trafficking. A central question in photoreceptor cell biology concerns post-biosynthesis transport of membrane-associated proteins and the mechanisms of targeting to the outer segments for disk assembly. A rapid and efficient transport system from the ER to the proximal outer segment is essential for cell maintenance and survival. Here, we address the targeting and transport of polypeptides associated with rod and cone phototransduction. Principal components in the rod cascade are: the photoreceptor molecule (rhodopsin), its G-protein (transducin), a cGMP phosphodiesterase (PDE6), and the cation channel/exchanger complex (CNG/NCKX) (Table 1A). Corresponding cone polypeptides are related closely in sequence, structure and function (Table 1B). The regulatory components (GRK1, GC1, GCAP1, and GAP) are shared in rods and cones (Table 1C), but arrestins have rod- and cone-specific versions. Visual pigments, GRKs, and arrestins are single subunit proteins (although they may form homodimers or multimers), whereas transducin, PDE6 and cation channels are hetero-multimeric complexes. These polypeptides are either integral membrane proteins (visual pigments, GCs, channel/exchanger complex), peripherally membrane-associated (T, PDE6 subunits) or associated with integral membrane proteins (arrestin/rhodopsin., GCAPs/GCs). Membrane-associated proteins are considered non-diffusible with the exception of arrestins and possibly transducins, under certain conditions of light-induced translocation (Lobanova et al., 2007; Nair et al., 2005; Rosenzweig et al., 2007; Strissel, Sokolov, Trieu, & Arshavsky, 2006).

2. ER processing of nascent, prenylated proteins

Membrane association of transducin, PDE6, and GRK1 occurs posttranslationally and is mediated by N-terminal acylation (Tα) or C-terminal prenylation (Tγ, PDE6 catalytic subunits, GRK1). The Tα subunits are acylated heterogeneously (Johnson et al., 1994; Neubert, Johnson, Hurley, & Walsh, 1992), the Tγ subunit, PDE6α subunit, and GRK1 are farnesylated (C_{15} side chain) (Anant et al., 1992; Inglese, Koch, Caron, & Lefkowitz, 1992; Lai, Perez-Sala, Cañada, & Rando, 1990; Qin, Pittler, & Baehr, 1992), and PDE6β and cone PDE6α′ subunits (Li, Volpp, & Applebury, 1990) are geranylgeranylated (C_{20} side chain). Prenyl side chains are synthesized in all mammals via the mevalonate pathway and attached to newly synthesized cytosolic proteins carrying a C-terminal CaaX box motif (C, cysteine; a, aliphatic amino acid; X, any amino acid) (Hancock, Cadwallader, Paterson, & Marshall, 1991; Magee & Seabra, 2005; McTaggart, 2006). The prenyl chain is attached to the cysteine of the CaaX motif via a thioether bond by cytosolic prenyl transferases (Zhang & Casey, 1996). Prenylated proteins are synthesized in the cytosol, dock to the endoplasmic reticulum (ER) and are further processed by ER-associated proteins (Gelb et al., 2006). The ER-resident processing machinery removes the C-terminal tripeptide aaX and carboxymethylates the C-terminal cysteine, thereby producing a hydrophobic anchor suitable for protein–membrane or protein–protein interaction. A key question concerns mechanisms of targeting and transport of ER-anchored prenylated proteins to the cilium followed by intraflagellar transport through the cilium to nascent outer segments. A current hypothesis is that prenylated proteins docked to the ER require vesicular transport. Transfer to vesicles is thought to be mediated by cytosolic prenyl binding proteins featuring a hydrophobic groove accommodating prenyl side chains (Gelb et al., 2006; McTaggart, 2006), perhaps mediated by small GTP binding proteins. One such protein is PrBP/δ, formerly named PDE6δ (see below).

3. Biosynthesis of integral membrane proteins and post-Golgi transport

Transmembrane proteins are synthesized by ER-associated ribosomes and exported to the Golgi apparatus, finally emerging in vesicles from the trans-Golgi network (TGN) (reviewed by (Lippincott-Schwartz, Roberts, & Hirschberg, 2000)). A well investigated example is the rod photoreceptor transport of the G-protein coupled receptor (GPCR), rhodopsin, a hepta-spanning polypeptide (Deretic, 2006). Rhodopsin transport occurs sequentially, including budding of vesicular transport carriers carrying rhodopsin from the TGN, vectorial translocation of the carrier through the inner segment towards the basal body involving molecular motors, fusion of the carriers with the plasma membrane near the cilium, and intraflagellar transport through the cilium to the ROS disks. Mechanistic details are complex and incompletely understood. A C-terminal sorting motif (VXPX) regulates budding of rhodopsin containing transport carriers from the TGN (Deretic, 1998) whereupon the VXPX motif is recognized by ARF4, a small GTPase which regulates incorporation of rhodopsin into transport carriers (Deretic et al., 2005). The C-terminal cytoplasmic tail of rhodopsin was shown to interact with cytoplasmic dynein via the dynein light