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# Molecular complexes that direct rhodopsin transport to primary cilia

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ABSTRACT

Rhodopsin is a key molecular constituent of photoreceptor cells, yet understanding of how it regulates photoreceptor membrane trafficking and biogenesis of light-sensing organelles, the rod outer segments (ROS) is only beginning to emerge. Recently identified sequence of well-orchestrated molecular interactions of rhodopsin with the functional networks of Arf and Rab GTPases at multiple stages of intracellular targeting fits well into the complex framework of the biogenesis and maintenance of primary cilia, of which the ROS is one example. This review will discuss the latest progress in dissecting the molecular complexes that coordinate rhodopsin incorporation into ciliary-targeted carriers with the recruitment and activation of membrane tethering complexes and regulators of fusion with the periciliary plasma membrane. In addition to revealing the fundamental principals of ciliary membrane renewal, recent advances also provide molecular insight into the ways by which disruptions of the exquisitely orchestrated interactions lead to cilia dysfunction and result in human retinal dystrophies and syndromic diseases that affect multiple organs, including the eyes.



**Contents**



*Abbreviations:* ADRP, Autosomal Dominant Retinitis Pigmentosa; BBS, Bardet-Biedl Syndrome; BBSome, a conserved complex of BBS proteins; CTS, Ciliary Targeting Signal; DHA, Docosahexaenoic Acid; GAP, GTPase Activating Protein; GC1, Guanylyl Cyclase 1; GEF, Guanine Nucleotide Exchange Factor; IFT, Intraflagellar Transport; JBTS, Joubert Syndrome; MKS, Meckel Syndrome; MT, Microtubules; MTOC, Microtubule Organizing Center; NPHP, Nephronophthisis; PLA, Proximity Ligation Assay; OCT, Optical Coherence Tomography; RIS, Rod Inner Segment(s); ROS, Rod Outer Segment(s); RPGR, Retinitis Pigmentosa GTPase Regulator; RTC(s), Rhodopsin Transport Carrier(s); SNARE, Soluble N-ethylmaleimide-sensitive Factor Attachment Protein Receptor; TGN, Trans-Golgi Network.

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## 1. Introduction

Identifying physiological functions of human retinopathy-associated proteins is a long-term goal towards the treatment of blinding diseases. Rhodopsin is the main feature of retinal rod photoreceptors that plays an essential role not only in evoking visual signals, but also in shaping the necessary morphology of photoreceptor cells for the specific signaling processes (Arshavsky and Burns, 2012; Burns and Arshavsky, 2005; Deretic, 2006; Deretic and Wang, 2012; Palczewski, 2012). The phototransduction cascade that propagates visual excitation takes place in the light sensing organelle, the photoreceptor rod outer segment (ROS), which does not form in the absence of rhodopsin (Humphries et al., 1997). The last decade has seen a remarkable progress in our understanding of the functional effects of rhodopsin mutations and brought us closer to using this information to devise potential therapies for retinal dystrophies caused by rhodopsin mutations (Mendes et al., 2005; Rakoczy et al., 2011). However, much needs to be learned about the physiological roles of rhodopsin beyond its regulation of the phototransduction cascade in photoreceptor cells. For instance, mutations in the C-terminal domain of rhodopsin cause some of the most severe forms of Autosomal Dominant Retinitis Pigmentosa (ADRP) (Berson et al., 2002; Bessant et al., 1999). This suggests that photoreceptor cells have particularly low tolerance for injuries caused by this category of rhodopsin mutants, even in the presence of generally sufficient quantities of wild type rhodopsin. Moreover, rhodopsin variants that contain mutations within the C-terminal domain can function as light receptors, but fail to rescue ROS morphogenesis in rhodopsin knockout mice that otherwise lack ROS (Concepcion and Chen, 2010; Concepcion et al., 2002). So what is wrong with the photoreceptor cells expressing rhodopsin carrying the C-terminal ADRP mutations? A broad search for answers to this question has recently brought to light a number of physiological interactions that act in concert to localize rhodopsin efficiently and exclusively to the ROS.

## 2. The origin of the rod outer segment (ROS)

### 2.1. ROS is a modified primary cilium

The ROS is a light-sensing organelle that arises through a distinctive conversion of the plasma membrane of the sensory cilium, also called the primary cilium. Primary, or non-motile cilia are specialized projections that are found on the cell membranes of almost all eukaryotic cell types where they function to capture a wide range of extracellular signals (Singla and Reiter, 2006). They are exquisitely organized to enclose sensory receptors that are specifically targeted to, and highly concentrated in, the ciliary membranes. Because of the exceptional unidirectional membrane flow, rhodopsin trafficking represents an extreme case of ciliary receptor targeting. The recent appreciation of the high conservation of intracellular trafficking complexes that direct membrane delivery to specific intracellular destinations has provided new insight into the molecular mechanisms of ciliary membrane targeting. For example, it facilitated elucidation of the molecular mechanisms of a broad range of human diseases collectively known as ciliopathies, which are caused by dysfunctional formation and dysfunction of primary cilia that contribute to both retinal degeneration and cystic kidneys and are often associated with obesity, polydactyly and sensory impairments (Arts et al., 2007; Blacque and Leroux, 2006; Cui et al., 2011; Fliegauf et al., 2007; Gerdes et al., 2009; Otto et al., 2005; Wiens et al., 2010).

To fulfill the specialized role in light capture and propagation of visual signals, rhodopsin and associated proteins involved in phototransduction are sequestered in the light-sensing membranes of the ROS. The connection between the ROS and the cell body, or the rod inner segment (RIS), is often called the connecting cilium. Although this is topographically correct considering photoreceptor morphology, it is functionally inaccurate as the entire ROS is in fact a sensory cilium. ROS has no biosynthetic organelles, thus rhodopsin is synthesized in the RIS and delivered to the ROS on membranous carriers that can be readily detected in amphibian photoreceptors in the immediate proximity of the cilia (Fig. 1A). A comparison between rod photoreceptor “connecting” cilia and the green algae flagella reveals great structural similarities (Rosenbaum et al., 1999) (Fig. 1B and C). Early studies of rhodopsin trafficking have determined that the rhodopsin-bearing vesicles deliver the newly synthesized protein to the base of the cilia (Papermaster et al., 1985, 1986) (Fig. 1D), and that they fuse with a specialized domain elaborated by the RIS plasma membrane, the periciliary ridge complex (Peters et al., 1983) (PRC, Fig. 1E). These studies were originally performed on amphibians because of their extensive ROS membrane turnover compared to mammalian photoreceptors. *Xenopus laevis* and *Rana berlandieri* photoreceptors synthesize and transport $\sim 3\ \mu m^2$ and $\sim 1.5\ \mu m^2$ of membrane per minute, respectively, vs. $0.1\ \mu m^2$ of membrane per minute synthesized by rat photoreceptors (Besharse, 1986). Additionally, due to their larger size, light-sensing membranes in amphibians contain $6 \times 10^4$ molecules of rhodopsin vs. 2000 molecules of rhodopsin in rats. Although mammalian ROS membrane turnover is much slower, they also have a region that is functionally equivalent to the frog PRC, which is called the periciliary membrane complex (PMC) (Maerker et al., 2008; Yang et al., 2010).

Early studies using GFP-fusion proteins, to address general principals of intracellular trafficking in living cells, indicated that post-Golgi transport involves formation of large complex membrane intermediates, rather than small vesicles, that often stretch into tubules as they move along the cytoskeletal elements (Hirschberg et al., 1998). Similarly, conventional EM analysis revealed that the membranes that carry rhodopsin are not small vesicles of defined size and shape (Deretic and Papermaster, 1991). These rather pleiomorphic structures are thus more accurately named rhodopsin transport carriers (RTCs) (Deretic, 2006). On their way to the cilium RTCs have to meet the challenge of the infinitesimally small fusion area combined with an extremely high membrane influx, which requires a supremely regulated process that enables them to deliver rhodopsin to the exact periciliary location in the photoreceptor cell and nowhere else. This task is further complicated by the densely packed mitochondria (m, Fig. 1A