Vision Research 75 (2012) 5–10

Contents lists available at SciVerse ScienceDirect

Vision Research

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

Molecular assemblies that control rhodopsin transport to the cilia

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ARTICLE INFO

Article history:
Received 18 June 2012
Accepted 25 July 2012
Available online 7 August 2012

Keywords:
Arf GAPs
Cilium
Rabs
Rhodopsin
Trafficking

ABSTRACT

This review will focus on the conserved molecular mechanisms for the specific targeting of rhodopsin and rhodopsin-like sensory receptors to the primary cilia. We will discuss the molecular assemblies that control the movement of rhodopsin from the central sorting station of the cell, the trans-Golgi network (TGN), into membrane-enclosed rhodopsin transport carriers (RTCs), and their delivery to the primary cilia and the cilia-derived sensory organelle, the rod outer segment (ROS). Recent studies reveal that these processes are initiated by the synergistic interaction of rhodopsin with the active form of the G-protein Arf4 and the Arf GTPase activating protein (GAP) ASAP1. During rhodopsin progression, ASAP1 serves as an activation platform that brings together the proteins necessary for transport to the cilia, including the Rab11a–Rabin8–Rab8 complex involved in ciliogenesis. These specialized molecular assemblies, through successive action of discrete modules, cooperatively determine how rhodopsin and other rhodopsin-like signaling receptors gain access to primary cilia.

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1. ROS is a modified primary cilium

Primary cilia are specialized membrane projections that are found on most eukaryotic cell types. Although their architecture varies, from a simple membrane outgrowth to elaborate specialized organelles, such as the retinal photoreceptor rod outer segments (ROS), their common function is to capture extracellular signals. Together with other components of the signal transduction complexes, sensory receptors are highly concentrated in ciliary membranes allowing for exceptional sensitivity to external stimuli. In the case of vision, the architecture and the molecular composition of the ROS provide for the optimum performance underlying extraordinary light sensitivity, i.e. the light receptor rhodopsin and associated phototransduction machinery concentrated in the stacked disk membranes ensure the capture of a single photon of light (Burns & Arshavsky, 2005; Rieke & Baylor, 1998).

The challenge in maintaining the distinctive light sensitivity of the ROS is partially met by the tight control of the protein entrance across the base, the connecting cilium, which is equivalent to the transition zone of primary (non-motile) cilia. Rhodopsin constitutes the main ciliary-targeted cargo protein in rod photoreceptors. Its delivery to ROS is an outstanding case of ciliary receptor transport, with connecting cilia trafficking ~1000 rhodopsin molecules per second (Besharse, 1986). Light sensitivity is maintained through the continuous renewal of the light sensing rhodopsin-laden membranes that are shed and engulfed by the adjacent retinal pigment epithelium, combined with the light-dependent entry and exit of the cytosolic components of the signal transduction machinery and the selective delivery of the lipid-modified peripheral membrane proteins (Calvert et al., 2006; Insinna & Besharse, 2008; Najafi, Maza, & Calvert, 2011; Wright et al., 2011; Zhang et al., 2011).

The selective inclusion of ROS-specific molecular complexes and the exclusion of the macromolecules that function in other parts of the cell are ongoing processes throughout the life of the photoreceptor, which often go awry when the key proteins required for the function and maintenance of this specialized organelle are affected by disease-causing mutations. The transition zone of the photoreceptor cilia houses a network of highly conserved proteins affected by a wide range of human diseases and developmental disorders known as ciliopathies. These include Senior–Loken, Jeune and Bardet–Biedel Syndrome (BBS), which are characterized by both retinal degeneration and cystic kidneys, frequently combined with obesity, polydactyly and sensory impairments (Blacque & Leroux, 2006; Fliegauf, Benzing, & Omran, 2007; Gerdes, Davis, & Katsanis, 2009).

Abbreviations: RIS(s), rod inner segment(s); ROS(s), rod outer segment(s); RTC(s), rhodopsin transport carrier(s); RP, retinitis pigmentosa; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; TGN, trans-Golgi network.

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0042-6989/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.visres.2012.07.015

2. Rhodopsin trafficking to the ciliary base

Like all membrane proteins, rhodopsin follows the intracellular path from the site of its synthesis, the endoplasmic reticulum (ER) to the Golgi complex responsible for N-linked oligosaccharide modifications, sorting and transport. The ER and the Golgi are localized in the myoid part of the rod inner segment (RIS) (Fig 1A). After exiting the Golgi complex, rhodopsin reaches the TGN where it is specifically incorporated into rhodopsin transport carriers (RTCs) (Deretic, 2010), which traverse the mitochondria-rich ellipsoid region on their way to the base of the cilia. All primary cilia are contiguous with, but separated from the plasma membrane by the periciliary diffusion barrier. The exact nature of the periciliary diffusion barrier in photoreceptor cells is unclear at present, but is likely to combine specific lipid ordering, septin rings and transition zone protein complexes (Besharse, 1986; Chih et al., 2012; Garcia-Gonzalo et al., 2011; Hu et al., 2010; Nachury, Seeley, & Jin, 2010; Vieira et al., 2006). The periciliary membrane and the specialized membrane domain called the periciliary ridge complex (PRC) (Papermaster, Schneider, & Besharse, 1985; Peters et al., 1983), function as a site for docking and fusion of RTCs.

3. ASAP1 as a component of a putative protein coat that regulates rhodopsin trafficking

Precious little is known about how membrane cargo moves from the TGN to the specialized organelles like primary cilia. Earlier studies have identified ubiquitous protein complexes that coat membranes and direct movement of cargo between intracellular organelles. For example, membrane transport between the ER and the Golgi, as well as the intra-Golgi transport, are mediated by the COPI and COPII families of coat proteins (Lee et al., 2004). Our studies suggest that rhodopsin post-TGN trafficking to the cilia is regulated by a new, specialized system that differs from the well-studied assemblies that control the distribution of proteins within a cell. This molecular assembly functions as an effector of Arf4 at the TGN, where it forms a ciliary targeting module comprised of (i) the Arf GTPase activating protein (GAP) ASAP1, (ii) the small GTPase Rab11 and (iii) the Rab11/Arf effector FIP3 (Mazelova, Astuto-Gribble, et al., 2009) (Fig 1B). Arf4 is a member of the Arf family of G-proteins that regulate membrane transport (Donaldson & Jackson, 2011), whereas Rab11 belongs to the Rab family of GTPases that together with protein coats, membrane tethers and SNAREs regulate intracellular membrane targeting (Cai, Reinisch, & Ferro-Novick, 2007). FIP3 is a dual Arf/Rab11 effector that may serve to coordinate the activities of Arfs and Rabs (Hales et al., 2001; Hickson et al., 2003). FIP3 also interacts with ASAP1 (Inoue et al., 2008), thus linking all known components of the ciliary targeting module.

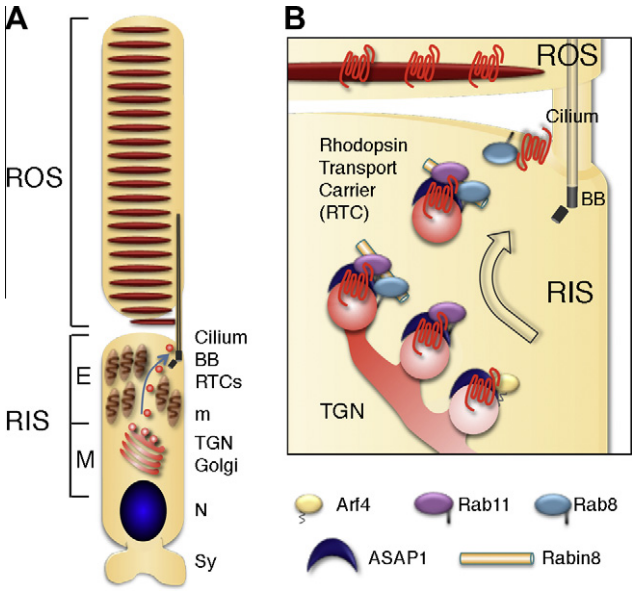

Fig. 1. (A) Diagram of a photoreceptor cell. Primary cilium originates from the basal body (BB) in the rod inner segment (RIS) and the axoneme elaborates the rod outer segment (ROS). The transition zone is a "connecting cilium", the gateway to the ROS. Golgi and the TGN are localized in the myoid region (M) of the RIS. RTCs that bud from the TGN are targeted to the base of the cilium (arrow), through the ellipsoid region (E) filled with mitochondria (m). N, nucleus; Sy, synapse. (B) Molecular interactions taking place during rhodopsin progression to the ciliary base. At the TGN, activated Arf4 interacts with rhodopsin and they recruit ASAP1 into the ternary complex. ASAP1 likely initiates membrane deformation through its BAR domain while mediating GTP-hydrolysis on Arf4, which then dissociates from the TGN. Next, ASAP1 selectively binds Rab11a, which also associates with rhodopsin. ASAP1 and Rab11a recruit Rabin8 and Rab8. On RTCs, ASAP1 serves as a scaffold for the Rab11a/Rabin8/Rab8 complex, which controls the activation of Rab8. Activated Rab8 regulates RTCs fusion and the delivery of rhodopsin across the diffusion barrier surrounding the cilium. Rhodopsin then proceeds through the transition zone (or the connecting cilium) into the ciliary axoneme that forms the ROS.

ASAP1 is a multifunctional scaffold protein that acts as a regulator of rhodopsin trafficking through GTP hydrolysis on Arf4 and functions as an Arf4 effector that regulates RTC budding (Mazelova, Astuto-Gribble, et al., 2009). Arf4 mutant I46D that selectively abolishes ASAP1-mediated GTP hydrolysis disrupts RTC budding, causing rhodopsin mislocalization and rapid retinal degeneration in transgenic animals (Mazelova, Astuto-Gribble, et al., 2009). The functional domains of ASAP1 include the Arf GAP domain, pleckstrin homology (PH), SH3, proline-rich, and N-terminal BAR domain (Nie & Randazzo, 2006; Randazzo & Hirsch, 2004). The phosphorylation of the C-terminal SH3 and proline-rich domains, involved in protein–protein interactions, affects the conformation and the activity of ASAP1 (Nie & Randazzo, 2006; Randazzo & Hirsch, 2004). The N-terminal BAR (Bin/amphiphysin/Rvs) domain (Peter et al., 2004), mediates membrane tubulation and homodimerization of ASAP1, and acts as an autoinhibitor of its GAP activity (Jian et al., 2009; Nie et al., 2006).

Our studies indicate that targeting of ciliary cargo presented in the context of Arf4 involves a stepwise assembly of ASAP1-associated complexes. This mechanism of action is distinctly different from that of the BBSome, a coat protein complex that also acts as an effector of Arf family proteins (Arl6), which functions in ciliary sorting of the GPCR Sstr3, although it lacks the capacity to deform the membrane (Jin et al., 2010). These differences parallel the differences in the assembly of the COPI and COPII coat protein complexes involved in bi-directional ER-Golgi trafficking (Lee et al., 2004). ASAP1 resembles the Sec23 component of the COPII coat, which acts as a GAP for the Arf-family GTPase Sar1, and controls the forward direction of vesicle traffic (Lord et al., 2011). The en-block assembly of the BBSome resembles the assembly of the COPI coat involved in retrograde trafficking. The release of the COPII coat is regulated by the phosphorylation of the Sec23/Sec24 complex, so it is possible that the phosphorylation of the C-terminal protein–protein interaction domains of ASAP1 also regulates its release from RTCs.

Recently, a combination of proteomic, structural and comparative genomic studies has revealed that membrane-deforming machineries, which control intracellular trafficking by generating vesicles and tubules carrying cargo proteins, belong to three families: (i) protocoatomer-derived, which function in endo- and exocytosis (clathrin, COPI and COPII), (ii) ESCRT, which function in the generation of multivesicular bodies and (iii) BAR domain-associated complexes, which function in the Golgi-to-endosome trafficking as well as in endo- and phagocytosis (amphiphysin, FBP-17) (Field, Sali, & Rout, 2011). In this context, ASAP1 could