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

Dusanka Deretic [a,b,*], Jing Wang [a]

[a] Department of Surgery, Division of Ophthalmology, University of New Mexico, Albuquerque, NM 87131, United States
[b] Department of Cell Biology and Physiology, University of New Mexico, Albuquerque, NM 87131, United States

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.

Published by Elsevier Ltd.

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.

* Corresponding author at: Department of Surgery, Division of Ophthalmology, University of New Mexico, School of Medicine, Basic Medical Sciences Building, Rm. 377, 915 Camino de Salud, NE, Albuquerque, NM 87131, United States. Fax: +1 505 272 6029.

E-mail address: dderetic@salud.unm.edu (D. Deretic).

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.

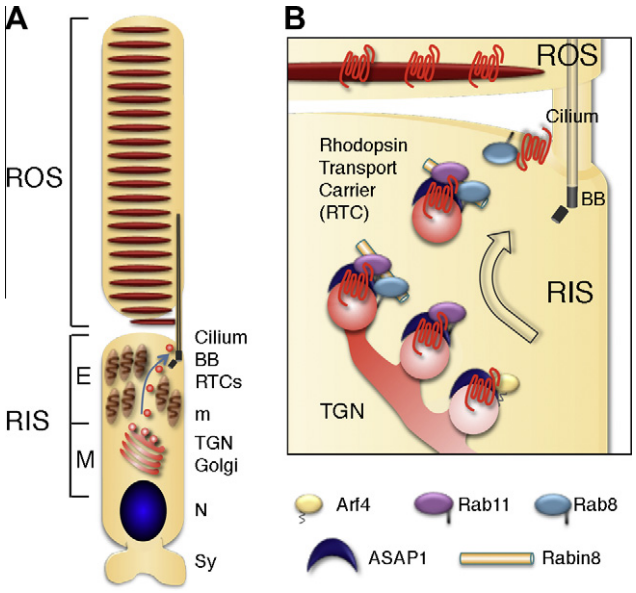

Fig. 1. (A) Diagram of a photoreceptor cell. Primary cilium originates from the basal body (BB) in the rod inner segment (RIS) and the axoneme elaborates the rod outer segment (ROS). The transition zone is a "connecting cilium", the gateway to the ROS. Golgi and the TGN are localized in the myoid region (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

be viewed as a component of a BAR domain-associated complex that regulates ciliary targeting of sensory receptors such as rhodopsin. ASAP1 could also be a component of a membrane-deforming protein coat. Its coat-like properties include the regulation by a member of the Arf family (Arf4), oligomerization and membrane curvature-inducing activity, and formation of a heterotrimeric complex with Rab11 and FIP3 (Inoue et al., 2008), which also acts as an oligomer. FIP3 may form a coat with ASAP1, or may link the ASAP1-based coat to the dynein motor (Horgan et al., 2010) for transport of RTCs to the base of the cilium.

4. Rhodopsin has two functional ciliary targeting signals, the VxPx and the FR, which are recognized by Arf4 and ASAP1, respectively

One of the main functions of the canonical coat complexes is the recognition of intracellular targeting signals in cargo proteins (Springer, Spang, & Schekman, 1999). Rhodopsin possesses the C-terminal VxPx targeting motif that regulates Arf4 binding and rhodopsin targeting in vitro and in vivo (Concepcion, Mendez, & Chen, 2002; Deretic et al., 1998, 2005; Green et al., 2000; Li et al., 1996; Tam et al., 2000). The highly conserved VxPx motif is also functional in polycystins 1 and 2, and the cyclic nucleotide-gated channel CNGB1b subunit (Geng et al., 2006; Jenkins et al., 2006; Ward et al., 2011). The second ciliary targeting signal, the FR motif, which is present in several other GPCRs (Corbit et al., 2005), lies within the cytoplasmic helix H8 of rhodopsin (Fig 2). Our recent studies indicate that the FR motif binds to ASAP1, implicating Arf4 and ASAP1 as the recognition module for the VxPx- and FR-containing cargo. We find that, unlike the WT, the rhodopsin FR-AA mutant defective in ASAP1 binding does not localize to cilia in the heterologous expression system, in epithelial IMCD3 cells. In this aspect, rhodopsin FR-AA mutant is comparable to smoothened Ciliary Localization Deficient (CLDSmo) mutant (Corbit et al., 2005). Interestingly, the GPCR Sstr3 that is sorted by the BBSome also contains a homologous motif that is apparently not employed for its ciliary targeting (Berbari et al., 2008). This difference may reflect the cell specificity, or the existence of parallel pathways to cilia characterized by their dependency on the BBSome or ASAP1.

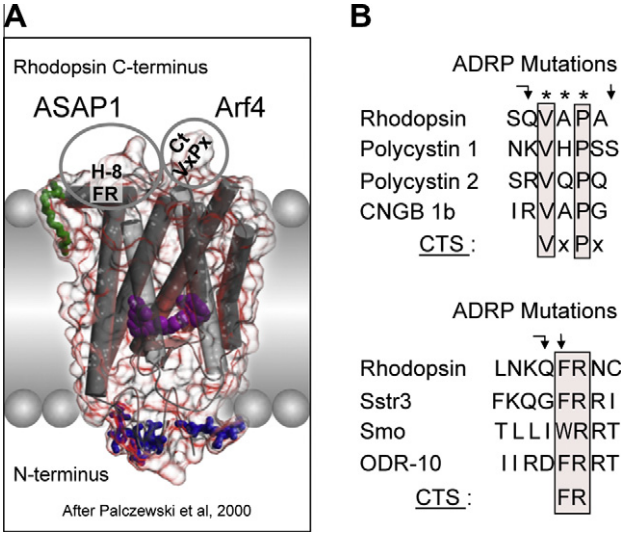

Fig. 2. (A) Schematic of bovine rhodopsin with protein backbone shown with a transparent surface, chromophore in purple, palmitic acid residues in green and oligosaccharides in blue (after Palczewski et al. (2000)). The cytoplasmic C-terminal VxPx motif, which is the Arf4 binding site, is circled. The FR motif, which binds ASAP1 is within α helix H-8 (circled). (B) GPCRs containing the VxPx (top) and FR targeting sequences (bottom) are aligned. The VxPx C-terminal ciliary targeting signal (CTS) of rhodopsin is a hot spot for ADRP mutations, which are indicated in the figure (asterisk denotes a single amino acid substitution, arrow an insertion and bent arrow a truncation). Truncation mutation and insertion disrupting the rhodopsin CTS FR also result in ADRP. The sequence alignment of GPCRs containing the FR motif is according to Corbit et al. (2005). However, we changed the alignment of Sstr3 to reflect the presence of the FR signal, which lies three amino acids downstream of the FK signal previously identified by Corbit et al. Interestingly, the FK signal has been mutagenized and found non-functional by Mykytyn and colleagues (Berbari et al., 2008), which leaves the role of the FR signal in the ciliary targeting of Sstr3 undetermined.

5. Regulation of the putative protein coat

In addition to signal recognition, coat complexes also deform membranes. Our experiments suggest that activated Arf4 and ASAP1 are necessary and sufficient for ciliary cargo recognition in vitro, however membrane lipids probably affect complex formation under physiological conditions. This is highly likely because ASAP1 acts as a coincidence detector for acidic phospholipids and activated Arfs, and functions as an Arf effector by increasing membrane curvature (Nie et al., 2006). The formation of the rhodopsin–Arf4–ASAP1 complex in the presence of acidic phospholipids at the TGN likely results in membrane deformation and packaging of rhodopsin into budding RTCs, a conclusion supported by our finding that ASAP1 stimulates RTC budding in vitro (Mazelova, Astuto-Gribble, et al., 2009).

ASAP1 functions as a temporal regulator of ciliary traffic through GTP hydrolysis on Arf4. A conformational change involving the Arf–ASAP1 complex is required to disable the autoinhibition of GAP activity blocked by the BAR domain (Jian et al., 2009; Luo et al., 2007). This autoinhibition can be alleviated by phosphatidylinositol 4,5 bisphosphate ($PI(4,5)P_2$) and/or the binding of FIP3 to the BAR domain of ASAP1 (Inoue et al., 2008; Jian et al., 2009). Since FIP3 is a component of the ciliary targeting module that regulates rhodopsin trafficking, it could serve to activate the GAP activity of ASAP1 in photoreceptor cells. An appealing possibility is that $PI(4,5)P_2$ determines the binding partner for ASAP1, since it regulates both its GAP activity and interaction with FIP3. Our studies implicate $PI(4,5)P_2$ as a crucial component necessary for RTC fusion (Deretic et al., 2004) and this function could involve the regulation of the assembly/disassembly of the putative ASAP1/FIP3 coat.

The role of rhodopsin as a cargo protein may be to stabilize the inhibitory conformation of the BAR domain of ASAP1, precluding premature GTP hydrolysis on Arf4. A cargo-induced pause could stall the complex and prolong the effector function of ASAP1, facilitating the collection of cargo into ciliary-targeted RTCs. This implies that in the absence of the ciliary cargo at the TGN, ASAP1-mediated GTP hydrolysis leads to the release of Arf4 from the membrane. This concept is supported by our study showing that Arf4 is absent from the photoreceptor TGN when its access to rhodopsin is blocked (Mazelova, Astuto-Gribble, et al., 2009).

6. ASAP1 acts as a scaffold for the Rab11a–Rabin8–Rab8 ciliogenesis cascade

The Rab11a–Rabin8–Rab8 cilia-generating module is a regulatory complex that governs ciliary targeting and ciliogenesis (Bryant et al., 2010; Knodler et al., 2010; Westlake et al., 2011). The guanine nucleotide exchange factor (GEF) for Rab8, Rabin8, colocalizes with ASAP1 and Rab11 at the photoreceptor TGN. Rabin8 specifically associates with ASAP1 and the inactive form of Rab8, indicating that in photoreceptor cells ASAP1 acts as a scaffold for the Rab11a–Rabin8–Rab8 ciliogenesis cascade (Fig 1B). Interestingly, retinal photoreceptors possess an additional Rab8 GEF named Retinitis Pigmentosa GTPase Regulator (RPGR). Mutations in RPGR that reduce its GEF activity cause photoreceptor degeneration in

X-linked retinitis pigmentosa (XLRP) (Murga-Zamalloa et al., 2010). The presence of two Rab8 GEFs suggests that the additional level of Rab8 control may be needed for the extraordinary ciliary traffic that supports continuous ciliary membrane expansion in photoreceptor cells.

Our data suggest that Rab11a–Rabin8–Rab8 complex in addition to ciliogenesis also regulates ciliary access and acts as a ciliary destination module. This module allows the cargo that is vetted by the Arf4/ASAP1 cargo recognition module to pass across the periciliary diffusion barrier. Interestingly, our studies show that rhodopsin binds Rab11 and Rab8 directly, similar to other ciliary sensory receptors (Follit et al., 2010; Ward et al., 2011). This binding might strengthen cargo interaction with the ciliary destination module. Both Rab11a and Rab8a interact with the Sec6/8 membrane tethering complex (Bryant et al., 2010). The Sec6/8 complex is localized at the base of the photoreceptor cilium (Mazelova, Ransom, et al., 2009), consistent with its role as an effector for the ciliary cargo destination module. The final steps in RTC fusion at the base of the cilium involve SNAREs Syntaxin 3 and SNAP-25. Surprisingly, docosahexaenoic acid (DHA), an essential polyunsaturated fatty acid of the ROS, increases Syntaxin 3 and SNAP-25 pairing, thus regulating ciliary membrane expansion (Mazelova, Ransom, et al., 2009). Once in the ciliary membrane, rhodopsin likely moves towards the ROS through different molecular assemblies that include kinesin microtubule motors and the ubiquitous IFT machinery (Bhowmick et al., 2009; Insinna et al., 2009; Keady, Le, & Pazour, 2011; Lopes et al., 2010; Pazour et al., 2002).

7. Retinopathy-associated proteins and degenerative diseases

Mutations in the rhodopsin gene that cause autosomal dominant retinitis pigmentosa (ADRP) (http://www.retina-international.com/sci-news/rhomut.htm) affect both the VxPx and the FR targeting signals that are essential for the correct delivery to the ciliary membrane (Fig 2). Disruptions of the VxPx motif lead to some of the most severe forms of ADRP (Berson et al., 2002; Bessant et al., 1999). Our studies suggest that the FR targeting signal that interacts with ASAP1 critically affects ciliary access of rhodopsin in the heterologous expression system, in epithelial cells. The interaction of the rhodopsin FR ciliary targeting signal with ASAP1 in retinal photoreceptors remains to be confirmed, however ADRP mutations affecting the FR signal suggest that it also plays a role in ROS biogenesis.

One of the regulators of RTC trafficking to the ciliary base, Rab8 has recently emerged as a crucial regulator of ciliogenesis and ciliary targeting (Bryant et al., 2010; Deretic et al., 1995; Knodler et al., 2010; Moritz et al., 2001; Omori et al., 2008; Westlake et al., 2011; Yoshimura et al., 2007). Rab8 directly interacts with: (i) the ciliary cargo such as rhodopsin (our studies), Polycystin 1 and fibrocystin (Follit et al., 2010; Ward et al., 2011), (ii) the BBSome complex affected by mutations causing the Bardet–Biedel Syndrome (Nachury et al., 2007), (iii) RPGR, which is mutated in XLRP (Murga-Zamalloa et al., 2010) and (iv) CEP-290 (Kim, Krishnaswami, & Gleeson, 2008; Tsang et al., 2008) that organizes NPHP, MKS and JBTS complexes at the transition zone, where they restrict the diffusion of membrane proteins across the ciliary base (Craige et al., 2010; Garcia-Gonzalo et al., 2011; Sang et al., 2011; van Reeuwijk, Arts, & Roepman, 2011). Mutations in NPHP, MKS and JBTS proteins cause nephronophthisis, Meckel syndrome and Joubert syndrome. NPHP and MKS proteins interact with IFT complexes and support the ciliary transport of rhodopsin, but not peripherin (Zhao & Malicki, 2011). Finally, in rhodopsin trafficking Rab8 appears to be functionally linked to Tulp1, and mutations in TULP1 gene cause autosomal recessive RP and LCA (Hagstrom et al., 2012). Thus, the molecular assemblies that control rhodopsin transport to the cilia that are described here exert the principal organizing function for the proteins involved in cilia structure and function, which account for the largest group among the genes linked to photoreceptor degeneration. Future studies of molecular assemblies that regulate the biogenesis of cilia and cilia-derived sensory organelles are likely to reveal the additional involvement of the specific members of the regulatory machinery described here in ciliopathies and other degenerative diseases.

Acknowledgments

We thank lab members and collaborators for their valuable contributions and many colleagues for their generous gifts of reagents and fruitful discussions. We also thank Kris Palczewski for the image of bovine rhodopsin. Supported by the NIH Grant EY-12421.

References

Berbari, N. F., Johnson, A. D., Lewis, J. S., Askwith, C. C., & Mykytyn, K. (2008). Identification of ciliary localization sequences within the third intracellular loop of G protein-coupled receptors. *Molecular Biology of the Cell, 19*(4), 1540–1547.

Berson, E. L., Rosner, B., Weigel-DiFranco, C., Dryja, T. P., & Sandberg, M. A. (2002). Disease progression in patients with dominant retinitis pigmentosa and rhodopsin mutations. *Investigative Ophthalmology and Visual Science, 43*(9), 3027–3036.

Besharse, J. C. (1986). Photosensitive membrane turnover: Differentiated membrane domains and cell–cell interaction. In R. Adler & D. Farber (Eds.), *The retina: A model for cell biological studies* (pp. 297–352). New York: Academic Press.

Bessant, D. A., Khaliq, S., Hameed, A., Anwar, K., Payne, A. M., Mehdi, S. Q., et al. (1999). Severe autosomal dominant retinitis pigmentosa caused by a novel rhodopsin mutation (Ter349Glu). Mutations in brief no. 208. *Human Mutation (Online), 13*(1), 83.

Bhowmick, R., Li, M., Sun, J., Baker, S. A., Insinna, C., & Besharse, J. C. (2009). Photoreceptor IFT complexes containing chaperones, guanylyl cyclase 1 and rhodopsin. *Traffic, 10*(6), 648–663.

Blacque, O. E., & Leroux, M. R. (2006). Bardet–Biedl syndrome: An emerging pathomechanism of intracellular transport. *Cellular and Molecular Life Sciences, 63*(18), 2145–2161.

Bryant, D. M., Datta, A., Rodriguez-Fraticelli, A. E., Peranen, J., Martin-Belmonte, F., & Mostov, K. E. (2010). A molecular network for de novo generation of the apical surface and lumen. *Nature Cell Biology, 12*(11), 1035–1045.

Burns, M. E., & Arshavsky, V. Y. (2005). Beyond counting photons: Trials and trends in vertebrate visual transduction. *Neuron, 48*(3), 387–401.

Cai, H., Reinisch, K., & Ferro-Novick, S. (2007). Coats, tethers, Rabs, and SNAREs work together to mediate the intracellular destination of a transport vesicle. *Developmental Cell, 12*(5), 671–682.

Calvert, P. D., Strissel, K. J., Schiesser, W. E., Pugh, E. N., Jr., & Arshavsky, V. Y. (2006). Light-driven translocation of signaling proteins in vertebrate photoreceptors. *Trends in Cell Biology, 16*(11), 560–568.

Chih, B., Liu, P., Chinn, Y., Chalouni, C., Komuves, L. G., Hass, P. E., et al. (2012). A ciliopathy complex at the transition zone protects the cilia as a privileged membrane domain. *Nature Cell Biology, 14*(1), 61–72.

Concepcion, F., Mendez, A., & Chen, J. (2002). The carboxyl-terminal domain is essential for rhodopsin transport in rod photoreceptors. *Vision Research, 42*(4), 417–426.

Corbit, K. C., Aanstad, P., Singla, V., Norman, A. R., Stainier, D. Y., & Reiter, J. F. (2005). Vertebrate Smoothened functions at the primary cilium. *Nature, 437*(7061), 1018–1021.

Craige, B., Tsao, C. C., Diener, D. R., Hou, Y., Lechtreck, K. F., Rosenbaum, J. L., et al. (2010). CEP290 tethers flagellar transition zone microtubules to the membrane and regulates flagellar protein content. *Journal of Cell Biology, 190*(5), 927–940.

Deretic, D. (2010). Post-Golgi trafficking and ciliary targeting of rhodopsin. In D. A. Dartt (Ed.). *Encyclopedia of the eye* (Vol. 3, pp. 480–487). Oxford, UK: Academic Press.

Deretic, D., Huber, L. A., Ransom, N., Mancini, M., Simons, K., & Papermaster, D. S. (1995). Rab8 in retinal photoreceptors may participate in rhodopsin transport and in rod outer segment disk morphogenesis. *Journal of Cell Science, 108*(Pt. 1), 215–224.

Deretic, D., Schmerl, S., Hargrave, P. A., Arendt, A., & McDowell, J. H. (1998). Regulation of sorting and post-Golgi trafficking of rhodopsin by its C-terminal sequence QVS(A)PA. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 10620–10625.

Deretic, D., Traverso, V., Parkins, N., Jackson, F., Rodriguez De Turco, E. B., & Ransom, N. (2004). Phosphoinositides, ezrin/moesin and rac1 regulate fusion of rhodopsin transport carriers in retinal photoreceptors. *Molecular Biology of the Cell, 15*, 359–370.

Deretic, D., Williams, A. H., Ransom, N., Morel, V., Hargrave, P. A., & Arendt, A. (2005). Rhodopsin C terminus, the site of mutations causing retinal disease, regulates trafficking by binding to ADP-ribosylation factor 4 (ARF4). *Proceedings*

of the National Academy of Sciences of the United States of America, 102(9), 3301–3306.
Donaldson, J. G., & Jackson, C. L. (2011). ARF family G proteins and their regulators: Roles in membrane transport, development and disease. *Nature Reviews Molecular Cell Biology, 12*(6), 362–375.
Field, M. C., Sali, A., & Rout, M. P. (2011). Evolution: On a bender – BARs, ESCRTs, COPs, and finally getting your coat. *Journal of Cell Biology, 193*(6), 963–972.
Fliegauf, M., Benzing, T., & Omran, H. (2007). When cilia go bad: Cilia defects and ciliopathies. *Nature Reviews Molecular Cell Biology, 8*(11), 880–893.
Follit, J. A., Li, L., Vucica, Y., & Pazour, G. J. (2010). The cytoplasmic tail of fibrocystin contains a ciliary targeting sequence. *Journal of Cell Biology, 188*(1), 21–28.
Garcia-Gonzalo, F. R., Corbit, K. C., Sirerol-Piquer, M. S., Ramaswami, G., Otto, E. A., Noriega, T. R., et al. (2011). A transition zone complex regulates mammalian ciliogenesis and ciliary membrane composition. *Nature Genetics, 43*(8), 776–784.
Geng, L., Okuhara, D., Yu, Z., Tian, X., Cai, Y., Shibazaki, S., et al. (2006). Polycystin-2 traffics to cilia independently of polycystin-1 by using an N-terminal RVxP motif. *Journal of Cell Science, 119*(Pt 7), 1383–1395.
Gerdes, J. M., Davis, E. E., & Katsanis, N. (2009). The vertebrate primary cilium in development, homeostasis, and disease. *Cell, 137*(1), 32–45.
Green, E. S., Menz, M. D., LaVail, M. M., & Flannery, J. G. (2000). Characterization of rhodopsin mis-sorting and constitutive activation in a transgenic rat model of retinitis pigmentosa. *Investigative Ophthalmology and Visual Science, 41*(6), 1546–1553.
Hagstrom, S. A., Watson, R. F., Pauer, G. J., & Grossman, G. H. (2012). Tulp1 is involved in specific photoreceptor protein transport pathways. *Advances in Experimental Medicine and Biology, 723*, 783–789.
Hales, C. M., Griner, R., Hobdy-Henderson, K. C., Dorn, M. C., Hardy, D., Kumar, R., et al. (2001). Identification and characterization of a family of Rab11-interacting proteins. *Journal of Biological Chemistry, 276*(42), 39067–39075.
Hickson, G. R., Matheson, J., Riggs, B., Maier, V. H., Fielding, A. B., Prekeris, R., et al. (2003). Arfophilins are dual Arf/Rab 11 binding proteins that regulate recycling endosome distribution and are related to *Drosophila* nuclear fallout. *Molecular Biology of the Cell, 14*(7), 2908–2920.
Horgan, C. P., Hanscom, S. R., Jolly, R. S., Futter, C. E., & McCaffrey, M. W. (2010). Rab11-FIP3 binds dynein light intermediate chain 2 and its overexpression fragments the Golgi complex. *Biochemical and Biophysical Research Communications.*
Hu, Q., Milenkovic, L., Jin, H., Scott, M. P., Nachury, M. V., Spiliotis, E. T., et al. (2010). A septin diffusion barrier at the base of the primary cilium maintains ciliary membrane protein distribution. *Science.*
Inoue, H., Ha, V. L., Prekeris, R., & Randazzo, P. A. (2008). Arf GTPase-activating protein ASAP1 interacts with Rab11 effector FIP3 and regulates pericentrosomal localization of transferrin receptor-positive recycling endosome. *Molecular Biology of the Cell, 19*(10), 4224–4237.
Insinna, C., & Besharse, J. C. (2008). Intraflagellar transport and the sensory outer segment of vertebrate photoreceptors. *Developmental Dynamics, 237*(8), 1982–1992.
Insinna, C., Humby, M., Sedmak, T., Wolfrum, U., & Besharse, J. C. (2009). Different roles for KIF17 and kinesin II in photoreceptor development and maintenance. *Developmental Dynamics*.
Jenkins, P. M., Hurd, T. W., Zhang, L., McEwen, D. P., Brown, R. L., Margolis, B., et al. (2006). Ciliary targeting of olfactory CNG channels requires the CNGB1b subunit and the kinesin-2 motor protein, KIF17. *Current Biology, 16*(12), 1211–1216.
Jian, X., Brown, P., Schuck, P., Gruschus, J. M., Balbo, A., Hinshaw, J. E., et al. (2009). Autoinhibition of Arf GTPase-activating protein activity by the BAR domain in ASAP1. *Journal of Biological Chemistry, 284*(3), 1652–1663.
Jin, H., White, S. R., Shida, T., Schultz, S., Aguiar, M., & Nachury, M. V. (2010). The conserved Bardet–Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. *Cell, 141*, 1208–1219.
Keady, B. T., Le, Y. Z., & Pazour, G. J. (2011). IFT20 is required for opsin trafficking and photoreceptor outer segment development. *Molecular Biology of the Cell, 22*(7), 921–930.
Kim, J., Krishnaswami, S. R., & Gleeson, J. G. (2008). CEP290 interacts with the centriolar satellite component PCM-1 and is required for Rab8 localization to the primary cilium. *Human Molecular Genetics, 17*(23), 3796–3805.
Knodler, A., Feng, S., Zhang, J., Zhang, X., Das, A., Peranen, J., et al. (2010). Coordination of Rab8 and Rab11 in primary ciliogenesis. *Proceedings of the National Academy of Sciences of the United States of America.*
Lee, M. C., Miller, E. A., Goldberg, J., Orci, L., & Schekman, R. (2004). Bi-directional protein transport between the ER and Golgi. *Annual Review of Cell and Developmental Biology, 20*, 87–123.
Li, T., Snyder, W. K., Olsson, J. E., & Dryja, T. P. (1996). Transgenic mice carrying the dominant rhodopsin mutation P347S: Evidence for defective vectorial transport of rhodopsin to the outer segments. *Proceedings of the National Academy of Sciences of the United States of America, 93*(24), 14176–14181.
Lopes, V. S., Jimeno, D., Khanobdee, K., Song, X., Chen, B., Nusinowitz, S., et al. (2010). Dysfunction of heterotrimeric kinesin-2 in rod photoreceptor cells and the role of opsin mislocalization in rapid cell death. *Molecular Biology of the Cell, 21*(23), 4076–4088.
Lord, C., Bhandari, D., Menon, S., Ghassemian, M., Nycz, D., Hay, J., et al. (2011). Sequential interactions with Sec23 control the direction of vesicle traffic. *Nature, 473*(7346), 181–186.
Luo, R., Ahvazi, B., Amariei, D., Shroder, D., Burrola, B., Losert, W., et al. (2007). Kinetic analysis of GTP hydrolysis catalysed by the Arf1-GTP–ASAP1 complex. *Biochemical Journal, 402*(3), 439–447.
Mazelova, J., Astuto-Gribble, L., Inoue, H., Tam, B. M., Schonteich, E., Prekeris, R., et al. (2009). Ciliary targeting motif VxPx directs assembly of a trafficking module through Arf4. *EMBO Journal, 28*(3), 183–192.
Mazelova, J., Ransom, N., Astuto-Gribble, L., Wilson, M. C., & Deretic, D. (2009). Syntaxin 3 and SNAP-25 pairing, regulated by omega-3 docosahexaenoic acid, controls the delivery of rhodopsin for the biogenesis of cilia-derived sensory organelles, the rod outer segments. *Journal of Cell Science, 122*(Pt. 12), 2003–2013.
Moritz, O. L., Tam, B. M., Hurd, L. L., Peranen, J., Deretic, D., & Papermaster, D. S. (2001). Mutant rab8 impairs docking and fusion of rhodopsin-bearing post-Golgi membranes and causes cell death of transgenic *Xenopus rods*. *Molecular Biology of the Cell, 12*(8), 2341–2351.
Murga-Zamalloa, C. A., Atkins, S. J., Peranen, J., Swaroop, A., & Khanna, H. (2010). Interaction of retinitis pigmentosa GTPase regulator (RPGR) with RAB8A GTPase: Implications for cilia dysfunction and photoreceptor degeneration. *Human Molecular Genetics, 19*(18), 3591–3598.
Nachury, M. V., Loktev, A. V., Zhang, Q., Westlake, C. J., Peranen, J., Merdes, A., et al. (2007). A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell, 129*(6), 1201–1213.
Nachury, M. V., Seeley, E. S., & Jin, H. (2010). Trafficking to the ciliary membrane: How to get across the periciliary diffusion barrier? *Annual Review of Cell and Developmental Biology, 26*, 59–87.
Najafi, M., Maza, N. A., & Calvert, P. D. (2011). Steric volume exclusion sets soluble protein concentrations in photoreceptor sensory cilia. *Proceedings of the National Academy of Sciences of the United States of America, 109*(1), 203–208.
Nie, Z., Hirsch, D. S., Luo, R., Jian, X., Stauffer, S., Cremesti, A., et al. (2006). A BAR domain in the N terminus of the Arf GAP ASAP1 affects membrane structure and trafficking of epidermal growth factor receptor. *Current Biology, 16*(2), 130–139.
Nie, Z., & Randazzo, P. A. (2006). Arf GAPs and membrane traffic. *Journal of Cell Science, 119*(Pt. 7), 1203–1211.
Omori, Y., Zhao, C., Saras, A., Mukhopadhyay, S., Kim, W., Furukawa, T., et al. (2008). Elipsa is an early determinant of ciliogenesis that links the IFT particle to membrane-associated small GTPase Rab8. *Nature Cell Biology, 10*(4), 437–444.
Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., et al. (2000). Crystal structure of rhodopsin: A G protein-coupled receptor. *Science, 289*(5480), 739–745.
Papermaster, D. S., Schneider, B. G., & Besharse, J. C. (1985). Vesicular transport of newly synthesized opsin from the Golgi apparatus toward the rod outer segment. Ultrastructural immunocytochemical and autoradiographic evidence in *Xenopus retinas*. *Investigative Ophthalmology and Visual Science, 26*(10), 1386–1404.
Pazour, G. J., Baker, S. A., Deane, J. A., Cole, D. G., Dickert, B. L., Rosenbaum, J. L., et al. (2002). The intraflagellar transport protein, IFT88, is essential for vertebrate photoreceptor assembly and maintenance. *Journal of Cell Biology, 157*(1), 103–113.
Peter, B. J., Kent, H. M., Mills, I. G., Vallis, Y., Butler, P. J., Evans, P. R., et al. (2004). BAR domains as sensors of membrane curvature: The amphiphysin BAR structure. *Science, 303*(5657), 495–499.
Peters, K. R., Palade, G. E., Schneider, B. G., & Papermaster, D. S. (1983). Fine structure of a periciliary ridge complex of frog retinal rod cells revealed by ultrahigh resolution scanning electron microscopy. *Journal of Cell Biology, 96*(1), 265–276.
Randazzo, P. A., & Hirsch, D. S. (2004). Arf GAPs: Multifunctional proteins that regulate membrane traffic and actin remodelling. *Cellular Signalling, 16*(4), 401–413.
Rieke, F., & Baylor, D. A. (1998). Single-photon detection by rod cells of the retina. *Reviews of Modern Physics, 70*(3), 1027–1036.
Sang, L., Miller, J. J., Corbit, K. C., Giles, R. H., Brauer, M. J., Otto, E. A., et al. (2011). Mapping the NPHP–JBTS–MKS protein network reveals ciliopathy disease genes and pathways. *Cell, 145*(4), 513–528.
Springer, S., Spang, A., & Schekman, R. (1999). A primer on vesicle budding. *Cell, 97*(2), 145–148.
Tam, B. M., Moritz, O. L., Hurd, L. B., & Papermaster, D. S. (2000). Identification of an outer segment targeting signal in the COOH terminus of rhodopsin using transgenic *Xenopus laevis*. *Journal of Cell Biology, 151*(7), 1369–1380.
Tsang, W. Y., Bossard, C., Khanna, H., Peranen, J., Swaroop, A., Malhotra, V., et al. (2008). CP110 suppresses primary cilia formation through its interaction with CEP290, a protein deficient in human ciliary disease. *Developmental Cell, 15*(2), 187–197.
van Reeuwijk, J., Arts, H. H., & Roepman, R. (2011). Scrutinizing ciliopathies by unraveling ciliary interaction networks. *Human Molecular Genetics, 20*(R2), R149–R157.
Vieira, O. V., Gaus, K., Verkade, P., Fullekrug, J., Vaz, W. L., & Simons, K. (2006). FAPP2, cilium formation, and compartmentalization of the apical membrane in polarized Madin-Darby canine kidney (MDCK) cells. *Proceedings of the National Academy of Sciences of the United States of America, 103*(49), 18556–18561.
Ward, H. H., Brown-Glaberman, U., Wang, J., Morita, Y., Alper, S. L., Bedrick, E. J., et al. (2011). A conserved signal and GTPase complex are required for the ciliary transport of polycystin-1. *Molecular Biology of the Cell, 22*(18), 3289–3305.
Westlake, C. J., Baye, L. M., Nachury, M. V., Wright, K. J., Ervin, K. E., Phu, L., et al. (2011). Primary cilia membrane assembly is initiated by Rab11 and transport protein particle II (TRAPPII) complex-dependent trafficking of Rabin8 to the centrosome. *Proceedings of the National Academy of Sciences of the United States of America, 108*(7), 2759–2764.

Wright, K. J., Baye, L. M., Olivier-Mason, A., Mukhopadhyay, S., Sang, L., Kwong, M., et al. (2011). An ARL3-UNC119-RP2 GTPase cycle targets myristoylated NPHP3 to the primary cilium. *Genes and Development, 25*(22), 2347–2360.

Yoshimura, S., Egerer, J., Fuchs, E., Haas, A. K., & Barr, F. A. (2007). Functional dissection of Rab GTPases involved in primary cilium formation. *Journal of Cell Biology, 178*(3), 363–369.

Zhang, H., Constantine, R., Vorobiev, S., Chen, Y., Seetharaman, J., Huang, Y. J., et al. (2011). UNC119 is required for G protein trafficking in sensory neurons. *Nature Neuroscience, 14*(7), 874–880.

Zhao, C., & Malicki, J. (2011). Nephrocystins and MKS proteins interact with IFT particle and facilitate transport of selected ciliary cargos. *EMBO Journal, 30*(13), 2532–2544.