Published in final edited form as:

Essays Biochem. 2018 December 07; 62(6): 753–763. doi:10.1042/EBC20180030.

Trafficking of ciliary membrane proteins by the intraflagellar transport/BBSome machinery

Jenna L. Wingfield¹, Karl-Ferdinand Lechtreck¹, Esben Lorentzen²

¹Department of Cellular Biology, University of Georgia, Athens, GA 30602

²Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10c, DK-8000 Aarhus C, Denmark

Abstract

Bardet-Biedl syndrome is a rare inherited disease caused by defects in the BBSome, an octameric complex of BBS proteins. The BBSome is conserved in most organisms with cilia, which are microtubule-based cell organelles that protrude from the cell surface and function in motility and sensing. Cilia assembly, maintenance, and function require intraflagellar transport (IFT), a bidirectional motility of multi-megadalton IFT trains propelled by molecular motors along the ciliary microtubules. IFT has been shown to transport structural proteins, including tubulin, into growing cilia. The BBSome is an adapter for the transport of ciliary membrane proteins and cycles through cilia via IFT. While both the loss and the abnormal accumulation of ciliary membrane proteins have been observed in *bbs* mutants, recent data converge on a model where the BBSome mainly functions as a cargo adapter for the removal of certain transmembrane and peripheral membrane proteins from cilia. Here, we review recent data on the ultrastructure of the BBSome and how the BBSome recognizes its cargoes and mediates their removal from cilia.

Keywords

BBSome; Cilia; Intraflagellar Transport (IFT); G-protein Coupled Receptor (GPCR); membrane proteins

Introduction

Bardet-Biedl syndrome ((BBS; Online Mendelian Inheritance in Man (OMIM) 209900)) is a genetically heterogenous recessive mendelian disorder characterized primarily by polydactyly, genital malformation, retinal degeneration, morbid obesity and kidney anomalies (1). This combination of features identifies BBS as a ciliopathy, a disease caused by defects in cilia (2). The overall prevalence of BBS is low with an incidence rate of ~1 in 150,000 but higher rates are observed in consanguine populations (3). Nevertheless, the condition attracts plenty of interest from researchers because blindness and obesity, two BBS pathologies, are much more common in the general population. *BBS3* mutations cause

Corresponding authors: Esben Lorentzen (el@mbg.au.dk) and Karl-Ferdinand Lechtreck (lechtrek@uga.edu).

Competing interests The authors declare that there are no competing interests associated with the manuscript

non-syndromic isolated blindness and SNPs in *BBS4* have been linked to common obesity (4, 5). While mutations in at least 21 genes cause BBS-like phenotypes in humans, the core BBS machinery consists of the octameric BBSome (BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, BBS9, and BBS18/BBIP10) and the small GTPase BBS3/Arl6 (6–9). The assembly of the BBSome requires the activity of the BBS/CCT (CCT: chaperonin containing TCP-1) complex encompassing three chaperonin-like BBS proteins (BBS6, BBS10, and BBS12) and CCT/TRiC (TriC: T-complex protein-1 ring complex) family chaperonins (10). While the BBS/CCT complex is present only in vertebrates, the core BBS machinery is widely conserved in organisms with cilia, including protists (10, 11).

The phylogenetic distribution of the BBS genes and the phenotype of BBS indicate that the function of the BBSome is closely linked to cilia and flagella. For the longest time, cilia were known primarily for their function in cell locomotion and fluid transport (12). Think of the undulating motion of sperm tails or the synchronous whip-like beat pattern of the cilia on the cells lining our airways, which generate a cleansing outward flow of mucus (13). The vast majority of mammalian cilia, however, are non-motile and possess sensory and signaling functions; such cilia are present on many cell types in virtually all tissues and organs (14). Besides the sensing of light and odor by structurally specialized sensory cilia in the eye and nose, non-motile cilia participate in several signaling pathways including Hedgehog (Hh) (15–18), Wnt ((19), Notch (20), Receptor Tyrosine Kinase (RTKs) (21), Hippo (22, 23), TGF- β signaling (20, 24, 25), and GPCR signaling (26). The above described phenotype of BBS patients suggests defects in several of these signaling pathways. For example, the Hh pathway is altered, which results in polydactyly, and altered GPCR signaling likely causes the obesity phenotype. Ciliary sensing and signaling relies on membrane-embedded receptors that cycle through the cilium during signaling. Thus, it is important to understand how cells establish, maintain and adjust the specific receptor and ion channel composition of the ciliary membrane.

The principal parts of cilia are the basal body, the transition zone, and the cilium proper, which projects from the cell body (Figure 1). The basal body is a modified centriole where nine triplet microtubules template the doublet microtubules of the ciliary axoneme. Microtubular doublets consist of a complete A-tubule and an incomplete B-tubule and are a hallmark of most cilia (27). They provide structural stability for the long and narrow cilia, drive cilia elongation, determine its length, and serve as tracks for intraflagellar transport (see below) and, in motile cilia, bind axonemal dyneins and other complexes that power ciliary motion (28). Where the MT doublets emerge from the basal body triplets marks the proximal end of the transition zone (TZ, see Figure 1). The TZ separates the ciliary compartment from the cell body and serves as a diffusion barrier with a size exclusion limit of ~50kDa and probably as a ciliary gate that regulates the influx and efflux of proteins from the cilium (29, 30). The ciliary membrane is continuous with the plasma membrane covering the cell body but possesses a specialized lipid and protein composition. The free exchange of proteins between the ciliary and cell body portions of the plasma membrane is thought to be prevented by rings of intramembranous particles in the TZ, referred to as the ciliary necklace (31, 32).

Since cilia lack ribosomes, ciliary proteins are synthesized in the cell body and posttranslationally moved through the TZ into the organelle. Toward this end, cells utilize intraflagellar transport (IFT), a bidirectional motility of multi-megadalton periodic IFT trains that import and export proteins from cilia (33). The IFT machinery is well conserved and consists of the IFT particles and motors (11, 34). The backbone of the trains is formed by strings of IFT particles consisting of at least 22 polypeptides organized into IFT-A, IFT-B1 and IFT-B2 subcomplexes (35). Heterotrimeric kinesin-2 moves the trains to the ciliary tip by anterograde IFT and IFT dynein pulls them back to the cells body by retrograde IFT. In most systems, BBSomes move on IFT trains through cilia and the BBSome can thus be considered an additional IFT subcomplex (36–39). However, while mutations in IFT components typically result in cilia loss or truncation, the effect of *bbs* mutations on cilia size and ultrastructure is often moderate (40). In some cases, structural defects of cilia have been shown to occur secondarily, i.e., the cilia-like outer segments of photoreceptor cells in *Bbs4*^{-/-} mice initially form normally but then degenerate as the animals age and the composition of the ciliary membrane deteriorates (41–43). Also, the genes encoding the BBS proteins are absent from some phylogenetic groups with the ability to assemble cilia (i.e., lower plants and diatoms)(11). Thus, it is widely accepted that the BBSome is mostly dispensable for cilia assembly *per se* but performs critical roles in the transport of ciliary membrane proteins.

Architecture and cargo specificity of the BBSome

The BBSome was identified and characterized as a protein complex containing eight subunits about 10 years ago (6, 8). Endogenously purified BBSomes appear to be stoichiometric complexes that are stable at high salt concentrations (2.5M NaCl; (7)). BBSome complexes have been notoriously difficult to reconstitute recombinantly but a recent study succeeded in preparing a stoichiometric and highly pure hexameric BBSome core complex lacking only BBS2 and BBS7 (44). This work allowed for elucidation of direct protein-protein interactions within the BBSome and a comprehensive analysis of interactions with ciliary trafficking motifs of cargo membrane proteins (44). A negative-stain electron microscopic reconstruction of the core BBSome structure was carried out at 23Å resolution and revealed a Y-shaped complex, although the position of individual BBS subunits could not be identified at this resolution (44). Taken together with several other architectural studies using methods such as visible immunoprecipitation, a detailed interaction map of the BBSome is emerging where BBS9 acts as the central component, directly contacting four or five other BBSome subunits (44, 45).

Sequence analysis revealed that the BBS5 subunit contains tandem repeat PH-domains with phosphoinositide-binding properties typical of membrane-associated proteins (6, 7). Additionally, BBS4 and BBS8 display sequence similarity to the COP-ε subunits of vesicle-coating complexes and the BBSome was indeed demonstrated to coat large synthetic liposomes *in vitro*. However, whereas canonical coating complexes such as COP and clathrin form small and largely spherical vesicle coats, the BBSome appears to form a planar coat and does not induce membrane curvature (7). This observation probably reflects the function of BBSomes in lateral transport of ciliary membrane proteins rather than in vesicular transport. However, in *T. brucei*, the BBSome was found to localize to the flagellar pocket

and to adjacent cytoplasmic vesicles, perhaps suggesting that it serves as a more canonical vesicle coat in this organism (46). Membrane recruitment of the BBSome requires the small GTPase Arl6/BBS3 in its active GTP-bound state (7). The crystal structure of the Arl6-BBS1 complex demonstrated that the N-terminal β -propeller of BBS1 displays a small but compact binding interface with Arl6-GTP but not Arl6-GDP as expected of an effector (47). The Arl6 interaction interface with BBS1 is evolutionarily highly conserved and human Arl6 binds both human and *C. reinhardtii* BBS1 with similar affinities (47). In addition to the Arl6-BBS1 structure, a crystal structure is available for the β -propeller domain of BBS9 (48), but there is currently no high-resolution structures published for larger BBSome complexes to help rationalize cargo-selection and the molecular similarities to COP and clathrin coating complexes.

In vivo imaging in various species revealed that BBSomes travel on IFT trains through cilia raising the question of how IFT and BBSome complexes interact (36–39). BBSomes and BBSome cargoes accumulate in *ift25*^{−/−} and *ift27*^{−/−} mutants suggesting that these IFT proteins mediate BBSome binding to retrograde IFT trains (49, 50). It has been proposed that the small GTPase IFT27 functions as a GEF to activate Arl6/BBS3 thus allowing recruitment of BBSomes to the ciliary membrane (38). The membrane-associated BBSome clusters at the ciliary tip, binds to cargoes, and exits the organelle by retrograde IFT (51) (Figure 1). Thus, it is unclear whether BBSomes bind to distinct IFT sites during anterograde and retrograde travel. Furthermore, the BBSome and its putative cargoes but not IFT27/25 accumulate in *Lztfl1*^{−/−} cells indicating that LZTFL1 mediates IFT/BBSome interactions (49).

Membrane association of the BBSome is in agreement with its a role in trafficking of membrane proteins. Indeed, phospholipase D (PLD) was observed to undergo BBSome-mediated IFT only when the N-terminally PLD lipid-anchor was intact (52). This observation suggests that BBSomes are arranged on IFT trains such that the cargo-interacting domains of BBS subunits are located in close proximity to the ciliary membrane. Additionally, pull-down experiments have identified interactions between BBSomes (or BBSome components) and numerous integral membrane proteins involved in signaling such as dopamine receptor 1 (D1), somatostatin receptor type 3 (SSTR3), the leptin receptor and the insulin receptor (7, 53–57). The ciliary trafficking motifs of these receptors have been mapped to either the third intracellular loop (3ICL) or to the C-terminal tail, both located at the intracellular side of the membrane (for review see (40, 58)). With recombinantly reconstituted BBSome core complexes in hand, Klink and colleagues carried out a thorough study of the binding affinities of known BBSome cargoes (44). Interestingly, Ax(S/A)xQ or (V/I)KARK sequences both from the 3ICL, and (F/Y/W)/(K/R) motifs at C-terminal tail were shown to interact directly with the BBSome (44). ASAGQ and VNAAK sequences are also present in the head domain of PLD, a confirmed BBSome cargo in *C. reinhardtii* (52). Fluorescence polarization experiments revealed affinities of the BBSome for ciliary trafficking motifs that varied from sub- μ M to double digit μ M although it cannot be ruled out that binding properties may differ somewhat in context of full-length membrane proteins in the native membrane environment. No interaction was observed between BBSomes and the VxPX motif of Polycystin-1 and Rhodopsin or the RVxP motif of Polycystin-2, suggesting that these motifs do not function in BBSome-mediated ciliary trafficking (44).

Structural studies of BBSomes in complex with ciliary trafficking sequences will provide a molecular basis of the diverse sequence recognition abilities of BBSomes.

The analysis of BBS in model organisms

The genes encoding the subunits of the BBSome and BBS3/Arl6 are present in most but not all organisms with cilia suggesting that the BBSome, while not essential for ciliogenesis, plays an important role related to cilia. Comparative analysis of various unicellular and multicellular models has greatly contributed to unraveling of the BBSomes functions and the etiology of the disease. Below, we summarize key insights obtained from these studies.

Chlamydomonas reinhardtii:

C. reinhardtii mutants in *BBS1*, *BBS4*, *BBS7*, and *BBS8* fail to phototax, a behavior that allows the cells to move towards or away from light via cilia motility (37, 59). The cilia are nearly full-length and motile and the frequency and velocity of IFT are only mildly affected in the *bbs* mutants (60). Two-color imaging showed BBS proteins co-migrating with IFT trains in cilia (37). Data mining of the ciliary proteome and quantitative mass spectrometry indicate that the BBSome is a substoichiometric component of the IFT machinery (IFT-B: BBSome = ~10 : 1) and live imaging of BBS4-GFP rescue cells demonstrates that not all IFT trains carry a BBSome (37). Thus, the BBSome is dispensable for IFT in *C. reinhardtii*. In contrast, BBSomes are present on virtually all IFT trains in murine olfactory cilia and in primary cilia (38, 39) although the BBSome is also substoichiometric to the IFT complex in primary cilia of kidney cells (51). In *C. reinhardtii*, PLD and an AMP-regulated protein kinase (AMPK) are abnormally accumulated in the ciliary membrane of *bbs* mutants (60). Although PLD and AMPK are not transmembrane proteins, they are predicted to be dual fatty acid modified explaining the association with the membrane (52). Cilia of the *bbs* mutants fail to perform phototactic steering movements and their axonemes are insensitive to changes in calcium concentration (59). PLD is active in *bbs* mutant cilia and signaling lipids such as DAG, generated by PLD, could activate protein kinases and ultimately phosphorylate axonemal proteins impairing phototactic steering (60). In wild-type cells, PLD enters cilia by diffusion and IFT; while in *bbs* mutants IFT of PLD is not observed suggesting that the BBSome functions as an adapter enabling the association of PLD and other proteins to IFT trains (52).

Paramecium tetraurelia:

Upon knock-down of BBS protein expression in the ciliate *Paramecium tetraurelia*, a K⁺ channel and the TRP channel PKD2 are lost from the ciliary membrane causing prolonged backward swimming (61). Furthermore, cilia loss or reduced cilium length were observed in BBS7 and BBS9 RNAi cells. The data suggest a role for the BBSome in the delivery of certain ion channels to the cilium.

Trypanosoma brucei:

Knock-out of *BBS1*, *BBS4*, *BBS5*, *BBS7*, and *BBS9* in the pathogenic protist *Trypanosoma brucei*, the agent of sleeping sickness, reduced the virulence while flagellar assembly, motility and cell viability were unaffected (46). Loss of virulence was also observed in a

bbs1 mutant strain of the parasite *Leishmania major* (62). In *T. brucei*, BBS proteins localize to the TZ and flagellar pocket, the main site of exo- and endocytosis in these cells (63). While bulk endocytosis is unaffected in the BBSome mutants, the composition of certain surface proteins is significantly altered by both abnormal accumulation and reduction of proteins. The resulting changes in host–parasite interaction could explain the virulence defects. The group of mis-distributed proteins encompasses proteins that require sorting by ubiquitination and the BBSome interacts with ubiquitin in pull-downs from cell extracts (46). Thus, the BBSome could function in protein export from cilia by facilitating endocytic sorting of select membrane proteins at the base of the cilium.

Caenorhabditis elegans:

C. elegans lacks motile cilia. In wild-type worms, the sensory cilia of the neurons in the head and tail project through the cuticle into the environment. *C. elegans bbs* mutants have shorter than normal cilia with additional structural abnormalities and, as typical for many *C. elegans* mutants with short cilia, display defects in dye-filling (*dyf*), chemosensory (*che*) and osmosensory (*osm*). Indeed, BBS7 is encoded by the *osm-12* gene (36, 64). In *C. elegans bbs* mutants, IFT-A and IFT-B separate and move with the distinct velocities suggesting that the BBSome stabilizes the IFT-A/B interaction (65). *C. elegans* is unusual in employing two distinct kinesins for IFT: the conventional heterotrimeric kinesin-2 consisting of Kif3a, Kif3b, and KAP and the homodimeric OSM-3 (66, 67). OSM-3 is associated to IFT-B via IFT70 while the trimeric kinesin is thought to associate with IFT-A (68). Thus, when IFT-A and IFT-B separate they move with the velocity of the respective motor in the absence of the BBSome. In contrast, recent data suggest that the mammalian heterotrimeric kinesin is attached to the IFT-B complex (68, 69). It has been further proposed that the BBSome assembles IFT-A/B complexes and IFT trains at the flagellar base and tip (70). Of note, IFT25, IFT27 and LZTFL1, which in mammals mediate BBSome-IFT interaction, are absent in *C. elegans* and therefore distinct BBSome-IFT complex interactions could have evolved (49, 50). With respect to the role of the BBSome in the transport of ciliary membrane proteins, PKD-2, OSM-9 and ODR-10 accumulate in *C. elegans bbs4 bbs5* double mutants (71). Since previous studies failed to detect IFT movements of GFP-PKD2 (72), the data suggested that lysosome-directed degradative sorting of mono-ubiquitinated PKD2, rather than retrograde IFT, is disrupted. On the other hand, an enlargement of the periciliary membrane compartment observed in *C. elegans* mutants with defective endocytotic traffic is diminished in *bbs8* mutants suggesting a role of the BBSome in membrane delivery to the cilium (73). *C. elegans bbs* mutant animals are smaller than wild type, which is a likely result of increased insulin and neuropeptide secretion (74).

Danio rerio:

Morpholino-based suppression of *bbs2*, *bbs4*, *bbs5*, *bbs6*, *bbs7* or *bbs8* in zebrafish embryos shortens the motile cilia of in the Kupffer's vesicle (KV) causing defects in left-right patterning (75). Interestingly, the retrograde transport of melanosomes, pigment filled vesicles, in the cytoplasm of skin cells is strongly reduced (75). This transport employs cytoplasmic dynein rather than IFT dynein putatively suggesting a role of the BBSome in mediating particular cargo-dynein interactions outside of cilia.

Mus musculus:

Bbs mutant mice, including *Bbs*^{M390R/M390R} knock-in mice mimicking the most common *Bbs* mutation in humans (~27% of all characterized cases; (76)), largely recapitulate the phenotype of BBS patients including obesity, blindness, anosmia and polydactyly; kidney cysts, a main feature of BBS in humans, were not observed (77). Further, *Bbs* mutant animals develop hydrocephalus and males are infertile. Cilia in the mutant animals have a broad range of defects. Sperm flagella fail to develop, primary cilia are often shorter, and motile cilia show swellings (77). The outer segments in the eye are initially present but then progressively degenerate suggesting that some of the ciliary defects are caused secondarily, e.g., by the increasing deterioration of the ciliary protein composition (41, 78). In summary, loss of BBSome function does not interfere with global cilia assembly but interferes with cilia signaling (42).

Bbs mutant mice are less active, eat more, and become obese, gaining three times the total body fat of control mice (78). While over-proliferation and altered metabolism of adipocytes could contribute to obesity in BBS, the main drivers likely are neurologically induced changes in food uptake and energy expenditure (79, 80). Indeed, dietary restrictions prevent obesity and downstream features such as hypertension and diabetes in BBS patients. Conditional truncation of cilia of the PomC neurons in the hypothalamus results in increased food intake, reduced activity and weight gain in adult mice (81). Recently, point mutations in ADCY3 encoding the ciliary adenylylate cyclase 3 have been linked to inherited obesity and type 2 diabetes in humans (82–84). How cilia and the BBSome regulate satiety is not completely understood. In a cilia-independent process, BBS1 has been implicated in the transport of the leptin receptor to the plasma membrane; the hormone leptin is produced by adipocyte tissue and reduces appetite (85). Further, SSTR3 and melanin-concentrating hormone receptor 1 (MCHR1) are absent from the primary cilia of neuronal cells of *Bbs*^{2-/-} and *Bbs*^{4-/-} mice (26). On the other hand, retrieval of the activated dopamine receptor 1 (D1) from neuronal primary cilia is affected in *Bbs* mutants (53). SSTR3, MCHR1, and D1 are thought to contribute to the control of feeding behavior, satiety, energy expenditure and body weight. The BBSome has been also implicated in the ciliary transport of Hh signaling proteins (86). However, the phenotype of *Bbs* mutants indicates a comparatively mild disturbance of Hh signaling. The presence of tissue- and organ specific BBS protein isoforms and BBS complexes in mice suggests that BBSomes are specialized for diverse transport functions in different cilia (10, 87).

Cell Biology of the BBSome

Data from several systems reveal that *bbs* mutations alter the composition of the ciliary membrane (58). Initially, the BBSome was thought to mediate the trafficking of transmembrane proteins, in particular of GPCRs, into cilia either by directing post-Golgi vesicles to the ciliary base or by mediating the passage of GPCRs from plasma membrane of the cell body through the TZ into the ciliary membrane (6, 7, 88). Indeed, cilia of *Paramecium bbs* knock-downs lack certain ion channels and the GPCRs SSTR3 and MCHR1 are essentially absent from neuronal cilia of *Bbs* mutant mice (26, 61). More recently, however, it was shown that the BBSome removes SSTR3 from cilia by ensuring

retrograde transport and transit through the TZ (51). How can this apparent conundrum be explained? Nager et al. (2017) observed that SSTR3 accumulates at the tip of cilia upon agonist binding and, when BBSome-dependent retrieval is impeded, SSTR3 is released from cilia by ectosome shedding thus leaving cilia devoid of the protein (89). Whether ectosome shedding sufficiently explains the near total absence of SSTR3 from cilia of cultured neuronal *Bbs* mutant cells is currently unclear.

A role of the BBSome in removing membrane proteins from cilia is well supported by direct *in vivo* imaging of BBSome cargoes but the precise mechanism of BBSome-dependent protein export is still under discussion. The analysis of the BBSome cargo PLD in *C. reinhardtii*, for example, suggests a simple model, in which PLD enters cilia by both diffusion and BBS/IFT transport but exits cilia only by BBSome-dependent IFT (52). PLD that enters cilia by diffusion is picked-up by BBS/IFT trains patrolling the cilium and carried out of the organelle. In *bbs* mutants, PLD continues to diffuse into cilia and accumulates over time since the exit is blocked. Only trace amounts of PLD are present in wild-type cilia, while >50% of the total PLD accumulate in *bbs* mutant cilia (60). This is surprising because the ciliary membrane accounts for only a fraction of the plasma membrane and suggests that the cilium is a sink for PLD and that the BBS/IFT system is required to extract PLD from this trap. The build-up of PLD in *C. reinhardtii bbs* cilia is a slow process requiring several hours, while PLD is removed rapidly (within a few minutes) from cilia when functional BBSomes are reintroduced (60). In comparison, signaling induced retrieval of GPCRs from cilia via the BBSome pathway is a rather slow process, requiring several hours (51).

BBSome-dependent retrograde transport could scavenge a diverse array of non-ciliary proteins leaking into cilia and/or regulate the cilia concentration of specific group of ciliary signaling proteins. The former is supported by data from *bbs* mutant mice, which accumulate an apparently unrelated set of non-ciliary protein in the outer segments (41). The latter is supported by the observation that the transport of only a limited number of proteins, mostly signaling proteins, is directly affected by *bbs* mutations. PLD, for example, is a negative regulator of phototaxis in *C. reinhardtii* and BBSome-controlled traffic of PLD could regulate phototaxis (52).

During the BBSome-dependent retrieval of GPCRs from mammalian cilia both cargo-BBSome and BBSome/cargo complex-IFT interactions are regulated (53). Prior to their activation by ligand binding, SSTR3 and Smoothened (Smo) are poor cargoes of BBS/IFT and largely diffuse in the ciliary membrane (90). As characteristic for GPCRs, ligand binding (sst and SAG, respectively) alters the conformation of the cytoplasmic face of the receptor, which allows the BBSome to bind, either directly or via additional adapters such as GPR161 (51). Prior to retrograde IFT, BBSomes and its cargoes cluster at the ciliary tip in a process regulated by the GTPases BBS3/Arl6 and probably IFT27. The clustering of GPCR/BBSome/IFT material is followed processive transport to the ciliary base. While the *C. reinhardtii* model suggests that BBS/IFT trains simply pull cargoes through the TZ, live imaging in mammalian cells indicates a step-wise exit procedure, in which the GPCRs first pass the Y-shaped linkers and then transfer across the transitional fiber into the plasma membrane of the cell body (51) (Figure 1).

After exiting the ciliary compartments, GPCRs and other ciliary membrane proteins will be removed from the plasma membrane by clathrin-dependent endocytosis (91). Studies in *Trypanosoma*, *C. elegans* and zebrafish implicate the BBSome in endocytosis near the cilia base (46, 71, 92). The ciliary base is surrounded by the periciliary membrane, a portion of the cell body plasma membrane with specialized lipid and protein content (93). The periciliary membrane appears to serve as a loading dock for membrane protein delivery and removal from cilia. Often, this region is recessed from the cell surface, e.g., in form of a flagellar pocket (20, 46). Proteins enter this periciliary compartment in post-Golgi vesicles or from the plasma membrane either by direct lateral movements or via the recycling endosome (Figure 1)(94, 95). The specific roles of the BBSome in periciliary membrane dynamics or vesicular cargo selection are currently unclear.

In summary, the BBSome promotes protein export from cilia by ensuring transport to the ciliary base via IFT, passage through the barrier of the TZ, and probably removal from the periciliary membrane compartment.

Acknowledgments

Funding

E.L. acknowledges the Novo Nordisk Foundation (grant no. NNF15OC0014164) for funding and K.-F. L. acknowledges support by the National Institutes of Health (grant No. R01GM110413). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abbreviations

BBS	Bardet-Biedl Syndrome
IFT	Intraflagellar Transport
MT	Microtubule
TZ	transition zone
PLD	Phospholipase D
AMPK	AMP-regulated protein kinase
GPCR	G-protein Coupled Receptor
Smo	Smoothed
SSTR3	Somatostatin Receptor 3
MCHR1	Melanin-Concentrating Hormone Receptor 1
D1	Dopamine receptor 1.

References

1. Sheffield VC. The blind leading the obese: the molecular pathophysiology of a human obesity syndrome. *Trans Am Clin Climatol Assoc* 2010;121:172–81; discussion 81–2. [PubMed: 20697559]

2. Brown JM, Witman GB. Cilia and Diseases. *Bioscience* 2014;64(12):1126–37. [PubMed: 25960570]
3. Moore SJ, Green JS, Fan Y, Bhogal AK, Dicks E, Fernandez BA, et al. Clinical and genetic epidemiology of Bardet-Biedl syndrome in Newfoundland: a 22-year prospective, population-based, cohort study. *Am J Med Genet A* 2005;132(4):352–60.
4. Benzinou M, Walley A, Lobbens S, Charles MA, Jouret B, Fumeron F, et al. Bardet-Biedl syndrome gene variants are associated with both childhood and adult common obesity in French Caucasians. *Diabetes* 2006;55(10):2876–82. [PubMed: 17003356]
5. Pretorius PR, Aldahmesh MA, Alkuraya FS, Sheffield VC, Slusarski DC. Functional analysis of BBS3 A89V that results in non-syndromic retinal degeneration. *Hum Mol Genet* 2011;20(8):1625–32. [PubMed: 21282186]
6. Nachury MV, Loktev AV, Zhang Q, Westlake CJ, Peranen J, Merdes A, et al. A core complex of BBS proteins cooperates with the GTPase Rab8 to promote ciliary membrane biogenesis. *Cell* 2007;129(6):1201–13. [PubMed: 17574030]
7. Jin H, White SR, Shida T, Schulz S, Aguiar M, Gygi SP, et al. The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. *Cell* 2010;141(7):1208–19. [PubMed: 20603001]
8. Loktev AV, Zhang Q, Beck JS, Searby CC, Scheetz TE, Bazan JF, et al. A BBSome subunit links ciliogenesis, microtubule stability, and acetylation. *Dev Cell* 2008;15(6):854–65. [PubMed: 19081074]
9. Scheidecker S, Etard C, Pierce NW, Geoffroy V, Schaefer E, Muller J, et al. Exome sequencing of Bardet-Biedl syndrome patient identifies a null mutation in the BBSome subunit BBIP1 (BBS18). *J Med Genet* 2013.
10. Seo S, Baye LM, Schulz NP, Beck JS, Zhang Q, Slusarski DC, et al. BBS6, BBS10, and BBS12 form a complex with CCT/TRiC family chaperonins and mediate BBSome assembly. *Proc Natl Acad Sci U S A* 2010;107(4):1488–93. [PubMed: 20080638]
11. van Dam TJ, Townsend MJ, Turk M, Schlessinger A, Sali A, Field MC, et al. Evolution of modular intraflagellar transport from a coatomer-like progenitor. *Proc Natl Acad Sci U S A* 2013;110(17):6943–8. [PubMed: 23569277]
12. Dobell C, Leeuwenhoek Av. Antony van Leeuwenhoek and his “Little animals”; being some account of the father of protozoology and bacteriology and his multifarious discoveries in these disciplines New York,: Harcourt, Brace and company; 1932 vii, 435, 1 p. p.
13. Fliegauf M, Benzing T, Omran H. When cilia go bad: cilia defects and ciliopathies. *Nat Rev Mol Cell Biol* 2007;8(11):880–93. [PubMed: 17955020]
14. Sloboda RD. Preface. Widespread interest in the primary cilium. *Methods Cell Biol* 2009;94:xv–xvi. [PubMed: 19963094]
15. Pazour GJ, Dickert BL, Vucica Y, Seeley ES, Rosenbaum JL, Witman GB, et al. Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J Cell Biol* 2000;151(3):709–18. [PubMed: 11062270]
16. Huangfu D, Liu A, Rakeman AS, Murcia NS, Niswander L, Anderson KV. Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature* 2003;426(6962):83–7. [PubMed: 14603322]
17. Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF. Vertebrate Smoothed functions at the primary cilium. *Nature* 2005;437(7061):1018–21. [PubMed: 16136078]
18. Haycraft CJ, Banizs B, Aydin-Son Y, Zhang Q, Michaud EJ, Yoder BK. Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. *PLoS Genet* 2005;1(4):e53. [PubMed: 16254602]
19. Wallingford JB, Mitchell B. Strange as it may seem: the many links between Wnt signaling, planar cell polarity, and cilia. *Genes Dev* 2011;25(3):201–13. [PubMed: 21289065]
20. Clement CA, Ajbro KD, Koefoed K, Vestergaard ML, Veland IR, Henriques de Jesus MP, et al. TGF-beta signaling is associated with endocytosis at the pocket region of the primary cilium. *Cell Rep* 2013;3(6):1806–14. [PubMed: 23746451]
21. Christensen ST, Clement CA, Satir P, Pedersen LB. Primary cilia and coordination of receptor tyrosine kinase (RTK) signalling. *J Pathol* 2012;226(2):172–84. [PubMed: 21956154]

22. Habbig S, Bartram MP, Muller RU, Schwarz R, Andriopoulos N, Chen S, et al. NPHP4, a cilia-associated protein, negatively regulates the Hippo pathway. *J Cell Biol* 2011;193(4):633–42. [PubMed: 21555462]
23. Kim M, Kim M, Lee MS, Kim CH, Lim DS. The MST1/2-SAV1 complex of the Hippo pathway promotes ciliogenesis. *Nat Commun* 2014;5:5370. [PubMed: 25367221]
24. Christensen ST, Morthorst SK, Mogensen JB, Pedersen LB. Primary Cilia and Coordination of Receptor Tyrosine Kinase (RTK) and Transforming Growth Factor beta (TGF-beta) Signaling. *Cold Spring Harb Perspect Biol* 2017;9(6).
25. Monnich M, Borgeskov L, Breslin L, Jakobsen L, Rogowski M, Doganli C, et al. CEP128 Localizes to the Subdistal Appendages of the Mother Centriole and Regulates TGF-beta/BMP Signaling at the Primary Cilium. *Cell Rep* 2018;22(10):2584–92. [PubMed: 29514088]
26. Berbari NF, Lewis JS, Bishop GA, Askwith CC, Mykityn K. Bardet-Biedl syndrome proteins are required for the localization of G protein-coupled receptors to primary cilia. *Proc Natl Acad Sci U S A* 2008;105(11):4242–6. [PubMed: 18334641]
27. Witman GB, Carlson K, Berliner J, Rosenbaum JL. *Chlamydomonas* flagella. I. Isolation and electrophoretic analysis of microtubules, matrix, membranes, and mastigonemes. *J Cell Biol* 1972;54(3):507–39. [PubMed: 4558009]
28. Mizuno N, Taschner M, Engel BD, Lorentzen E. Structural studies of ciliary components. *J Mol Biol* 2012;422(2):163–80. [PubMed: 22683354]
29. Kee HL, Dishinger JF, Blasius TL, Liu CJ, Margolis B, Verhey KJ. A size-exclusion permeability barrier and nucleoporins characterize a ciliary pore complex that regulates transport into cilia. *Nat Cell Biol* 2012;14(4):431–7. [PubMed: 22388888]
30. Reiter JF, Blacque OE, Leroux MR. The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. *EMBO Rep* 2012;13(7):608–18. [PubMed: 22653444]
31. Gilula NB, Satir P. The ciliary necklace. A ciliary membrane specialization. *J Cell Biol* 1972;53(2):494–509. [PubMed: 4554367]
32. Rohatgi R, Snell WJ. The ciliary membrane. *Curr Opin Cell Biol* 2010;22(4):541–6. [PubMed: 20399632]
33. Kozminski KG, Johnson KA, Forscher P, Rosenbaum JL. A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proc Natl Acad Sci U S A* 1993;90(12):5519–23. [PubMed: 8516294]
34. Jekely G, Arendt D. Evolution of intraflagellar transport from coated vesicles and autogenous origin of the eukaryotic cilium. *Bioessays* 2006;28(2):191–8. [PubMed: 16435301]
35. Taschner M, Lorentzen E. The Intraflagellar Transport Machinery. *Cold Spring Harb Perspect Biol* 2016;8(10).
36. Blacque OE, Reardon MJ, Li C, McCarthy J, Mahjoub MR, Ansley SJ, et al. Loss of *C. elegans* BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev* 2004;18(13):1630–42. [PubMed: 15231740]
37. Lechtreck KF, Johnson EC, Sakai T, Cochran D, Ballif BA, Rush J, et al. The *Chlamydomonas reinhardtii* BBSome is an IFT cargo required for export of specific signaling proteins from flagella. *J Cell Biol* 2009;187(7):1117–32. [PubMed: 20038682]
38. Liew GM, Ye F, Nager AR, Murphy JP, Lee JS, Aguiar M, et al. The intraflagellar transport protein IFT27 promotes BBSome exit from cilia through the GTPase ARL6/BBS3. *Dev Cell* 2014;31(3):265–78. [PubMed: 25443296]
39. Williams CL, McIntyre JC, Norris SR, Jenkins PM, Zhang L, Pei Q, et al. Direct evidence for BBSome-associated intraflagellar transport reveals distinct properties of native mammalian cilia. *Nat Commun* 2014;5:5813. [PubMed: 25504142]
40. Nachury MV. The molecular machines that traffic signaling receptors into and out of cilia. *Curr Opin Cell Biol* 2018;51:124–31. [PubMed: 29579578]
41. Datta P, Allamargot C, Hudson JS, Andersen EK, Bhattarai S, Drack AV, et al. Accumulation of non-outer segment proteins in the outer segment underlies photoreceptor degeneration in Bardet-Biedl syndrome. *Proc Natl Acad Sci U S A* 2015;112(32):E4400–9. [PubMed: 26216965]

42. Mykytyn K, Mullins RF, Andrews M, Chiang AP, Swiderski RE, Yang B, et al. Bardet-Biedl syndrome type 4 (BBS4)-null mice implicate Bbs4 in flagella formation but not global cilia assembly. *Proc Natl Acad Sci U S A* 2004;101(23):8664–9. [PubMed: 15173597]
43. Hsu Y, Garrison JE, Kim G, Schmitz AR, Searby CC, Zhang Q, et al. BBSome function is required for both the morphogenesis and maintenance of the photoreceptor outer segment. *PLoS Genet* 2017;13(10):e1007057. [PubMed: 29049287]
44. Klink BU, Zent E, Juneja P, Kuhlee A, Raunser S, Wittinghofer A. A recombinant BBSome core complex and how it interacts with ciliary cargo. *Elife* 2017;6.
45. Katoh Y, Nozaki S, Hartanto D, Miyano R, Nakayama K. Architectures of multisubunit complexes revealed by a visible immunoprecipitation assay using fluorescent fusion proteins. *J Cell Sci* 2015;128(12):2351–62. [PubMed: 25964651]
46. Langousis G, Shimogawa MM, Saada EA, Vashisht AA, Spreafico R, Nager AR, et al. Loss of the BBSome perturbs endocytic trafficking and disrupts virulence of *Trypanosoma brucei*. *Proc Natl Acad Sci U S A* 2016;113(3):632–7. [PubMed: 26721397]
47. Mourao A, Nager AR, Nachury MV, Lorentzen E. Structural basis for membrane targeting of the BBSome by ARL6. *Nat Struct Mol Biol* 2014;21(12):1035–41. [PubMed: 25402481]
48. Knockenhauer KE, Schwartz TU. Structural Characterization of Bardet-Biedl Syndrome 9 Protein (BBS9). *J Biol Chem* 2015;290(32):19569–83. [PubMed: 26085087]
49. Eguether T, San Agustin JT, Keady BT, Jonassen JA, Liang Y, Francis R, et al. IFT27 links the BBSome to IFT for maintenance of the ciliary signaling compartment. *Dev Cell* 2014;31(3):279–90. [PubMed: 25446516]
50. Keady BT, Samtani R, Tobita K, Tsuchya M, San Agustin JT, Follit JA, et al. IFT25 links the signal-dependent movement of Hedgehog components to intraflagellar transport. *Dev Cell* 2012;22(5):940–51. [PubMed: 22595669]
51. Ye F, Nager AR, Nachury MV. BBSome trains remove activated GPCRs from cilia by enabling passage through the transition zone. *J Cell Biol* 2018;217(5):1847–68. [PubMed: 29483145]
52. Liu P, Lechtreck KF. The Bardet-Biedl syndrome protein complex is an adapter expanding the cargo range of intraflagellar transport trains for ciliary export. *Proc Natl Acad Sci U S A* 2018.
53. Domire JS, Green JA, Lee KG, Johnson AD, Askwith CC, Mykytyn K. Dopamine receptor 1 localizes to neuronal cilia in a dynamic process that requires the Bardet-Biedl syndrome proteins. *Cell Mol Life Sci* 2011;68(17):2951–60. [PubMed: 21152952]
54. Seo S, Guo DF, Bugge K, Morgan DA, Rahmouni K, Sheffield VC. Requirement of Bardet-Biedl syndrome proteins for leptin receptor signaling. *Hum Mol Genet* 2009;18(7):1323–31. [PubMed: 19150989]
55. Gerdes JM, Christou-Savina S, Xiong Y, Moede T, Moruzzi N, Karlsson-Edlund P, et al. Ciliary dysfunction impairs beta-cell insulin secretion and promotes development of type 2 diabetes in rodents. *Nat Commun* 2014;5:5308. [PubMed: 25374274]
56. Starks RD, Beyer AM, Guo DF, Boland L, Zhang Q, Sheffield VC, et al. Regulation of Insulin Receptor Trafficking by Bardet Biedl Syndrome Proteins. *PLoS Genet* 2015;11(6):e1005311. [PubMed: 26103456]
57. Su X, Driscoll K, Yao G, Raed A, Wu M, Beales PL, et al. Bardet-Biedl syndrome proteins 1 and 3 regulate the ciliary trafficking of polycystic kidney disease 1 protein. *Hum Mol Genet* 2014;23(20):5441–51. [PubMed: 24939912]
58. Mourao A, Christensen ST, Lorentzen E. The intraflagellar transport machinery in ciliary signaling. *Curr Opin Struct Biol* 2016;41:98–108. [PubMed: 27393972]
59. Pazour GJ, Sineshchekov OA, Witman GB. Mutational analysis of the phototransduction pathway of *Chlamydomonas reinhardtii*. *J Cell Biol* 1995;131(2):427–40. [PubMed: 7593169]
60. Lechtreck KF, Brown JM, Sampaio JL, Craft JM, Shevchenko A, Evans JE, et al. Cycling of the signaling protein phospholipase D through cilia requires the BBSome only for the export phase. *J Cell Biol* 2013;201(2):249–61. [PubMed: 23589493]
61. Valentine MS, Rajendran A, Yano J, Weeraratne SD, Beisson J, Cohen J, et al. Paramecium BBS genes are key to presence of channels in Cilia. *Cilia* 2012;1(1):16. [PubMed: 23351336]

62. Price HP, Paape D, Hodgkinson MR, Farrant K, Doehl J, Stark M, et al. The Leishmania major BBSome subunit BBS1 is essential for parasite virulence in the mammalian host. *Mol Microbiol* 2013;90(3):597–611. [PubMed: 23998526]
63. Dean S, Moreira-Leite F, Varga V, Gull K. Cilium transition zone proteome reveals compartmentalization and differential dynamics of ciliopathy complexes. *Proc Natl Acad Sci U S A* 2016;113(35):E5135–43. [PubMed: 27519801]
64. Mukhopadhyay S, Lu Y, Shaham S, Sengupta P. Sensory signaling-dependent remodeling of olfactory cilia architecture in *C. elegans*. *Dev Cell* 2008;14(5):762–74. [PubMed: 18477458]
65. Ou G, Blacque OE, Snow JJ, Leroux MR, Scholey JM. Functional coordination of intraflagellar transport motors. *Nature* 2005;436(7050):583–7. [PubMed: 16049494]
66. Pan X, Ou G, Civelekoglu-Scholey G, Blacque OE, Endres NF, Tao L, et al. Mechanism of transport of IFT particles in *C. elegans* cilia by the concerted action of kinesin-II and OSM-3 motors. *J Cell Biol* 2006;174(7):1035–45. [PubMed: 17000880]
67. Prevo B, Scholey JM, Peterman EJG. Intraflagellar transport: mechanisms of motor action, cooperation, and cargo delivery. *FEBS J* 2017;284(18):2905–31. [PubMed: 28342295]
68. Mohamed MAA, Stepp WL, Okten Z. Reconstitution reveals motor activation for intraflagellar transport. *Nature* 2018;557(7705):387–91. [PubMed: 29743676]
69. Funabashi T, Katoh Y, Okazaki M, Sugawa M, Nakayama K. Interaction of heterotrimeric kinesin-II with IFT-B-connecting tetramer is crucial for ciliogenesis. *J Cell Biol* 2018.
70. Wei Q, Zhang Y, Li Y, Zhang Q, Ling K, Hu J. The BBSome controls IFT assembly and turnaround in cilia. *Nat Cell Biol* 2012;14(9):950–7. [PubMed: 22922713]
71. Xu Q, Zhang Y, Wei Q, Huang Y, Li Y, Ling K, et al. BBS4 and BBS5 show functional redundancy in the BBSome to regulate the degradative sorting of ciliary sensory receptors. *Sci Rep* 2015;5:11855. [PubMed: 26150102]
72. Qin H, Burnette DT, Bae YK, Forscher P, Barr MM, Rosenbaum JL. Intraflagellar transport is required for the vectorial movement of TRPV channels in the ciliary membrane. *Curr Biol* 2005;15(18):1695–9. [PubMed: 16169494]
73. Kaplan OI, Doroquez DB, Cevik S, Bowie RV, Clarke L, Sanders AA, et al. Endocytosis genes facilitate protein and membrane transport in *C. elegans* sensory cilia. *Curr Biol* 2012;22(6):451–60. [PubMed: 22342749]
74. Lee BH, Liu J, Wong D, Srinivasan S, Ashrafi K. Hyperactive neuroendocrine secretion causes size, feeding, and metabolic defects of *C. elegans* Bardet-Biedl syndrome mutants. *PLoS Biol* 2011;9(12):e1001219. [PubMed: 22180729]
75. Yen HJ, Tayeh MK, Mullins RF, Stone EM, Sheffield VC, Slusarski DC. Bardet-Biedl syndrome genes are important in retrograde intracellular trafficking and Kupffer's vesicle cilia function. *Hum Mol Genet* 2006;15(5):667–77. [PubMed: 16399798]
76. Cox KF, Kerr NC, Kedrov M, Nishimura D, Jennings BJ, Stone EM, et al. Phenotypic expression of Bardet-Biedl syndrome in patients homozygous for the common M390R mutation in the BBS1 gene. *Vision Res* 2012;75:77–87. [PubMed: 22940089]
77. Davis RE, Swiderski RE, Rahmouni K, Nishimura DY, Mullins RF, Agassandian K, et al. A knockin mouse model of the Bardet-Biedl syndrome 1 M390R mutation has cilia defects, ventriculomegaly, retinopathy, and obesity. *Proc Natl Acad Sci U S A* 2007;104(49):19422–7. [PubMed: 18032602]
78. Nishimura DY, Fath M, Mullins RF, Searby C, Andrews M, Davis R, et al. Bbs2-null mice have neurosensory deficits, a defect in social dominance, and retinopathy associated with mislocalization of rhodopsin. *Proc Natl Acad Sci U S A* 2004;101(47):16588–93. [PubMed: 15539463]
79. Marion V, Stoetzel C, Schlicht D, Messaddeq N, Koch M, Flori E, et al. Transient ciliogenesis involving Bardet-Biedl syndrome proteins is a fundamental characteristic of adipogenic differentiation. *Proc Natl Acad Sci U S A* 2009;106(6):1820–5. [PubMed: 19190184]
80. Aksanov O, Green P, Birk RZ. BBS4 directly affects proliferation and differentiation of adipocytes. *Cell Mol Life Sci* 2014;71(17):3381–92. [PubMed: 24500759]

81. Davenport JR, Watts AJ, Roper VC, Croyle MJ, van Groen T, Wyss JM, et al. Disruption of intraflagellar transport in adult mice leads to obesity and slow-onset cystic kidney disease. *Curr Biol* 2007;17(18):1586–94. [PubMed: 17825558]
82. Grarup N, Moltke I, Andersen MK, Dalby M, Vitting-Seerup K, Kern T, et al. Loss-of-function variants in ADCY3 increase risk of obesity and type 2 diabetes. *Nat Genet* 2018;50(2):172–4. [PubMed: 29311636]
83. Saeed S, Bonnefond A, Tamanini F, Mirza MU, Manzoor J, Janjua QM, et al. Loss-of-function mutations in ADCY3 cause monogenic severe obesity. *Nat Genet* 2018;50(2):175–9. [PubMed: 29311637]
84. Siljee JE, Wang Y, Bernard AA, Ersoy BA, Zhang S, Marley A, et al. Subcellular localization of MC4R with ADCY3 at neuronal primary cilia underlies a common pathway for genetic predisposition to obesity. *Nat Genet* 2018;50(2):180–5. [PubMed: 29311635]
85. Guo DF, Cui H, Zhang Q, Morgan DA, Thedens DR, Nishimura D, et al. The BBSome Controls Energy Homeostasis by Mediating the Transport of the Leptin Receptor to the Plasma Membrane. *PLoS Genet* 2016;12(2):e1005890. [PubMed: 26926121]
86. Zhang Q, Seo S, Bugge K, Stone EM, Sheffield VC. BBS proteins interact genetically with the IFT pathway to influence SHH-related phenotypes. *Hum Mol Genet* 2012;21(9):1945–53. [PubMed: 22228099]
87. Pretorius PR, Baye LM, Nishimura DY, Searby CC, Bugge K, Yang B, et al. Identification and functional analysis of the vision-specific BBS3 (ARL6) long isoform. *PLoS Genet* 2010;6(3):e1000884. [PubMed: 20333246]
88. Jin H, Nachury MV. The BBSome. *Curr Biol* 2009;19(12):R472–3. [PubMed: 19549489]
89. Nager AR, Goldstein JS, Herranz-Perez V, Portran D, Ye F, Garcia-Verdugo JM, et al. An Actin Network Dispatches Ciliary GPCRs into Extracellular Vesicles to Modulate Signaling. *Cell* 2017;168(1–2):252–63 e14. [PubMed: 28017328]
90. Ye F, Breslow DK, Koslover EF, Spakowitz AJ, Nelson WJ, Nachury MV. Single molecule imaging reveals a major role for diffusion in the exploration of ciliary space by signaling receptors. *Elife* 2013;2:e00654. [PubMed: 23930224]
91. Pal K, Hwang SH, Somatilaka B, Badgandi H, Jackson PK, DeFea K, et al. Smoothed determines beta-arrestin-mediated removal of the G protein-coupled receptor Gpr161 from the primary cilium. *J Cell Biol* 2016;212(7):861–75. [PubMed: 27002170]
92. Leitch CC, Lodh S, Prieto-Echague V, Badano JL, Zaghoul NA. Basal body proteins regulate Notch signaling through endosomal trafficking. *J Cell Sci* 2014;127(Pt 11):2407–19. [PubMed: 24681783]
93. Vieira OV, Gaus K, Verkade P, Fullekrug J, Vaz WL, Simons K. FAPP2, cilium formation, and compartmentalization of the apical membrane in polarized Madin-Darby canine kidney (MDCK) cells. *Proc Natl Acad Sci U S A* 2006;103(49):18556–61. [PubMed: 17116893]
94. Monis WJ, Faundez V, Pazour GJ. BLOC-1 is required for selective membrane protein trafficking from endosomes to primary cilia. *J Cell Biol* 2017;216(7):2131–50. [PubMed: 28576874]
95. Milenkovic L, Scott MP, Rohatgi R. Lateral transport of Smoothed from the plasma membrane to the membrane of the cilium. *J Cell Biol* 2009;187(3):365–74. [PubMed: 19948480]

Unresolved Questions and concluding points

- Why do certain ciliary membrane proteins, even within a specific protein family such as GPCRs, require the BBSome for ciliary export while others do not?
- How are BBS/IFT interactions regulated to modulate the content of signaling proteins in cilia?
- In mammalian *Bbs* mutants, how is the protein content of various types of cilia altered and how are these changes linked to the phenotypes of BBS, particularly obesity?
- Which of the BBS subunits specifically recognize the different ciliary trafficking motifs and what is the molecular basis of the interactions?

Summary

- Cells control ciliary membrane content both by BBSome export and ectocytosis in both the unicellular alga *C. reinhardtii* and the multicellular human.
- BBSomes function as exporters of activated GPCRs and peripheral membrane proteins.
- BBSomes act as adaptors for IFT trains by recognizing ciliary export signals on a variety of membrane proteins, allowing them to be carried past the transition zone, into the cell body/plasma membrane.
- The accumulation of proteins at the ciliary tip is important for the retrieval of membrane proteins by the BBSome; emphasizing the importance of this specialized portion of the cilium.

Figure 1: Model of BBSome-mediated trafficking of ciliary membrane proteins

BBSome cargoes originating from the Golgi or endosome travel to the periciliary membrane (darker gray). Cargoes enter the cilium membrane (lighter gray) by either diffusion or IFT. Once in the cilium, cargoes can be recognized by BBSome. **BBSome cargoes Box:** Both transmembrane and peripheral membrane proteins are transported in a BBSome-dependent manner. Lipid modifications that allow for membrane association (i.e. palmitoylation and myristoylation), are shown in pink. **BBSome cargo recognition Box:** Simple Pick-Up: Moving BBSome-IFT trains encountering a membrane-associated cargo bind to its ciliary export sequence and carry it along and out of the cilium. Cargo Activation by Ligand Binding: Binding of an extracellular ligand (pink circle) induces conformational changes (indicated by a pink star) in the cytoplasmic domain of transmembrane receptors. This allows for receptors to be accumulated at the ciliary tip and by recognized by BBSomes, forming a BBSome-coat which can then undergo ciliary export by retrograde IFT trains.

BBSome-cargo complexes undergo processive retrograde IFT trafficking, cross through the transition zone (red Y-linkers) and re-enter the plasma membrane. Ye et al. (2018) suggest that transmembrane cargoes frequently dissociate from the BBSome in the space between the TZ and transition fibers (red triangles) and diffuse (green dashed line) back into the cilium proper (51). Only a fraction of these make it past the transition fibers to the periciliary membrane (smaller green arrow). Cargos can then undergo endocytosis for further processing by the cell.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript