The BBSome controls IFT assembly and turnaround in cilia

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The bidirectional movement of intraflagellar transport (IFT) particles, which are composed of motors, IFT-A and IFT-B subcomplexes, and cargoes, is required for the biogenesis and signaling of cilia1,2. A successful IFT cycle depends on the proper assembly of the massive IFT particle at the ciliary base and its turnover from anterograde to retrograde transport at the ciliary tip. However, how IFT assembly and turnover are regulated in vivo remains elusive. From a whole-genome mutagenesis screen in Caenorhabditis elegans, we identified two hypomorphic mutations in dyf-2 and bbs-1 as the only mutants showing normal anterograde IFT transport but defective IFT turnaround at the ciliary tip. Further analyses revealed that the BBSome (refs 3,4), a group of conserved proteins affected in human Bardet-Biedl syndrome5 (BBS), assembles IFT complexes at the ciliary base, then binds to the anterograde IFT particle in a DYF-2- (an orthologue of human WDR19) and BBS-1-dependent manner, and lastly reaches the ciliary tip to regulate proper IFT recycling. Our results identify the BBSome as the key player regulating IFT assembly and turnover in cilia.

Phylogenetically conserved IFT machinery mediates the bidirectional movement of IFT cargoes that are required for the biogenesis, maintenance and signalling of cilia1. At the ciliary tip, IFT particles need to be remodelled to enable the motor exchange, cargo release/loading and turnaround6. In Chlamydomonas, IFT-A and IFT-B dissociate after being transported to the flagellar tip and then IFT-B reassociates with IFT-A before retrograde IFT (ref. 7). To dissect the molecular pathway controlling IFT turnaround at the ciliary tip, we initiated a genome-wide ethyl methanesulphonate (EMS) mutagenesis screen in C. elegans to search for mutants with abnormal ciliary accumulation of the GFP-tagged IFT-B component OSM-6 (the orthologue of human IFT52). We reasoned that defective IFT turnaround should lead to IFT-B accumulation at the ciliary tip and compromise cilia formation.

In C. elegans, mutants with abnormal cilia formation cannot take up fluorescent dye and are thus called dye-filling defective6 (Dyf). We screened ~60,000 haploid genomes and retrieved 608 Dyf mutants. Of these, 158 Dyf mutants showed strong OSM-6::GFP accumulation in cilia. However, 154 of 158 mutants showed severely truncated cilia and no IFT transport, either anterograde or retrograde, which suggest mutations in essential IFT structural genes. In contrast, four recessive mutants, jhu555, jhu590, jhu598 and jhu616, exhibited frequent IFT movements and normal (or only slightly truncated) cilia, indicative of defective IFT recycling at the ciliary tip.

Mapping of jhu616 identified a glycine-to-arginine (G361R) substitution at the highly conserved WD40 domain of the DYF-2 protein (Fig. 1a,b), a known IFT component that is the orthologue of human WDR19 (also known as IFT144 in Chlamydomonas)9. Introducing a wild-type dyf-2 gene fully rescued jhu616 (Supplementary Fig. S1a,b). GFP-tagged DYF-2G361R mutant protein cannot efficiently enter the cilia and tends to accumulate around the ciliary base (Supplementary Fig. S1c). Interestingly, dyf-2(jhu616) mutants differ from the reported dyf-2(m160) null mutants. The former possess almost normal length cilia as well as active IFT movements, whereas the latter show severely truncated cilia and completely disrupted IFT transport9 (Fig. 1d,e), indicating that G361R mutation is a hypomorphic allele that does not affect the main role of DYF-2 as an IFT structural protein. Remarkably, kymograph analyses revealed that, in dyf-2(jhu616), the IFT-B component OSM-6 showed characteristic anterograde IFT movement, but lost almost all retrograde IFT movements (Fig. 1e). The severely impaired retrograde IFT explains the accumulation of OSM-6-GFP at the ciliary tip and suggests a key function for the DYF-2 WD40 domain in regulating IFT-B recycling at the ciliary tip.

In C. elegans, two kinesins, the canonical heterotrimeric kinesin-II and homodimeric OSM-3 (the orthologue of human KIF17), coordinate anterograde IFT transport10,11. The amphid and phasmid cilia in C. elegans contain two segments, middle doublet and distal singlet segments (Fig. 1c). Kinesin-II and OSM-3 cooperate in moving

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Figure 1 The G361R mutation in the conserved WD40 domain of the DYF-2 protein abolishes retrograde IFT transport of OSM-6. (a) Schematic of the DYF-2 protein and mutation sites. The m160 null allele possesses a nonsense mutation at the first exon. (b) DYF-2 G361 is highly conserved across species. Cr, Chlamydomonas reinhardtii; Ce, Caenorhabditis elegans; Dm, Drosophila melanogaster; Mm, Mus musculus; Hs, Homo sapiens. (c) In C. elegans, amphids in the head and phasmids in the tail are the primary ciliated sensory organs. Amphid and phasmid ciliary axonemes consist of a doublet middle segment and a singlet distal segment. Amphid channel cilia consist of 10 cilia and phasmid channel cilia consist of two cilia. (d) Fluorescence micrographs of cilia labelled with OSM-6::GFP. Compared with the severely truncated cilia in dyf-2(m160) animals, cilia in dyf-2(jhu616) are almost normal. OSM-6::GFP strongly accumulates at the ciliary tip in dyf-2(jhu616). The arrows and arrowheads indicate the tips and bases of cilia, respectively. Scale bar, 5 μm. (e) Kymograph analyses of OSM-6::GFP movement in wild-type, dyf-2(jhu616) and dyf-2(m160) cilia. Kymographs (left panels) and corresponding schematics (right panels) show visible OSM-6 anterograde (blue lines) and retrograde (red lines) IFT motilities. Compared with wild-type controls, the retrograde IFT motilities of dyf-2(jhu616) and dyf-2(m160) are almost normal. OSM-6::GFP strongly accumulates at the ciliary tip in dyf-2(jhu616). Where no IFT movement can be detected in dyf-2(m160) null mutants.

the same IFT particle along the middle doublet at 0.7 μm s⁻¹, and then OSM-3 kinesin alone moves the IFT particle along the distal singlet at a faster rate of 1.3 μm s⁻¹. If IFT-A and IFT-B dissociate, IFT-A will be transported by kinesin-II at 0.5 μm s⁻¹ and IFT-B will be transported by OSM-3 at 1.3 μm s⁻¹ in middle segments. In dyf-2(jhu616), we found that the IFT velocity of OSM-6::GFP in middle segments is
comparable to the wild-type rate (Supplementary Table S1), indicating that the integrity of anterograde IFT particles is normal.

To further determine whether IFT-A and IFT-B associate in dyf-2(jhu616), we performed a bimolecular fluorescence complementation (BiFC) assay. The BiFC assay was developed for direct visualization of protein–protein interactions in the same macromolecular complex in their natural environment of living cells. As shown in Supplementary Fig. S2a, the IFT-A component CHE-11 and the IFT-B component IFT-20 showed fluorescence complementation in either wild-type or dyf-2(jhu616) animals, indicative of the association between IFT-A and IFT-B subcomplexes. In marked contrast, no fluorescence complementation was observed in the dyf-2(m160) null mutant, which is also consistent with the previous finding that IFT integrity is totally disrupted in dyf-2(m160) null worms.

As for OSM-6, in dyf-2(jhu616) animals, all other IFT-B components examined also showed active anterograde movements, but lost most of the retrograde IFT movements and accumulated at the ciliary tip (Fig. 2a–c,e,f).

In *Chlamydomonas*, IFT144 (the homologue of DYF-2) is an IFT-A component. A temperature-sensitive mutation in the ift144 gene (fla15) results in the accumulation of IFT-B and retrograde IFT motor dynein in the bulges of normal-length flagella as well as reduced retrograde IFT velocities and frequencies. fla15 mutants resemble other IFT-A mutants in *Chlamydomonas*. Similarly, in ift144ma7 mice that carry a partial loss-of-function IFT144 protein, cilia still form normally but accumulate IFT-B at the ciliary tip and lack detectable IFT-A proteins inside cilia. As dynein and IFT-A components are the key players of the retrograde IFT machinery, the accumulation of IFT-B in fla15 flagella or ift144ma7 cilia is probably due to the defective retrograde IFT machinery. To examine whether the accumulation of IFT-B in dyf-2(jhu616) is also due to the defective retrograde transport, we examined the IFT-A marker CHE-11 (the orthologue of human IFT140), the retrograde IFT motor dynein light chain XBX-1 (the orthologue of D2LIC) and the IFT-A associated kinesin-II motor KAP-1. Remarkably, we found that dynein as well as other IFT-A markers localize properly to cilia and show no accumulation at the ciliary tip, and most importantly, all of these markers exhibited normal bidirectional IFT transport (Fig. 2d,g,h). The comparable anterograde IFT rates between IFT-A and IFT-B components confirm again that IFT-A and IFT-B do associate together in anterograde transport (Supplementary Table S1). Taken together, these findings indicate that, in contrast to fla15 *Chlamydomonas* or ift144ma7 mice, the retrograde IFT machinery in dyf-2(jhu616) is still functional, and the ciliary accumulation of IFT-B in dyf-2(jhu616) is because IFT-B components fail to be assembled into the functional IFT-A–dynein machinery before retrograde IFT transport. Thus, our observations reveal a role for the WD40 domain of DYF-2 as the key factor in reassembling the IFT-B subcomplex into IFT-A–dynein retrograde machinery.

To further understand why only IFT reassembly at the ciliary tip is specifically affected in dyf-2(jhu616), we examined other proteins that were reported to associate with the IFT complex. The BBSome proteins are a group of eight conserved proteins whose etiologies are associated with cilia dysfunction and the autosomal recessive ciliopathy Bardet–Biedl syndrome. The role of the BBSome in the context of cilia has remained controversial. Knocking down of BBS5 in *Chlamydomonas* leads to the absence of flagella. However, *bbs4* *Chlamydomonas* mutants show normal flagella but defective IFT transport. *bbs* knockdown zebrafish show delayed dynein-dependent intracellular retrograde transport of melanosome and defective ciliogenesis in the ciliated Kupffer’s vesicle. Knockout mouse models of various *bbs* genes fail to form sperm flagella, but form primary cilia in other organs examined. However, the observations that *bbs* knockout mice exhibit mislocalization of rhodopsin in photoreceptors, misshaped kidney cilia and abnormal bulges inside ependymal and airway cilia also suggest a role for the BBSome in IFT transport in vivo alone with kinesin-II and IFT-B moving alone with OSM-3 (refs 10, 31). It is thus suggested that the BBSome holds IFT-A and IFT-B together during IFT transport to offset the mechanical competing force generated between the faster motor OSM-3 and the slower motor kinesin-II (ref. 32).

Surprisingly, in dyf-2(jhu616) animals, we found that all BBS proteins examined strongly accumulated around the ciliary base. Some of them (BBS-1 and BBS-4) totally lost the ciliary localization; the others (BBS-2, -5, -7, -8 and -9) showed only very dim ciliary staining when compared with wild-type animals (Fig. 3). Most importantly, all BBS proteins completely lost IFT movement, indicating the complete dissociation between the BBSome and the moving IFT particles (Fig. 3). To determine whether worm BBS proteins do form a complex, we performed BiFC analyses. In wild-type animals, fluorescence complementation can be observed in BBS-1–BBS-7 and BBS-1–BBS-9 pairs, indicative of the coexistence of these three BBS proteins in the same complex (Supplementary Fig. S3a). This finding is also consistent with the prediction that mammalian BBS1, 7 and 9 localize close to each other in the BBSome (ref. 3). The fluorescence complementation between BBS proteins was totally disrupted in the *bbs* null mutants examined (Supplementary Fig. S3b), which further suggests that worm BBS proteins form a macromolecular complex in their native environment. Strikingly, in dyf-2(jhu616) mutants, the BBSome still formed, but was restricted to the ciliary base (Supplementary Fig. S3b,c).

In dyf-2(jhu616) animals, the fact that IFT-A and IFT-B still associate in anterograde IFT in the absence of the entire BBSome demonstrates that the BBSome does not act as an indispensable component for the IFT machinery to stabilize the binding of IFT-A–IFT-B during IFT transport. This conclusion is also supported by the observation made in *Chlamydomonas* that the BBSome is substoichiometric to the IFT particle during IFT transport and, thus, not an integral part of IFT machinery. Combined with the observation that IFT-A and IFT-B dissociate in *bbs* null mutants, we proposed that the in vivo function for the BBSome is to regulate the assembly of the IFT particles at the ciliary base. After IFT particles are assembled at the ciliary base, the BBSome binds to the IFT particle like a cargo but not a structural component. We further reasoned that the BBSome may play a similar role in IFT reassembly and turnaround at the ciliary tip. In dyf-2(jhu616), although the BBSome lost the association with anterograde IFT particles, it is still functional at the ciliary base and results in normal IFT assembly and anterograde transport; the absence of the BBSome at the ciliary tip leads to defective IFT reassembly and the accumulation of IFT-B components at the ciliary tip (Fig. 5).

The key evidence supporting the mechanical competition model for the BBSome is that if either one of the two kinesin motors is absent in *bbs* null mutants, IFT-A and IFT-B will reassociate again
Figure 2 dyf-2(jhu616) animals show compromised IFT turnaround at the ciliary tip. (a-h) Fluorescence micrographs of cilia labelled with various IFT markers (left panels) and corresponding kymographs (right panels). In dyf-2(jhu616), the IFT-B components OSM-5 (the orthologue of human IFT88; a), CHE-2 (the orthologue of human IFT80; b) and CHE-13 (the orthologue of human IFT57; c), the IFT-B-associated kinesin motor OSM-3/KIF17 (e) and the OSM-3 activator DYF-1 (the homologue of human TTC30B; f) show consistent ciliary tip accumulation similar to OSM-6 (Fig. 1d) and exhibit severely abrogated retrograde IFT movement (a-c,e,f, right panels). In contrast, the IFT-A components CHE-11 (the orthologue of human IFT140; d) and the IFT-A-associated kinesin-II subunit KAP-1 (g) show no ciliary accumulation and exhibit both anterograde and retrograde IFT motilities at characteristic wild-type rates (Supplementary Table S1). Note that kinesin-II mediates IFT motility only in the middle segments (g). As expected, the dynein light chain XBX-1 that is responsible for retrograde IFT movement shows no ciliary accumulation and moves normally in both directions (h). Scale bar, 5 μm.

We then examined bbs-7; osm-3 double mutants. OSM-3 is the anterograde motor for IFT-B. We found that in ~50% of bbs-7; osm-3 animals, IFT-B components show no anterograde IFT movement at all; in the remaining half of them, only a few IFT-B-containing particles can be observed in anterograde transport (Supplementary Fig. S4a). Furthermore, in a given time period, equivalent numbers of IFT-B-containing and IFT-A-containing particles can be observed in wild-type, bbs-7 or osm-3 single-mutant animals, whereas the average number of IFT-B-containing particles (~4 particles min⁻¹) is only about one tenth of that of IFT-A-containing ones (~40 particles min⁻¹) in bbs-7; osm-3 animals (Supplementary Fig. S4c). In all bbs-7; osm-3 animals examined, IFT-B proteins show few retrograde IFT particles and still accumulate at the ciliary tip (Supplementary Fig. S4a,b). Taken together, these results demonstrate that in bbs-7; osm-3 double mutants, most IFT-B cannot associate with IFT-A even in the absence of the mechanical competing force. These observations indicate that the main role for the BBSome in cilia is to efficiently assemble IFT particles at both the ciliary base and tip.

To further verify our model, we mapped the other three Dyf mutants. jhu555, jhu590 and jhu598 were mapped to bbs-9, bbs-7 and bbs-1, respectively. Kymograph analyses indicated that IFT-A and IFT-B separate and move at different rates in anterograde IFT in bbs-7(jhu590)
Figure 3 Lack of a functional BBSome in dyf-2(jhu616) cilia. (a) BBS proteins show either absent (BBS-1 and BBS-4) or a markedly reduced (BBS-2, -5, -7, -8 and -9) level of ciliary localization in dyf-2(jhu616) animals. (b-f) No IFT movements are detected for residual ciliary BBS-2, BBS-5, BBS-7, BBS-8 or BBS-9 in dyf-2(jhu616) cilia. Scale bar, 5 μm.

(enceodes BBS-7 with a nonsense mutation at the Gln 546 site) and bbs-9(jhu555) (encodes BBS-9 with a nonsense mutation at Gln 171 site) mutants just like in other reported bbs null mutants (Supplementary Table S1). IFT-B components tend to accumulate in various bbs mutants, as revealed in published papers. However, the underlying mechanism for the accumulation of IFT-B in bbs null animals differs from that in dyf-2(jhu616). IFT-A, the essential player for retrograde IFT, cannot be transported into cilia distal segments to reach the ciliary tip in bbs null mutants. The absence of IFT-A at the ciliary tip would naturally lead to the IFT-B accumulation in bbs null mutants due to the defective retrograde IFT transport. In contrast, in dyf-2(jhu616) animals, IFT-A can reach the ciliary tip and perform retrograde IFT, but IFT-B fails to reassociate with IFT-A before retrograde IFT, which leads to the accumulation of IFT-B at the ciliary tip.

Remarkably, the defects of bbs-1(jhu598) animals completely phenocopy the observations in dyf-2(jhu616): IFT-A and IFT-B associate in anterograde but not retrograde IFT and IFT-B accumulates at the ciliary tip (Fig. 4b,c and Supplementary Table S1). The BiFC assay also confirmed that IFT-A can still associate with IFT-B in bbs-1(jhu598) (Supplementary Fig. S2b). Sequencing results indicated that bbs-1(jhu598) encodes a BBS-1 mutant with a glycine-to-aspartic-acid change at the highly conserved Gly 207 site (G207D; Fig. 4a). Compared with the strong ciliary targeting of the wild-type BBS-1 protein, GFP-tagged BBS-1G207D accumulated only around the ciliary base (Supplementary Fig. S5a). Overexpression of BBS-1G207D caused a mild dominant-negative effect in the dye-filling assay (Supplementary Fig. S5b). As in dyf-2(jhu616) animals, all BBSome proteins strongly accumulate around the ciliary base and show no IFT movement in bbs-1(jhu598) (Fig. 4d and Supplementary Fig. S5c–g). We then built a dyf-2(jhu616); bbs-1(jhu598) double mutant. In dyf-2(jhu616); bbs-1(jhu598) animals, IFT-A proteins still show wild-type ciliary localization, whereas IFT-B accumulation is more severe and extends to most of the cilium, and as expected, BBS proteins strongly accumulate around the ciliary base and show no IFT movement (Supplementary Fig. S5h.i).

We next assessed DYF-2 ciliary localization in bbs-1(jhu598) animals. Compared with the wild-type worms, the level of ciliary targeting of DYF-2 was significantly reduced in bbs-1(jhu598) mutants, whereas the remaining ciliary DYF-2 still showed normal IFT movement in both directions (Fig. 4e,f). These data reminded us of the behaviour of the DYF-2G361R protein (Supplementary Fig. S1). It seems that the dissociation between the BBSome and the IFT particle may also affect the efficient incorporation of DYF-2 into anterograde IFT particles. To test this possibility, we determined whether DYF-2 and BBS-1 interact with each other. Results from BiFC analyses suggest that DYF-2 localizes
Figure 4 The BBSome and DYF-2 coordinate IFT assembly and turnaround at the ciliary tip. (a) The BBS-1 Gly 207 site is conserved across species. Cr, Chlamydomonas reinhardtii; Ce, Caenorhabditis elegans; Dm, Drosophila melanogaster; Mm, Mus musculus; Hs, Homo sapiens. (b) In bbs-1(jhu598), the IFT-B components (OSM-6 and DYF-2) and the IFT-A-associated kinesin-II KAP-1 and the retrograde dynein motor OSM-3, but not the IFT-A component CHE-11 and Dm, Drosophila melanogaster species. Cr, Chlamydomonas reinhardtii; Mm, Mus musculus; Hs, Homo sapiens. (c) In bbs-1(jhu598), the IFT-B components (OSM-6 and DYF-2) and the IFT-A-associated kinesin-II KAP-1 and the retrograde dynein motor OSM-3, but not the IFT-A component CHE-11 and (d) In bbs-1(jhu598), all BBSome subunits show absent or a greatly reduced level of ciliary targeting. (e) The level of DYF-2 protein ciliary targeting is also greatly reduced in bbs-1(jhu598) cilia. (f) The residual ciliary DYF-2 protein shows active bidirectional IFT movement. (g) In BiFC analyses, fluorescence complementation can be visualized between YC-tagged DYF-2 and either one of YN-tagged BBS-1, BBS-7 and BBS-9, but not between YC-tagged DYF-2(G341R) and YN-tagged BBS-1. (h) HEK293 cells were transiently transfected with HA-tagged WDR19 and Flag-tagged BBS1 constructs. At 48 h later, cells were subjected to immunoprecipitation using normal mouse IgG (mIgG) or anti-Flag antibody. Scale bars, 5 μm. Uncropped images of blots/gels are shown in Supplementary Fig. S6.
IFT machinery to the ciliary base, but also is indispensable for retrograde transport not only functions to recycle cargoes, which are used to build cilia and maintain sensory function, but also is indispensable for 

### Methods

Methods and any associated references are available in the online version of the paper.

**Note:** Supplementary Information is available in the online version of the paper.
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AUTHOR CONTRIBUTIONS

J.H. conceived the initial concept for the screen. J.H., Q.W., Y.L. and Y.Z. performed the screening and mapped the mutants. Q.W. and Y.Z. generated transgenic flies. K.L. identified by filling sensory neurons with BBS4 and Q.Z. carried out biochemical assays with the support of Y.Z., K.L. and J.H. wrote the manuscript with contributions from Q.W., Y.Z., Q.Z. and Q.Z.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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METHODS

Strains and mutant alleles. Nematodes were raised using standard conditions. N2 worms represented the wild type in all assays. To ensure that the expression levels of GFP markers were comparable between the wild-type and mutant strains, we introduced each GFP transgene from the wild-type worms into individual mutant worms by genetic crossing. Dye-filling assay or PCR was used to monitor the mutant genotype. All strains used in this paper are listed in Supplementary Table S2.

Dye-filling assay. Worms were washed off the culturing plate with M9 buffer (3 g l⁻¹ KH₂PO₄, 6 g l⁻¹ Na₂HPO₄, 5 g l⁻¹ NaCl and 1 mM MgSO₄), collected by centrifugation at 3000 × g for 1 min, washed once with M9 and then incubated in diluted DiI dye (Molecular Probes, 2 mg ml⁻¹ stock solution in dimethyl formamide, diluted 1:200 in M9 before use) for 1 h at room temperature. After incubation, the worms were washed at least three times with M9, transferred to an NGM plate without a bacterial lawn, and then observed under a fluorescence microscope (M2Bio, Zeiss).

EMS mutagenesis screen and mapping. N2 animals were mutagenized with EMS using standard procedures. F2 progeny from mutagenized animals were grown at 20 °C and screened using a dye-filling assay. The worms with a defective dye-filling phenotype were selected. Isolated mutants were backcrossed at least three times with N2 animals.

Microscopy and imaging. Animals were raised at 20 °C. The live adult worms were mounted on 5% agar pads by anaesthetization in 10 mM levamisole (diluted in M9 buffer), observed, and images were acquired under a Plan Apochromat x 60, 1.49 NA oil objective (Nikon) with an imaging microscope (Nikon TE 2000-U).

IFT measurement. We performed all IFT analyses in phasmids as described before. Worms containing GFP-tagged protein were anaesthetized in a drop of 10 mM levamisole in M9 on a 5% agar mount slide, and imaged immediately. IFT motility was observed using a Plan Apochromat x 100, 1.49 NA oil total internal reflection fluorescence objective (Nikon). Live-time imaging was recorded at 200 ms per frame using a CCD (charge-coupled device) camera (Photometrics QuantEM 512SC, Roper Industries). Kymographs were produced and the transport speed was calculated using MetaMorph software (MDS Analytical Technologies).

Cell culture, transient transfection and immunoprecipitation. HEK293 cells were cultured in DMEM with 10% fetal bovine serum. To express HA–WDR19 and Flag–BBS1, open reading frames were amplified from mouse IMCD3 cells using PCR with reverse transcription, inserted into appropriate vectors and confirmed by sequencing. HEK293 cells were transfected with a calcium phosphate precipitation approach using 3 μg HA-tagged WDR19 and Flag-tagged BBS1 constructs. At 48 h later, cells were lysed in lysis buffer (50 mM Tris–HCl, at pH 7.4, 150 mM NaCl, 1% NP-40 and 10% glycerol) and subjected to immunoprecipitation using normal mouse IgG or mouse anti-Flag antibody. The immunocomplexes were separated by SDS-PAGE and analysed using appropriate antibodies. Primary monoclonal antibodies against Flag (clone M2, Sigma, F1804, 1:10,000), HA (clone HA-7, Sigma, H3663, 1:1,000) and actin (AC-15, Sigma, A1975, 1:1,000) were used.

Visualization of IFT-A and IFT-B association and BBSome complex using the BiFC assay. The Venus-based BiFC assay was developed to detect the protein interactions in cilia in living worms. For this purpose, we replaced the heat-shock promoter in worm BiFC vectors with pCE–BiFC–VN173 and pCE–BiFC–VC155 with the worm cell-specific promoter of arl-13 using SphI and Xmal enzymes to visualize. To visualize IFT-A and IFT-B in cilia, complementary DNA of the worm IFT-A component CHE-11 was sub-cloned into the VN173 vector and cDNA encoding the worm IFT-B component IFT-20 was sub-cloned into the VC155 vector, and then these two plasmids were co-injected along with the co-injection marker pH4 [rol-6(su1006)] into wild-type worms (5 ng μl⁻¹ for each BiFC plasmid and 100 ng μl⁻¹ PRF4). Fluorescent signals were visualized using a YFP filter. The same strategy was used to obtain and verify the recombination fluorescence signal from CHE-11::YN–DYF-2::YC. To visualize the formation of the BBSome, BBS-1::YC and BBS-7::YN or YN::BBS-9 were used to generate transgenic animals.

Quantification and statistical analysis. The dye-filling ratio is the number of positively stained amphiads or phasmids divided by the total number of amphiads or phasmids checked. The statistical results were obtained from three independent experiments with n > 50 worms in each trial. To measure the IFT rates, the velocities of IFT particles were calculated using Metamorph software. n was at least 60 IFT particles. To measure the IFT frequency, the number of IFT particles was calculated from the kymograph image. The statistical results were obtained from three independent experiments with n > 20 cilia in each trial. The differences were considered significant when P was < 0.05 in a two-tailed unpaired Student t-test.

Primers. Primers used for identifying the mutants and cloning are listed in Supplementary Table S3.

Accession number. Wormbase ID: jhu616 (WBVar01429489) and jhu598 (WBVar01429488).

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**Figure S1**  
*jhu616* is a *dyf-2* allele. Introduction of a wild-type *dyf-2* gene fully rescues the Dyf phenotype (a) and the abnormal accumulation of OSM-6::GFP at the ciliary tip (b) of *dyf-2(jhu616)* animals. c, Although DYF-2*G361R* mutant protein displays severely reduced ciliary targeting, it still shows active bidirectional IFT movement comparable to that of wild-type DYF-2. Error bars indicate ±SEM. *n* in (a) represents the total animal number from 3 independent experiments. Bar, 5 μm.
Figure S2 IFT-A and IFT-B associate inside dyf-2(jhu616) and bbs-1(jhu598) cilia. Visualization of IFT-A and IFT-B subcomplex in living animals using bimolecular fluorescence complementation (BiFC) analysis. IFT-A component CHE-11 was tagged with YFP N-fragment (CHE-11::YN) and IFT-B component DYF-2 or IFT-20 was tagged with YFP C-fragment (DYF-2::YC or IFT-20::YC). a, Transgenic animals that expressing CHE-11::YN+IFT-20::YC show fluorescence complementation in wild-type and dyf-2(jhu616) cilia, but not in dyf-2(m160) null cilia, indicating that IFT-A and IFT-B still associate in dyf-2(jhu616) cilia. b, Transgenic animals that expressing CHE-11::YN+DYF-2::YC show fluorescence complementation in wild-type and bbs-1(jhu598) cilia, but not in bbs-1(ok1111) null cilia, indicating that IFT-A and IFT-B still associate in bbs-1(jhu598) cilia. Star indicates cilia base. Bar, 5 μm.
**Figure S3** No BBSome inside *dyf-2(jhu616)* cilia. **a,** Visualization of the BBSome complex in living animals using bimolecular fluorescence complementation (BiFC) analysis. N-terminal fragment of YFP (YN) and C-terminal fragment of YFP (YC) were used in BiFC analyses. No Fluorescence was detected when only expressed YN and YC together. **b,** In wild-type, BBS-1::YC-BBS-7::YN and BBS-1::YC-YN::BBS-9 show detectable fluorescence signal at cilia base and inside cilia; whereas in *dyf-2(jhu616)* and *dyf-2(m160)* null mutants, the fluorescence complementation can only be detected at cilia base, indicating the absence of the BBSome inside cilia. In *bbs null* mutants, no fluorescence signal was detected, indicating the disruption of the BBSome. In BiFC analyses, the same transgenes were crossed into various mutants to make sure the same expressing level. **c,** Kymograph analysis indicated that BBS-1::YC-BBS-7::YN or BBS-1::YC-YN::BBS-9 containing particles move at normal IFT anterograde and retrograde rates inside wild-type cilia but not inside *dyf-2(jhu616)* cilia. Star indicates cilia base. Bar, 5 μm.
Figure S4 The association between IFT-A and IFT-B is severely compromised in bbs-7; osm-3 double mutants.  
a, In kymograph analyses on bbs-7; osm-3 cilia, IFT-A component CHE-11 shows normal bidirectional movement, whereas IFT-B protein only show few anterograde movement.  
b, IFT-B protein but not IFT-A protein accumulate at the ciliary tip in bbs-7; osm-3 mutants. Arrows indicate the accumulation.  
c, Few IFT-B component OSM-6 show anterograde IFT in bbs-7; osm-3 double mutants. For each mutant, n represents the total animal examined from 3 independent experiments. Error bars indicate ±SD. *p < 0.01.  
Bar, 5 μm.
Figure S5 Characterization of *bbs-1(jhu598)* and *bbs-1(jhu598); dyf-2(jhu616)* double mutants. **a**, *BBS-1*<sup>G207D</sup> loses ciliary targeting and accumulates around the ciliary base. **b**, Overexpression of *BBS-1*<sup>G207D</sup> causes a dominant-negative effect on ciliogenesis. *n* represents the total animal number from 3 independent experiments. **c-g**, No IFT movements are detected for BBS-2, BBS-5, BBS-7, BBS-8, or BBS-9 in *bbs-1(jhu598)* cilia. **h**, The accumulation of OSM-6::GFP extends to most part of the cilia in *bbs-1(jhu598); dyf-2(jhu616)* double mutants. The ciliary localization of IFT-A component CHE-11 in *bbs-1(jhu598); dyf-2(jhu616)* is normal. **i**, In *bbs-1(jhu598); dyf-2(jhu616)*, subunits of the BBSome show either absent (BBS-1, BBS-4) or dramatically reduced (BBS-2, BBS-7) ciliary localization. *p* < 0.01. Error bars indicate ±SEM. Bar, 5 μm.
Table S1. IFT velocities of various IFT markers in wild-type and mutants. Notably, we noticed that, for most IFT markers examined in *dyf-2(jhu616)* and *bbs-1(jhu598)* animals, the anterograde velocities are consistently faster in either middle segments (~0.8 μm/s) or distal segments (~1.4 μm/s); whereas the retrograde velocities are comparable to those in wild-type. The subtle difference in velocity indicates that although the coordination between two kinesin motors and the association between IFT-A and IFT-B are not disrupted in *dyf-2(jhu616)* and *bbs-1(jhu598)*, the composition and/or the mass of anterograde IFT particles may be different than that in wild-type.

Table S2. Worm strains used in this study.

Table S3. Primers used for identifying the mutants and cloning.

Supplementary Movies

Movie S1. IFT movement of GFP-tagged CHE-2 in *bbs-1(jhu598)* cilia.
Movie S2. IFT movement of GFP-tagged CHE-11 in *bbs-1(jhu598)* cilia.
Movie S3. IFT movement of GFP-tagged CHE-13 in *bbs-1(jhu598)* cilia.
Movie S4. IFT movement of GFP-tagged DYF-1 in *bbs-1(jhu598)* cilia.
Movie S5. IFT movement of GFP-tagged KAP-1 in *bbs-1(jhu598)* cilia.
Movie S6. IFT movement of GFP-tagged OSM-3 in *bbs-1(jhu598)* cilia.
Movie S7. IFT movement of GFP-tagged OSM-5 in *bbs-1(jhu598)* cilia.
Movie S8. IFT movement of GFP-tagged OSM-6 in *bbs-1(jhu598)* cilia.
Movie S9. IFT movement of GFP-tagged CHE-11 in *bbs-1(jhu598)* cilia.
Movie S10. IFT movement of GFP-tagged CHE-11 in *bbs-7(n1606)* cilia.
Movie S11. IFT movement of GFP-tagged OSM-6 in *bbs-7(n1606)* cilia.
Movie S12. IFT movement of GFP-tagged CHE-2 in *dyf-2(jhu616)* cilia.
Movie S13. IFT movement of GFP-tagged CHE-11 in *dyf-2(jhu616)* cilia.
Movie S14. IFT movement of GFP-tagged CHE-13 in *dyf-2(jhu616)* cilia.
Movie S15. IFT movement of GFP-tagged DYF-1 in *dyf-2(jhu616)* cilia.
Movie S16. IFT movement of GFP-tagged KAP-1 in *dyf-2(jhu616)* cilia.
Movie S17. IFT movement of GFP-tagged OSM-3 in *dyf-2(jhu616)* cilia.
Movie S18. IFT movement of GFP-tagged OSM-5 in *dyf-2(jhu616)* cilia.
Movie S19. IFT movement of GFP-tagged OSM-6 in *dyf-2(jhu616)* cilia.
Movie S20. IFT movement of GFP-tagged CHE-11 in *dyf-2(jhu616)* cilia.
Movie S21. IFT movement of GFP-tagged CHE-11 in *osm-3(mn391); bbs-7(n1606)* cilia.
Movie S22. IFT movement of GFP-tagged OSM-6 in *osm-3(mn391); bbs-7(n1606)* cilia.
Movie S23. IFT movement of GFP-tagged CHE-11 in *osm-3(mn391)* cilia.
Movie S24. IFT movement of GFP-tagged OSM-6 in *osm-3(mn391)* cilia.
Movie S25. IFT movement of GFP-tagged CHE-2 in wild-type cilia.
Movie S26. IFT movement of GFP-tagged CHE-11 in wild-type cilia.
Movie S27. IFT movement of GFP-tagged CHE-13 in wild-type cilia.
Movie S28. IFT movement of GFP-tagged DYF-1 in wild-type cilia.
Movie S29. IFT movement of GFP-tagged DYF-2 in wild-type cilia.
Movie S30. IFT movement of GFP-tagged DYF-2 in wild-type cilia.
Movie S31. IFT movement of GFP-tagged KAP-1 in wild-type cilia.
Movie S32. IFT movement of GFP-tagged OSM-3 in wild-type cilia.
Movie S33. IFT movement of GFP-tagged OSM-5 in wild-type cilia.
Movie S34. IFT movement of GFP-tagged OSM-6 in wild-type cilia.
Movie S35. IFT movement of GFP-tagged CHE-11 in wild-type cilia.