Cell. Mol. Life Sci. 63 (2006) 2145–2161
1420-682X/06/182145-17
DOI 10.1007/s00018-006-6180-x

Cellular and Molecular Life Sciences

Human Genome & Diseases: Review

Bardet-Biedl syndrome: an emerging pathomechanism of intracellular transport

O. E. Blacque and M. R. Leroux*

Department of Molecular Biology and Biochemistry, Simon Fraser University, 8888 University Drive, Burnaby, BC V5A 1S6 (Canada), Fax: +1 604 291 5583, e-mail: leroux@sfu.ca

Received 20 April 2006; received after revision 30 May 2006; accepted 15 June 2006
Online First 11 August 2006

Abstract. From a handful of uncloned genetic loci 6 years ago, great strides have been made in understanding the genetic and molecular aetiology of Bardet-Biedl syndrome (BBS), a rare pleiotropic disorder characterised by a multitude of symptoms, including obesity, retinal degeneration and cystic kidneys. Presently, 11 *BBS* genes have been cloned, with the likelihood that yet more *BBS* genes remain undiscovered. In 2003, a major breakthrough was made when it was shown that BBS is likely caused by defects in basal bodies and/or primary cilia. Since then, studies in numerous animal models of BBS have corroborated the initial findings and, in addition, have further refined the specific functions of BBS proteins. These include roles in establishing planar cell polarity (noncanonical Wnt signaling) in mice and zebrafish, modulating intraflagellar transport and lipid homeostasis in worms, and regulating intracellular trafficking and centrosomal functions in zebrafish and human tissue culture cells. From these discoveries, a common theme has emerged, namely that the primary function of BBS proteins may be to mediate and regulate microtubule-based intracellular transport processes.

Keywords. Bardet-Biedl syndrome, cilia, centrosomes, intracellular trafficking, intraflagellar transport.

Introduction

In 1866, four siblings with obesity, retinal degeneration and mental retardation were reported by Laurence and Moon [1]. Over 50 years later, Bardet and Biedl found individuals who, in addition to the Laurence-Moon symptoms, presented with polydactyly [2, 3]. It is now generally accepted that Laurence-Moon syndrome and Bardet-Biedl syndrome are not distinct disorders, but rather are allelic [4, 5]. Bardet-Biedl syndrome (BBS; OMIM 209900) is a highly pleiotropic human disorder, characterised by a multitude of symptoms. Beales et al. [4] proposed that clinical diagnosis of BBS requires four of six primary symptoms (i.e. obesity, rod-cone dystrophy, renal abnormalities, polydactyly, male hypogonadism and learning disabilities), or three primary symptoms and at least two secondary symptoms, which include diabetes mellitus, hepatic fibrosis, ataxia/poor coordination/imbalance, speech disorder/delay, polyuria/polydipsia (nephrogenic diabetes insipidus), mild spasticity (especially lower limbs), dental crowding/hypodontia/small roots/high arched palate, left ventricular hypertrophy/congenital heart disease, hearing loss, anosmia, and *situs inversus* (for full description of BBS symptoms see [4, 6, 7].

Although many BBS symptoms are detectable at birth, a number of them only become manifest from childhood onwards (e.g. retinal degeneration, genital abnormalities, obesity). Given the clinical hypervariability of BBS, correct diagnosis requires careful phenotypic analysis, and for those symptoms that develop after childhood, due consideration must be given to the age of the patient. Indeed, phenotypic overlap with disorders such as Meckel syndrome has resulted in a number of BBS patients being

* Corresponding author.

misdiagnosed [8]. Interestingly, a recent report describes a new BBS-like disorder termed MORM syndrome (mental retardation, truncal obesity, retinal dystrophy and micropenis) [9]. Although the genetic lesion responsible for MORM syndrome is not linked to any of the known BBS loci, the high degree of phenotypic overlap with BBS, together with the fact that additional *BBS* genes remain to be discovered, implies that an allelic association with BBS cannot be discounted for MORM syndrome.

BBS is a rare disorder, with a prevalence of ~1 : 120,000 for all live births in North America and Europe [10–12]. However, in isolated communities with increased frequency of consanguinity, such as certain fishing communities in Newfoundland and the Bedouin tribes of Kuwait and Saudi Arabia, the incidence of BBS can be as high as 1 : 13,000–1 : 17,000 [6, 13].

Gene identification

BBS is a multigenic disorder, with 11 genes now associated with the syndrome. Standard positional cloning techniques were used to identify the first 3 *BBS* genes, *BBS6* [14, 15], *BBS2* [16] and *BBS4* [17], and very recently, a similar approach uncovered *BBS10* [18]. High-density single-nucleotide polymorphism (SNP) microarray genotyping was used to identify the most recently discovered *BBS* gene, *BBS11* [19]. For the remaining 6 genes, bioinformatics- and genomics-based approaches were applied to probe uncloned BBS genetic loci for candidate *BBS* genes. *BBS1* [20] and *BBS7* [21] were identified based on limited protein sequence similarity to *BBS2*. *BBS8* [22] was identified on account of protein sequence similarity to *BBS4*. Finally, *BBS3* [23, 24], *BBS5* [25] and *BBS9* [26] were identified using comparative genomics analyses that exploited the fact that all known *BBS* genes have been lost from the genomes of certain organisms.

Globally, *BBS1* and *BBS10* are the most common BBS loci, accounting for 23–56% and 20% of BBS cases, respectively [18, 27–29]. Next is *BBS2* at 8–16%, followed by *BBS6* at 4–5%, with the remaining BBS loci each contributing < 4% of cases [*BBS3* (2–4%), *BBS4* (3%), *BBS5* (3%), *BBS7* (3.5%), *BBS8* (1–2%) and *BBS-9* (not determined)] [21, 30–33]. The reported variation in the contribution of certain *BBS* genes, in particular *BBS1*, which accounts for only 20–30% of cases in Caucasians [27], suggests that other *BBS* genes remain to be identified. Interestingly, with regard to the obesity phenotype, the frequency of the most common BBS mutation, M390R, is identical among obese and non-obese individuals in a Newfoundland population, indicating that BBS mutations are unlikely to function in the pathogenesis of non-syndromic obesity [34]. Consistent with these findings, Anderson and colleagues [35] found that *BBS6* mutations are not linked to obesity in a Danish population with juvenile onset obesity.

With the exception of BBS3, BBS6, BBS10 and BBS11, the primary sequences of the other seven BBS proteins have not provided significant functional clues. BBS1, BBS2 and BBS7 proteins have predicted β-propeller domains, which are relatively common motifs with multiple functions [36]. BBS4 and BBS8 harbour several tetratricopeptide repeat domains (TPRs), which are found in many different proteins and play important roles in protein-protein interactions [37]. BBS5 possesses two DM16 repeat motifs of unknown function, and BBS9 was previously annotated as human B1 protein whose function is not known. In contrast, *BBS3* encodes ADP-ribosylation-like protein 6 (ARL6), a small GTPase of the Ras superfamily with likely regulatory functions [23, 24]. The entire BBS6 protein and limited regions within BBS10 show sequence homology to archaeal chaperonins and the eukaryotic CCT (chaperonin containing TCP-1) proteins, suggesting possible roles in protein folding or assembly [18, 38]. Finally, *BBS11* is a member of the TRIM family, and the encoded protein has E3 ubiquitin ligase activity that suggests a role in the ubiquitin/proteasome system [19]. Interestingly, as will be discussed later on, all of the *BBS* genes are widely distributed in ciliated organisms such as *Chlamydomonas reinhardtii*, *Caenorhabditis elegans* and *Drosophila melanogaster*, with the exception of the chaperonin-like *BBS6* and *BBS10* genes, which appear to have emerged more recently in vertebrates [18, 38].

Genetic inheritance of BBS

The genetic heterogeneity and familial phenotypic variability of BBS has sparked considerable interest in its mode of inheritance. Classically, it appears that BBS is inherited in an autosomal recessive manner, with two mutations at a single locus sufficient to cause the disease. However, over the last 5 years, several studies have indicated that BBS can be transmitted in a complex, non-Mendelian fashion, requiring mutations at more than one locus. In the seminal study, Katsanis et al. [39] screened 163 BBS families for mutations in *BBS2* and *BBS6* and found four pedigrees with individuals possessing three *BBS* alleles, namely a pair of homozygous mutations in one *BBS* gene and a third mutation in a second *BBS* gene. Since *BBS* gene mutations are rare in the general population, the probability of discovering four pedigrees with three *BBS* alleles is extremely small, indicating, therefore, that the third alleles are likely linked to disease pathogenicity in these individuals. Consistent with a triallelic mode of inheritance, Katsanis and colleagues also identified unaffected individuals with two nonsense alleles of *BBS2* (Q59X/Y22X), indicating that two null

mutations in one *BBS* gene are not always sufficient to cause the syndrome.

Since this preliminary study, oligogenic (triallelic and tetra-allelic) inheritance has been reported for *BBS1*, *BBS3*, *BBS4*, *BBS6*, *BBS7* and *BBS10* [24, 27, 31, 40]. Complex inheritance is also suggested by the identification of an excess of heterozygous BBS individuals where only one mutant allele was discovered, demonstrating that additional *BBS* gene mutations are necessary for manifestation of the disease phenotype [8, 25, 27, 29, 31, 39, 41, 42]. Nevertheless, it is important to note that only one of the above studies identified unaffected individuals homozygous for known pathogenic *BBS* gene mutations (i.e. M390R allele of *BBS1*) [27], indicating that an oligogenic model of disease causality may be overly simplistic. Indeed, a number of studies have presented data suggesting that most additional third and fourth BBS alleles function as modifiers of disease onset and penetrance. In this regard, a third mutation appears to exert an epistatic effect on two mutations at another BBS locus. Badano and colleagues [40] provided evidence that a single mutation in *BBS1*, *BBS2* or *BBS6* exerts an epistatic effect in individuals with two *BBS1* or two *BBS2* mutations, producing phenotypes of increased symptom severity and with earlier onset. Similarly, one of two affected sisters with homozygous *BBS1* M390R mutations suffered more severe symptoms presumably because of a third mutation in *BBS3* [24]. More recently, Badano and colleagues [43] identified a mutation in a non-*BBS* gene, MGC1203 (C430T), which appears to exert an enhanced effect on homozygous *BBS1* mutations in humans and zebrafish, resulting in a more severe phenotype. A functional link between MGC1203 and BBS proteins was demonstrated with the observation that *myc*-tagged MGC1203 immunoprecipitates all tested HA-tagged BBS proteins, and that endogenous MGC1203 colocalises with BBS proteins in tissues and cultured cells [43].

From the above studies, a number of conclusions can be drawn. First, it appears that BBS is most often inherited in a recessive manner. Indeed, for *BBS1*, greater than 80% of individuals display Mendelian inheritance [27], Second, *BBS2*, *BBS4* and *BBS6* appear to contribute most frequently to complex inheritance, with >70% of individuals possessing evidence of oligogenic inheritance [32]. Careful consideration of the data has led to a gradient model being proposed for BBS, where the penetrance, onset and severity of BBS are modified to varying extents by mutations at additional loci [32, 40]. At one end of the scale, two homozygous null mutations are sufficient to cause BBS, whereas at the other extreme, multiple mutations at multiple loci are required for phenotypic expression. In between, additional enhancer or suppressor mutations serve to modulate the phenotypic expressivity and/or penetrance of two *BBS* gene mutations at another locus. Although the evidence of complex inheritance for BBS is strong, the oligogenic model is still somewhat controversial. Indeed, two studies failed to detect any evidence of complex inheritance for BBS [28, 44], a finding that could possibly be explained by the small cohorts examined in these studies.

An important future goal will be to characterise the pathogenic contributions of individual alleles to the phenotypic expression of BBS. However, this will not be easily achieved for several reasons, including the fact that the full complement of *BBS* genes is not yet known. Elucidating the Mendelian and possible non-Mendelian characteristics of BBS is a most worthwhile endeavour, since it is hoped that the information gained will help to shed light on the inheritance mechanisms underlying truly complex disorders such as obesity and diabetes.

Basal body and/or cilia dysfunction underlies BBS

The first major breakthrough in understanding the molecular aetiology of BBS came in 2003, with the discovery that BBS is likely linked to dysfunction of basal bodies and/or cilia [22]. Specifically, Ansley and colleagues presented three major findings. First, they observed that a *myc*-epitope tagged BBS8 protein localised specifically at the centrosomes and basal bodies of cultured mammalian cells (Fig. 1a). Basal bodies are modified centriolar structures that direct the nucleation of the microtubule-based ciliary axoneme [45]. The BBS8 protein was also shown to interact with PCM1, a known basal body protein implicated in ciliogenesis [46]. Second, immunohistochemistry analyses demonstrated that the endogenous BBS8 protein localised within the ciliated cell layers of mice and human tissues, including the connecting cilium of the mouse retina and the ciliated columnar epithelial cells of human bronchial tissue. Lastly, four *Caenorhabditis elegans BBS* gene homologues (*bbs-1*, *bbs-2*, *bbs-7* and *bbs-8*) were shown to be expressed exclusively in cells possessing cilia (60 sensory neurons) (Fig. 2a), and to possess upstream X-box sequences that are known to bind the ciliogenic RFX transcription factor, DAF-19. These data were the first to substantiate the hypothesis by Rosenbaum and Witman [47] that BBS may be caused by ciliary dysfunction.

Shortly thereafter, a number of comparative genomics and bioinformatics studies provided further support for the cilia/basal body hypothesis for BBS. Comparative genomics revealed that *BBS* gene homologues are found only in the genomes of ciliated organisms, and not in those where cilia have been lost through evolutionary divergence. Specifically, by comparing the genomes of humans and the flagellated alga, *Chlamydomonas reinhardtii*, with that of the non-flagellated plant, *Arabidopsis thaliana*, a basal body and flagellar proteome of 688 genes was determined and found to contain most of the

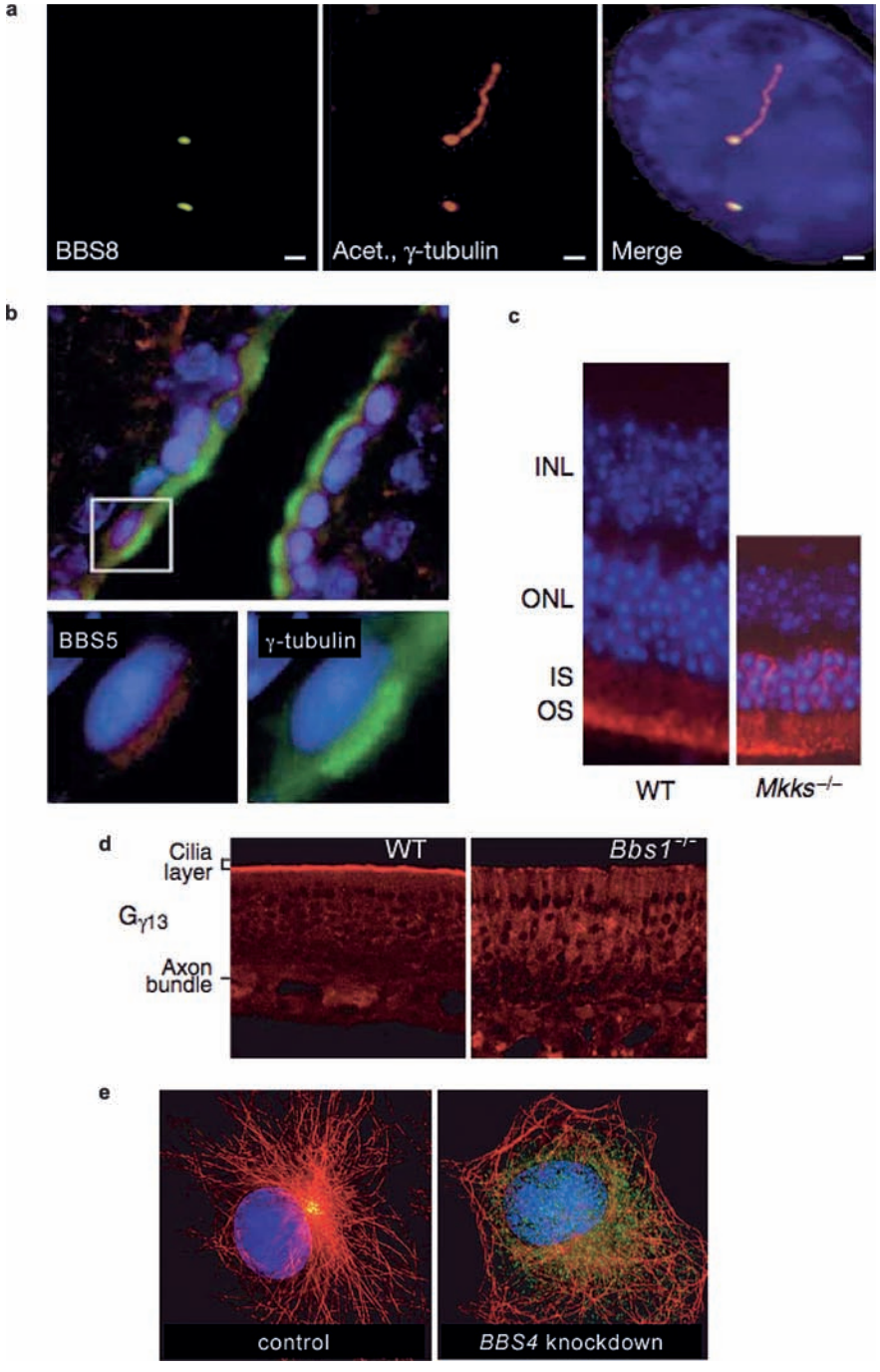

Figure 1. Mammalian BBS proteins localise near or at centriolar structures and are required for the proper localisation of basal body/ciliary proteins. (*a* and *b*) Mammalian BBS proteins localise at basal bodies and centrosomes. (*a*) In cultured mouse cells (i.e. ciliated NIH 3T3 fibroblast cells), BBS8 (green, left panel) localises at both the mother centriole (basal body emanating a primary cilium) and the daughter centriole (red; γ-tubulin staining), but not along the ciliary axoneme (red; acetylated α- tubulin staining) [22]. (*b*) In the ciliated cells of mouse tissue (i.e. the multi-ciliated ependymal cells that line the brain ventricles), BBS5 (red; lower left panel) co-localises with γ-tubulin (green; upper and lower right panels) at basal bodies [25]. The box in the upper panel indicates the region enlarged in the lower panels. (*c*) Rhodopsin mislocalisation in the photoreceptors of *Bbs6*$^{-/-}$ mice. In wild-type animals, rhodopsin (red) is confined to the outer segments (OS; modified ciliary structures), whereas in *Bbs6*$^{-/-}$ mice, rhodopsin is mislocalised, with a reduction of rhodopsin levels in the outer segments and additional signals within the inner segments (IS) and the outer nuclear layer (ONL) of retinal tissue [64]. (*d*) In mouse olfactory epithelium, the signaling protein Gγ13 (red) localises predominantly within the cilia layer of wild-type mice, whereas in *Bbs1*$^{-/-}$ animals, Gγ13 levels are highly reduced in the cilia layer, and mislocalised signals are observed within the cell bodies [61]. (*e*) Loss of *BBS4* gene function causes mislocalisation of PCM1 and defects in microtubule organisation. In control cells (left panel), PCM1 (green; both panels) localises predominantly at centrosomes and cytosolic microtubules (red; α-tubulin staining) display a distinct organisational pattern emanating from the microtubule organising center (MTOC). In *BBS4* RNAi knockdown COS-7 cells (right panel), PCM1 is dispersed throughout the cytosol and tubulin organisation from the MTOC is lost [56]. Figures reproduced with permission.

known *BBS* genes [25]. A separate study comparing the genomes of organisms with prototypical compartmentalised cilia (*Homo sapiens*, *Drosophila* and *C. elegans*) with those possessing non-compartmentalised or no cilia (*Saccharomyces cerevisiae*, *Arabidopsis*, *Dictyostelium*) led to the identification of approximately 200 genes required for prototypical cilia biogenesis, including at least four *BBS* genes [48]. Similar to that found in the study by Ansley and colleagues [22], bioinformatic screening for DAF-19-binding X-box sequences in *C. elegans* found that like known ciliogenic genes such as *osm-5* (IFT88), all of the nematode *bbs* genes possess canonical 14-bp X-box sequences in their upstream promoter regions [24, 25, 49, 50].

Further early evidence of a role for BBS proteins in cilia/basal body function came from the observation that BBS shares significant phenotypic overlap with other human disorders known to be firmly linked with defects in primary cilia function, including polycystic kidney disease (PKD), nephronophthisis, retinal dystrophy and *situs inversus*, and, recently, Senior-Loken syndrome and Meckel syndrome [47, 51–54]. Unlike their motile counterparts, primary cilia are non-motile and typically possess a 9+0 microtubule structure. Primary cilia are widely distributed in nature, being found on most mammalian cell types and cells of lower eukaryotes such as *C. elegans*. Research over the past decade has shown that primary cilia play very important sensory roles in olfaction, chemoreception, mechanosensation, photoreception and development [47, 51, 55].

Taken together, the above discoveries firmly established cilia/basal body dysfunction as an underlying cause of BBS. Over the last 3 years or so, numerous studies conducted in various experimental systems have built upon the initial findings and helped to further refine the genetic and cellular basis of BBS. Below follows a detailed account of the recent data, which corroborates the role of BBS proteins in cilia and basal body/centrosome processes, and strengthens the notion that these proteins serve as facilitators of intracellular trafficking systems.

BBS proteins are found within the ciliated cell layers of mammalian tissues, where they localise at basal bodies and centrosomes

Consistent with a cilia/basal body function, mammalian BBS proteins have been found to associate with ciliated tissues. Immunohistochemical analyses have revealed that BBS4 and BBS6 proteins are found preferentially within the ciliated border of renal tubules, the connecting cilium and inner/outer layers of retinal photoreceptors, as well as the ciliated epithelium of olfactory tissue [38, 56]. In addition, murine BBS5 and BBS6 was found within brain tissue cells, including the multi-ciliated ependymal cells that line brain ventricles [25, 38]. The probable involvement of BBS proteins at the embryonic node, which relies on cilia to specify left-right asymmetry in mammals [57, 58], is supported by in situ RNA hybridisation studies that demonstrate the expression of *BBS3*/*ARL6* in the ciliated embryonic node of early mouse embryos [59].

Immunolocalisation studies revealed that mammalian BBS proteins localise at the basal bodies and centrosomes of ciliated cells. As discussed above, BBS8 localizes to centrosomal and basal body structures in mammalian tissue culture cells (Fig. 1a) [22]. Mouse BBS5 was similarly detected at the basal bodies and centrosomes of ependymal cells, with perhaps very faint signals along the ciliary axonemes [25] (Fig. 1b). In human tissue cells, including ciliated IMCD3 cells, BBS4 and BBS6 proteins associate with both centrosomes and basal bodies, with additional signals for BBS6 at the midbody during cytokinesis [38, 56]. More precise immunolocalisation analysis of BBS6 using digital confocal microscopy found that BBS6 associates variably, and in a cell cycle-dependent manner, with the PCM tube structure that nearly envelops the centrosomal centrioles [38]. Interestingly, BBS4 does not localize to centrioles per se but rather exists in closely associated electron-dense structures termed centriolar satellites [56]. Notably, BBS4 and BBS6 proteins were not detected along ciliary axonemes, although they may be present there at low concentrations [38, 56].

Mouse models of BBS recapitulate the human phenotype

Null mouse models have thus far been presented for *BBS1*, *BBS2*, *BBS4* and *BBS6*, and in each case, the human BBS phenotype is at least partially recapitulated [60–64]. Phenotypes observed in the knockouts include obesity, retinal degeneration, kidney anomalies, neurosensory defects and behavioural problems.

Obesity

Although *Bbs* null mice are small in size at birth, they start to gain weight on their wild-type counterparts after weaning, becoming equal in size at 8–12 weeks, with many animals becoming obese in the weeks and months thereafter [60–64]. The increased weight of $Bbs^{-/-}$ mice is reported to be associated with increased centrally deposited adipose tissue [60, 62]. Interestingly, longitudinal feeding studies indicate that $Bbs^{-/-}$ animals eat more food (~10–20% more) than unaffected animals [60, 62, 63], a phenotype that may also be observed in human patients. $Bbs6^{-/-}$ mice were also shown to possess high arterial blood pressures and elevated serum hormone leptin levels [63]. Leptin is important in controlling long-term weight

regulation, and increased serum concentrations correlate with obesity in mice and humans [65].

Retinal degeneration

At 6–8 weeks, *Bbs* null mice display a moderate loss of the outer nuclear layer (ONL), with complete loss of photoreceptors at 7–10 months [60–64]. Photoreceptor loss appears to involve an apoptotic mechanism, since increased numbers of terminal transferase dUTP nick end labeling (TUNEL)-positive nuclei are seen in the ONL of 6-week-old *Bbs2*$^{-/-}$ and *Bbs4*$^{-/-}$ mice [60, 62].

Renal abnormalities

Although cystic kidney development is a primary feature of BBS patients, kidney defects have only been reported for *Bbs2*$^{-/-}$ mice. Specifically, two of three examined mice (5 months old) were found to possess bilateral multicystic kidneys [62].

Neurosensory defects

Most of the BBS mouse models have been reported as anosmic, as evidenced by an inability to find hidden food [62–64], or a reduced electro-olfactogram (EOG) response to odorant pulses [61]. Consistent with these observed olfactory defects in mice, a significant proportion of BBS patients present with anosmia. Kulaga and colleagues [61] found that 47% of BBS patients (n = 19) scored as fully or partially anosmic using the B-SIT 12-item smell identification test, and Iannacone and colleagues [66] identified anosmia in two BBS4 patients using the UPSIT 40-item smell test. In addition, *Bbs2*$^{-/-}$ and *Bbs6*$^{-/-}$ mice were found to possess decreased responses to acoustics and touch sensing [62–64].

Behavioural phenotypes

Bbs2$^{-/-}$, *Bbs4*$^{-/-}$ and *Bbs6*$^{-/-}$ mice were reported as more lethargic, less vocal to handling and possessing a significantly reduced level of social dominance [62, 63]. It appears that these behavioural abnormalities are not due to defects in muscle or lower motor neuron function [62, 63]. Interestingly, in humans, BBS is associated with numerous behavioural phenotypes, including withdrawn behaviour, anxious/depressed mood, thought disturbance and socialising problems [67]. In addition, it has also been reported that compared with non-affected obese controls, BBS patients may have a reduced level of physical activity, which could significantly affect energy requirements [68]. Since BBS patients do not appear to possess defects in energy metabolism, it has been suggested that the neurological (e.g. learning difficulties) and physical (e.g. retinal dysfunction) handicap components of BBS, in combination with various social and environmental factors, predisposes BBS patients to aberrant control of food intake or physical activity [68]. However, a physiological basis for disregulated appetite control (e.g. satiety sensation) cannot a priori be discounted.

Other phenotypes

A number of human BBS symptoms were never observed within the various mouse models, including limb abnormalities (e.g. polydactyly), hepatic cysts and organ laterality defects such as *situs inversus*. This indicates that the *Bbs* knockout mice may not phenocopy all of the ailments associated with human BBS patients. However, although polydactyly is often prevalent in patients (e.g. 58% reported by Green and colleagues [6]), the skeletal anomaly shows variable expressivity [69]. Similarly, *situs inversus* has only been reported for very few patients [22, 70].

Bbs null mice exhibit variable and subtle defects in cilia structure

Motile cilia

Numerous studies have shown that with the exception of sperm flagella, motile cilia structures are generally unaffected in *Bbs* null mice. TEM of tracheal epithelium in *Bbs* mice showed that motile cilia structures appeared normal, as defined by a typical 9+2 microtubule arrangement [60, 62]. Similarly, using anti-β-tubulin immunofluorescence staining, cultured tracheal epithelial cells from *Bbs6*$^{-/-}$ and wild-type mice were found to develop the same number of cilia, with indistinguishable morphologies [63]. Finally, acetylated α-tubulin staining of respiratory epithelial sections revealed that the motile cilia layer of *Bbs1*$^{-/-}$, *Bbs4*$^{-/-}$ and *Bbs6*$^{-/-}$ mice is grossly normal [61, 64]. A major exception to the above findings is that the motile flagellum of spermatozoa was found to be completely absent in *Bbs2*$^{-/-}$, *Bbs4*$^{-/-}$ and *Bbs6*$^{-/-}$ mice [60, 62, 63]. Interestingly, flagella were never detected in the seminiferous tubules of mice of different ages, suggesting that sperm flagella are never formed in the absence of BBS protein function [62, 63]. Consistent with these observations, scanning and transmission electron microscopy (SEM and TEM) analysis of semen samples from two adult male BBS2 patients revealed severe defects in the acrosome, nucleus and axonemal structures of sperm cells [62].

Non-motile primary cilia

The evidence to date indicates that loss of *Bbs* gene function in mice can cause defects in primary cilia structure, ranging from unaffected to grossly distorted. SEM of re-

nal tubular cells in *Bbs4*$^{-/-}$ and *Bbs6*$^{-/-}$ mice showed that primary cilia are normal in number, length and morphology [60, 63]. Similar observations were made in *Bbs2*$^{-/-}$ mice, with the exception that some of the renal tubular cell cilia appeared abnormally tapered [62].

In contrast, immunostaining of ciliary axonemes in the olfactory epithelium showed that *Bbs1*$^{-/-}$, *Bbs4*$^{-/-}$ and *Bbs6*$^{-/-}$ mice possess a severely reduced cilia layer, potentially indicative of short ciliary structures [61, 64]. In addition, the olfactory epithelial cell ciliary axonemes of *Bbs1*$^{-/-}$ and *Bbs4*$^{-/-}$ mice appear to be partially depleted of stable microtubules [61]. Interestingly, acetylated α-tubulin staining demonstrated that the dendritic microtubules are also distorted, indicating that the microtubule structure of the entire olfactory bulb is severely abrogated. This phenotype correlates with the mislocalisation of PCM1, a protein required for microtubule organisation, which was observed to be trapped within the olfactory epithelium cell bodies of *Bbs1*$^{-/-}$ mice and the dendritic knobs of *Bbs4*$^{-/-}$ mice [61]. Notably, anti γ-tubulin staining showed that the dendritic knob basal body structures appear to be present and properly positioned in *Bbs1*$^{-/-}$ and *Bbs4*$^{-/-}$ mice.

Finally, *Bbs* null mice exhibit defects in photoreceptor outer segments, which are modified primary cilia with higher-order structures, consisting of parallel stacks of membranous disks. Before the complete apoptotic loss of photoreceptors in 5-month-old *Bbs2*$^{-/-}$ mice, the outer segments were observed to be highly disorganised, with little evidence of any parallel stacks [62]. In addition, photoreceptor cell-connecting cilia were rarely observed in these mice [62]. Similarly, young *Bbs4*$^{-/-}$ and *Bbs6*$^{-/-}$ mice were reported to possess attenuated photoreceptor cell outer segments [60, 63, 64]. However, it is important to note that photoreceptor outer segment loss in *Bbs* null mice is also accompanied by an apoptotic disintegration of the entire cell and the underlying outer nuclear layer [60, 62–64]. Accordingly, it is difficult to discern the direct contribution of BBS protein function abrogation to photoreceptor cilia loss, since this phenotype may arise indirectly through upregulation of apoptotic pathways.

Ciliary proteins are mislocalised in *Bbs* null mice

Although *Bbs* null mice harbour potentially marginal defects in primary cilia structure, it is clear that loss of BBS protein function severely abrogates the ciliary localisation of several proteins, including photoreceptor rhodopsin and olfactory epithelium signaling molecules. Anti-rhodopsin staining of retinas revealed that in wild-type animals, rhodopsin localises almost entirely to the outer segments, whereas in 5-month-old *Bbs2*$^{-/-}$, *Bbs4*$^{-/-}$ and *Bbs6*$^{-/-}$ mice, significant mislocalisation of rhodopsin is observed in the photoreceptor cell inner segments and cell bodies before the apoptotic loss of photoreceptors [62–64] (Fig. 1c). The olfactory signaling protein adenyl cyclase III (AC III) localises very specifically to the ciliary layer of wild-type olfactory epithelium, whereas in *Bbs1*$^{-/-}$ and *Bbs4*$^{-/-}$ mice, this ciliary staining is reduced, and a fraction of AC III appears mislocalised to the apical dendrites [61]. Similarly, another olfactory signaling molecule normally found in cilia, $G\gamma_{13}$, was not found within the olfactory sensory cilia of *Bbs1*$^{-/-}$ mice, and was reduced in *Bbs4*$^{-/-}$ mice sensory cilia, displaying mislocalisation within the cell bodies and dendrites [61] (Fig. 1d). Finally, the lipid-raft protein SLP3 was depleted from the cilia layer and adjacent dendritic knobs of *Bbs1*$^{-/-}$ mice and *Bbs4*$^{-/-}$ mice [61]. Taken together, the above findings suggest that transport of ciliary proteins to cilia is defective in the photoreceptor and olfactory epithelium cells of *Bbs* null mice. The loss or reduction of opsin and ROM1 transport across the connecting cilium to the rod outer segment is also observed in the mouse intraflagellar transport hypomorphic mouse mutant IFT88/Polaris/*tg737*, and is thought to be the mechanism leading to apoptosis and retinal degeneration [71].

BBS genes function within PCP pathways

Planar cell polarity (PCP, non-canonical Wnt signaling) proteins play important roles in determining the cell polarity of many cell types, including the ommatidia of the fly eye and the hair cells of the mouse inner ear [72, 73]. Specifically, PCP proteins such as Vangl2 perform important functions in convergent extension processes during gastrulation [74]. A recent study by Ross and colleagues [64] has revealed an intriguing connection between PCP and BBS genetic pathways. *Bbs1*$^{-/-}$, *Bbs 4*$^{-/-}$ and *Bbs6*$^{-/-}$ mice display phenotypic overlap with various PCP mutants, including exencephaly and incomplete neural tube closure, open eyelids and disrupted cochlear stereociliary bundles within the inner ear [64]. In addition, *BBS1* and *BBS6* mutations were found to exert an enhanced effect on *Vangl2* mutations in mice and zebrafish, resulting in increased embryonic lethality and enhanced stereociliary bundle defects [64].

To investigate further the functional link between Vangl2 and BBS proteins, immunolocalisation analysis of Vangl2 protein in mouse IMCD3 kidney cells was conducted and revealed that this PCP protein localises at the base of cilia, similar to BBS proteins [64]. A connection between cilia function and PCP is further supported by the fact that in the middle ear, a single microtubule-based cilium structure, termed the kinocilium, lies adjacent to the actin-based cochlear stereociliary bundle during early development. Interestingly, SEM analysis demonstrated that although the kinocilium of *Bbs6*$^{-/-}$ mice is morpho-

logically intact, it appears to be placed further from the stereociliary bundle than in wild-type mice [64]. Since BBS protein function is required for basal body and ciliary localisation of various proteins such as rhodopsin [62–64] and PCM-1 [56], Ross and colleagues hypothesised that BBS protein function is also required for the proper ciliary localisation of PCP proteins such as Vangl2. To confirm this, future studies will need to formally examine the localisation patterns of PCP proteins in loss-of-function vertebrate *BBS* gene mutants.

Consistent with the BBS6 data, a very recent study has shown that loss of *BBS10* function in zebrafish embryos causes mild abnormalities in gastrulation movement (convergence and extension), such as shortening of the rostrocaudal body axis, dorsal thinning, and broadening and kinking of the notochord [18]. Interestingly, another basal body and ciliary protein, inversin, which is linked to organ laterality defects and cystic kidneys, is reported to act as a molecular switch between the canonical and non-canonical Wnt signaling pathways [75]. Taken together, the BBS6, BBS10 and inversin data provide strong evidence that cilia and basal body-associated functions play important roles in PCP pathways. Such roles may involve intracellular transport of PCP components to their sites of action, which in some cases may be at, or near, ciliary structures (e.g. Vangl2).

BBS4 plays a role in microtubule organisation

Kim et al. [56] employed yeast two-hybrid and coimmunoprecipitation analyses to show that human BBS4 interacts with both PCM1, a microtubule-organising protein [76], and p150^{glued}, a subunit of dynactin that serves to link dynactin with the dynein molecular motor [77]. PCM1 is an integral component of centriolar satellites, which are electron-dense structures located in proximity to centrosomes. This protein appears to play essential roles in coordinating microtubule organisation around the microtubule organising centre (centrosomes) by promoting the anchoring of microtubules [76]. Consistent with the observed interaction between PCM1 and BBS4, both of these proteins were found to co-localise at centriolar satellites [56]. Furthermore, it was observed that dynein is required for proper BBS4 and PCM1 localisation, since abrogation of dynein function (using p50 dynamitin overexpression) in HeLa cells resulted in a loss of BBS4 perinuclear localisation and abnormal diffuse cytosolic signals for PCM1 [56]. Finally, RNA interference (RNAi) knockdown of *BBS4* resulted in the cytosolic dispersal of PCM1 in HeLa, HEK293 and COS-7 cells, and a loss of centrosomal microtubule anchoring in COS-7 cells [56] (Fig. 1e). Taken together, the above data suggest that BBS4 functions in dynein-mediated intracellular transport as an adaptor of dynactin, which serves to recruit PCM1 and potentially other associated cargo to the centriolar satellites [56].

Coimmunoprecipitation experiments in HEK293 cells subsequently revealed that BBS4 interacts with two Prader-Willi syndrome (PWS)-linked proteins, nectin and Fez1 [78]. Consistent with these observations, all three proteins were observed to co-localise in proximity to centrosomes. PWS is a pleiotropic disorder characterised by a multitude of symptoms, including neonatal hyptonia, global developmental delay and hyperphagia [78]. PWS also exhibits significant phenotypic overlap with BBS, with common symptoms including obesity, learning disabilities and hypogonadism. Analysis of nectin null mice revealed that these animals possess neural and axonal outgrowth defects. The authors conclude that nectin's function in mediating the cytoskeletal rearrangements required for neural outgrowth and extension is closely linked to important centrosome-associated activities, including axonal transport (Fez1) and microtubule organisation (BBS4) [78].

Loss of BBS6 function causes centrosomal and cytokinesis defects

Unlike BBS4, the centrosomal localisation of BBS6 in IMCD3 cells is independent of intact microtubules and dynein motor function, and RNAi knockdown of *BBS6* in COS-7 and NIH3T3 cells does not affect the organisation of microtubules [38]. Instead, silencing of *BBS6* transcripts produces three specific phenotypes, namely cytokinesis abnormalities (unresolved intracellular bridges), centrosomal deficiencies (abnormal number of centrosomes) and nuclear defects (bi- and multi-nucleated cells) [38]. Interestingly, RNAi knockdown of *BBS4* in HeLa cells also produced cell division abnormalities, including cells with multiple nuclei and abnormal numbers of centrosomes, but cells did not normally progress up to telophase/cytokinesis, as did the BBS6-depleted cells [56].

Although the group II chaperonin-like sequence homology of BBS6 suggests a role in protein folding, the BBS6 protein sequence has diverged significantly from other chaperonins, resulting in the possible loss of a functional ATP hydrolysis domain that is conserved in all chaperonins. In addition, cytosolic BBS6 does not appear to oligomerise in the manner observed for all chaperonins [38]. These observations suggest that BBS6 is perhaps not a bona fide chaperonin and that the putative centrosomal and intracellular bridge-resolving functions of BBS6 may not entail protein folding mechanisms [38]. Interestingly, the newly discovered BBS10 chaperonin-like protein is even more divergent, suggesting that both the BBS6 and BBS10 proteins have evolved new functions.

C. elegans bbs genes function within intraflagellar transport pathways

With the exception of *BBS6*, *BBS10* and *BBS11*, the remaining eight known human *BBS* genes have strong homologues in *C. elegans*, indicating that *BBS* gene function is conserved in lower organisms. Similar to that observed for mammalian *BBS* genes, all eight nematode *bbs* genes are expressed exclusively in the ciliated subset of cells [22, 24, 25; unpublished observation for *bbs-4*] (Fig. 2a). In *C. elegans*, 302 of the ~1000 adult hermaphrodite cells are neuronal, and of these, 60 possess ciliary structures that extend from the dendritic endings. Ciliated cells in the nematode primarily serve sensory (e.g. chemosensory) functions, with many of the ciliary structures having access to the outside environment via pore structures (sensillae) in the nematode cuticle [79]. Similar to mouse and human BBS proteins, green fluorescent protein (GFP)-tagged *C. elegans* BBS proteins are enriched at the base of cilia (basal bodies), which are also called transition zones (Fig. 2b). Unlike what has been observed for vertebrate BBS proteins, however, the nematode BBS::GFP proteins also localise along the entire length of ciliary axonemes [24, 49] (Fig. 2b). Furthermore, when live worms expressing GFP-tagged BBS proteins were examined using time-lapse video microscopy, fluorescent BBS::GFP-associated particles were observed to undergo intraflagellar transport [24, 49, 80; unpublished observations for BBS-4 and BBS-5] (Fig. 2c).

Intraflagellar transport (IFT) was initially described in *Chlamydomonas* flagella as a bidirectional motility of protein complexes, required to deliver various cargo for building and maintaining the structure and function of cilia and flagella [47, 51, 81]. Anterograde IFT (basal body to distal tip) is driven by microtubule plus-ended kinesin-2 motors, whereas retrograde IFT (distal tip back to basal body) recycles the IFT machinery and probably also other components by way of the IFT-dynein motor [47, 51]. Associated with the IFT motor complexes is the IFT particle, which is biochemically characterised by two distinct subcomplexes, A and B, consisting of at least 6 and 11 polypeptides, respectively [82, 83]. Motor-IFT particle assemblies constitute core IFT machinery, and disruption of any one component leads to severe abrogation of IFT, and consequently defects in cilia structure and function [47, 51]. Genetic studies in *Chlamydomonas* and *C. elegans* have determined that IFT subcomplex B mutants have severely abrogated or no ciliary structures, with the core IFT machinery excluded from the stumpy ciliary axonemes. These observations indicate that subcomplex B components play important roles in anterograde transport [47, 51]. In contrast, IFT subcomplex A mutants possess moderately truncated cilia, yet contain large accumulations of core IFT machinery, indicating that subcomplex A proteins likely function within retrograde IFT [47, 51].

Similar to IFT mutants, the loss of *C. elegans bbs-7* and *bbs-8* gene function was found to compromise the structure and function of sensory cilia [49]. Specifically, *bbs* mutant cilia are moderately truncated and possess defective chemosensory functions [49]. These findings are consistent with studies in *Chlamydomonas*, which showed that RNAi knockdown of *BBS5* produced algae with either no flagella or short flagella [25]. Furthermore, IFT was compromised in *C. elegans bbs* mutants: the numbers of transport events observed were reduced, and core IFT components often accumulated within the truncated ciliary axonemes [49; and see below]. Interestingly, these *bbs* mutant phenotypes are less severe than those observed for IFT particle mutants, indicating that in *C. elegans*, BBS proteins likely play a subtle role in IFT, as modulators of the core IFT machinery (see below). To date, IFT has not been demonstrated for mammalian BBS proteins. Indeed, mammalian BBS proteins have not been observed within ciliary axonemes, although as with *C. elegans*, they accumulate at basal bodies (and centrosomes). Although the overall transport-associated functions of BBS proteins in *C. elegans* and mammals are likely to be evolutionarily conserved, it will be of great interest to determine whether this BBS machinery has been largely co-opted for IFT in worms and whether the main functions of the mammalian BBS proteins are to support intracellular transport processes in the cell body.

Functional coordination of IFT motors by BBS proteins in *C. elegans*

Unlike *Chlamydomonas*, where only one anterograde IFT pathway via the canonical heterotrimeric kinesin-2 motor has been described, work by Snow and colleagues [84] demonstrated that *C. elegans* sensory cilia are built via two anterograde IFT pathways, with each pathway building distinct segments of the ciliary axoneme. Construction of ciliary middle segments (extending from the basal body) requires the cooperative actions of two kinesin-2 motors, kinesin-II and OSM-3-kinesin, whereas the building of distal segments (extending from the tips of middle segments) is driven solely by the homodimeric kinesin OSM-3 [84] (Fig. 2c). *C. elegans* middle segments are built of doublet microtubules, as with other 'canonical' ciliary axonemes, but interestingly, the nematode distal segments consist of singlet microtubules. This bipartite ciliary structure does not appear to be unique to *C. elegans*, as distal ciliary segments are found in other organisms. For example, the transition of outer doublet microtubules to singlet microtubules at the distal end is observed for flagella of mating *Chlamydomonas* cells as well as for sensory cilia of several vertebrate cell types, including pancreatic, renal and olfactory cells [85–88].

Examination of anterograde IFT in *bbs* mutants revealed that BBS proteins are required to functionally coordinate the two kinesin-2 motors that drive IFT in the middle segment. Using *in vivo* time-lapse microscopy-based IFT assays developed by Jonathan Scholey's laboratory at UC Davis [84, 89, 90], it was found that GFP-tagged kinesin-II and OSM-3-kinesin are separated within the middle segments of *bbs* mutant cilia, moving at their corresponding slow (~0.5 μm/sec) and fast (1.3 μm/sec) velocities, respectively [80] (Fig. 2c). This is in contrast to the middle segments of wild-type cilia, where the kinesin-2 motors function cooperatively, resulting in an intermediate velocity (~0.7 μm/sec) for both of these motors [84] (Fig. 2c). Furthermore, it was found that fluorescence-tagged IFT particle subcomplex A and B components are also separated within *bbs* mutant cilia, with subcom-

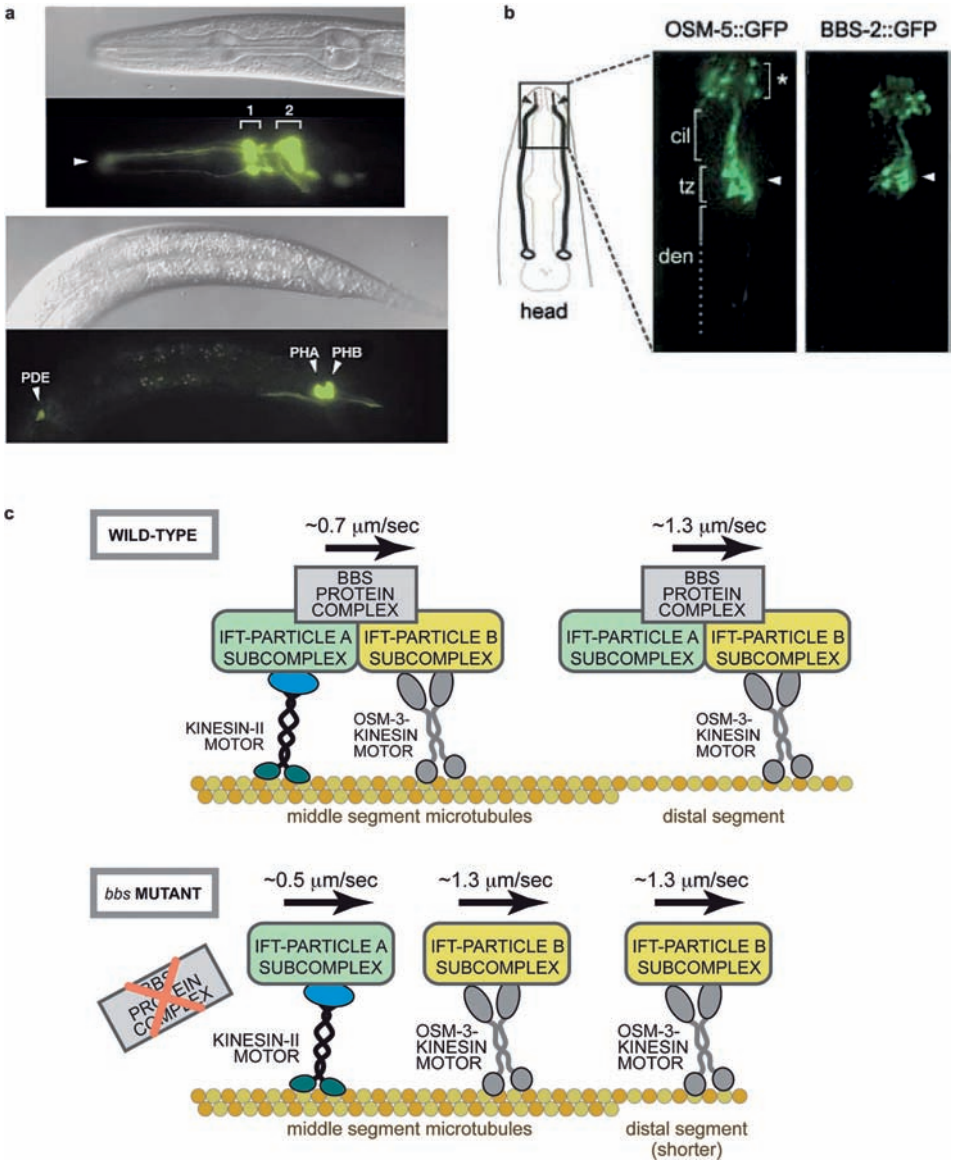

Figure 2. *C. elegans* BBS proteins undergo intraflagellar transport (IFT) and stabilise the anterograde IFT machinery. (*a*) In the nematode, *bbs* genes are expressed exclusively in cells with cilia, including the labial quadrant (1) and amphid (2) neurons in the head (upper panels), and the phasmid (PHA, PHB) and PDE neurons in the tail (lower panels) [22]. Shown are DIC and fluorescence images of a worm expressing a transcriptional GFP reporter to *bbs-8*. (*b*) Similar to IFT particle proteins (e.g. OSM-5), *C. elegans* BBS proteins (green) localise at the base of cilia (also called transition zones; tz; arrowhead) and along ciliary axonemes (cil), with little or no staining along the dendrite structures (den) [49]. Shown are one set of amphid neuronal cilia in the worm head. The asterisk denotes the ciliated endings of neurons found at the anterior end of the worm (e.g. IL1/2, OLL, CEP and BAG neurons). (*c*) Like IFT particle subcomplex proteins, BBS proteins are transported along wild-type ciliary middle segments at an intermediate rate (~0.7 μm/sec) by both heterotrimeric kinesin-II and homodimeric OSM-3-kinesin, and along distal segments at a fast rate (~1.3 μm/sec) by OSM-3-kinesin alone [80]. Loss of BBS protein function causes the separation of both kinesin-2 motors and IFT particle subcomplexes A and B, resulting in subcomplex A moving at a slow rate (~0.5 μm/sec) with heterotrimeric kinesin-2, and subcomplex B moving at a fast rate (~1.3 μm/sec) with OSM-3-kinesin [80]. Figures reproduced with permission.

plex A proteins moving at kinesin-II-associated velocities (~0.5 μm/sec) and subcomplex B proteins moving at OSM-3-kinesin-associated velocities (~1.3 μm/sec) (Fig. 2c). Together, these data indicate that *C. elegans* BBS proteins are required to stabilise the molecular associations between kinesin-II motors and IFT particle subcomplexes. Moreover, these observations suggest that within the macromolecular architecture of motor-IFT particle assemblies, subcomplex A is likely positioned close to the kinesin-II motor, whereas subcomplex B is placed close to the OSM-3-kinesin motor (Fig. 2c). Thus, the specific functional consequence of disrupting BBS protein function is that various IFT subcomplex A and B components are not transported correctly in the cilium and accumulate in the dendrite leading up to the base of the cilium, at the basal body (transition zone), at the midpoint between the middle and distal segments, and at the tip of the cilium [49].

Loss of zebrafish *BBS* gene function abrogates retrograde intracellular trafficking and disrupts Kupffer's vesicle cilia function

Direct roles for BBS proteins in cilia biogenesis and intracellular trafficking have recently been observed in *Danio rerio* (zebrafish). Using a morpholino-based approach to suppress the expression of zebrafish *bbs2*, *bbs4*, *bbs5*, *bbs6*, *bbs7*, *bbs8* and *bbs11*, two studies have found that morphant embryos have defects in the Kupffer's vesicle (KV) [19, 91]. This transient monocilia-containing spherical structure, which arises during gastrulation, is proposed to function comparably to the mouse node in determining left-right (l-r) asymmetry [92]. Specifically, 16–38% of *bbs* morphants have Kupffer's vesicles that were either absent altogether or possessed a severely reduced diameter [19, 91]. Unlike control morphants, where KV cilia length and number increase during development, *bbs* morphant KV cilia were observed to be reduced in number, shortened and occupied a diminishing KV space [91]. In addition, two genes important for establishing l-r asymmetry, *lefty-1* and *lefty-2*, were found to be misexpressed within the lateral plate mesoderm of *bbs6* and *bbs7* morphants, with 36% of animals displaying bilateral plate expression and 9% of animals exhibiting reversed right-sided plate expression [91]. Furthermore, 5–30% of *bbs* morphant embryos are predisposed to organ laterality defects, as evidenced by abnormal heart jogging and looping, and the frequency of these deficiencies correlated with the severity of KV defect, indicating that KV abnormalities are linked to altered organ laterality [91].

On account of the putative role of BBS proteins in intracellular transport pathways, intracellular trafficking was analysed in 5 day post fertilisation *bbs* morphant larvae using an established assay that assesses the microtubule-dependent redistribution of melanosome pigment organelles within melanophore cells. Using this system, all *bbs* morphants were found to possess defects in microtubule minus-end-directed retraction of melanosome pigments, indicating abnormalities in dynein-driven retrograde trafficking [19, 91]. Melanosome pigment retraction in *bbs* morphants was either slowed 2–4 times compared with control animals or failed to complete [19, 91] (Table 1). In contrast, microtubule plus-end-directed recovery to full pigment dispersal was normal in *bbs* morphants, indicating that loss of *bbs* function does not affect anterograde kinesin-2-driven melanosome trafficking [91]. The *bbs* morphant trafficking defect was phenocopied in wild-type animals treated with brefeldin A, which is a fungal metabolite that predominantly abrogates retrograde vesicle and organelle trafficking. Taken together, these zebrafish data indicate that BBS proteins play important organelle trafficking functions which are entirely consistent with the intracellular transport roles suggested by previous studies in *C. elegans* [49, 80], mice [60–64] and human tissue culture cells [38, 56].

Table 1. Loss of zebrafish BBS protein function slows retrograde trafficking of melanosome pigments. Using a morpholino (MO)-based approach, knockdown of *bbs* gene function delays retrograde trafficking of melanosomes (in zebrafish melanophore cells) from the cell periphery to the perinuclear region [19, 91]. In contrast, anterograde trafficking rates of melanosomes back to the cell periphery is indistinguishable in both the control and *bbs* morphant fish [91].

Treatment	Retrograde response time (min)	Anterograde response time (min)
Wild-type	1.7	4.1
Control – MO	1.6	–
bbs2 – MO	3.6	4.5
bbs4 – MO	3.5	4.3
bbs5 – MO	7.2	–
bbs6 – MO	3.3	4.5
bbs7 – MO	6.6	4.0
bbs8 – MO	4.3	–
bbs11 – MO	3.0	–

C. elegans bbs gene function regulates lipid storage

One of the most intriguing aspects of BBS, along with the Alström and MORM syndromes [9, 93], is an obesity phenotype that does not appear to be manifested in other 'classical' ciliopathies. A recent study by Mak and

colleagues [94] identified a genetic association between *bbs-1* and two conserved genes linked to lipid homeostasis and obesity, namely *kat-1*, a 3-ketoacyl-coenzyme A (coA) intestinal gene involved in fatty acid oxidation, and *tub-1*, a ciliated cell-specific gene whose loss of function causes obesity, retinal degeneration and hearing loss in mammals [95, 96]. Similar to *kat-1* single mutants, loss of *bbs-1* or *tub-1* function produced moderate levels of lipid accumulation within *C. elegans* intestinal cells. Consistent with a synergistic association, fat storage was dramatically increased in *kat-1;bbs-1* and *kat-1;tub-1* double mutants [94], a finding that was also observed for double mutants of *kat-1* and mutations in IFT particle subcomplex components such as *che-2* and *osm-5*. Transgenic expression of *bbs-1* within the ciliated neurons expressing *tax-4* in *kat-1;bbs-1* mutants suppressed the enhanced lipid accumulation phenotype, indicating that this lipid homeostasis pathway is regulated by a group of 15 cells which possess the potential to sense external (amphid neurons) and internal (PQR/AQR neurons) nutrient levels. Given these findings, the authors propose a model whereby ciliary genes such as *bbs-1* and *tub-1* form part of a neuroendocrine feedback loop that controls lipid homeostasis within intestinal cells. Based on the apparent roles of BBS proteins in intracellular transport, it is possible therefore that in humans, dysregulation of food consumption and obesity could arise by the mistrafficking of important receptors – for example, the cilia-localised 5-HT(6) serotonin receptor [97] – implicated in the modulation of energy metabolism [98].

Summary and conclusions

In the remarkably short time frame of 3 years, studies on Bardet-Biedl syndrome, a previously understudied rare genetic disorder, have provided significant insights into a multigene family of proteins implicated in a surprising range of human ailments, including obesity, retinal degeneration and cystic kidneys. As discussed below, the research findings have led us to conclude that BBS proteins function together, possibly as an oligomeric complex, in intracellular transport processes that are relevant to cilia function, microtubule organisation, cell division and PCP pathways.

BBS belongs to a growing list of human ciliopathies

BBS is a member of a growing number of human disorders caused by dysfunction of cilia and/or centrosomes or basal bodies. For some time now, it has been known that defects in cilia function underlie immotile cilia syndrome, polycystic kidney disease, *retinitis pigmentosa* and organ laterality disorders such as *situs inversus* (reviewed in [47, 51]). However, over the past few years, the number of developmental diseases and disorders linked to cilia dysfunction has grown further to include nephronophthisis [99], Joubert syndrome [100], hydrocephalus [101], Alström syndrome [93], Senior-Loken syndrome [52] and Meckel syndrome [53, 54]. Together, these findings underscore the diverse and important roles that cilia play during development and indicate that ciliary defects may contribute to relatively common phenotypes such as mental retardation (BBS, Joubert syndrome), obesity (Bardet-Biedl, Alström and MORM syndromes) and hypertension (BBS).

BBS proteins may form heteromeric complexes

Numerous studies indicate that *BBS* genes function within the same or parallel genetic pathways. First, BBS patients generally display almost identical phenotypes, regardless of the particular *BBS* gene mutated. Second, loss of *BBS* gene function in mice, *C. elegans* and zebrafish produces overlapping phenotypes [49, 60–64, 80, 91]. Third, mammalian BBS proteins co-localise at centrosomes and basal bodies. Fourth, the *C. elegans* BBS proteins all undergo IFT [24, 49], suggesting at the very least, close physical association of BBS components in moving IFT protein complexes. Together, these observations suggest that some, if not all, BBS proteins form part of large heteromeric complexes. Consistent with this notion is the recent finding that the protein product of MGC1203, a vertebrate-specific genetic modifier of BBS, immunoprecipitates no fewer than seven BBS proteins [43].

A single functional BBS complex could explain the genetics of BBS, such as its complex inheritance patterns in humans and the epistatic relationships of *BBS* genes in zebrafish [43, 91]. Although oligogenic inheritance is apparent for many *BBS* genes, very few unaffected individuals homozygous for pathogenic null mutations have been identified. In the context of a hypothetical BBS complex, this is perhaps not surprising, since the complete loss of any one BBS protein would likely have dire functional consequences on the entire BBS complex. Indeed, support of the latter has been observed in *C. elegans* null mutant models of BBS, where no enhancement of the phenotype (defects in cilia structure and IFT) is observed in double mutants versus single mutants [49]. On the contrary, in situations where *BBS* gene function is predicted to be reduced but not completely abolished (e.g. zebrafish morphants and missense/hypomorphic mutations in mice and humans [43, 91]), such alleles would be expected to exert varying effects on the integrity of BBS protein complexes, leading ultimately to subtle epistatic increases or decreases in the overall function of the complex.

BBS protein function is required to maintain cilia structure and function

As discussed in this review, loss of BBS protein function impacts variably on cilia structure. Data from the various BBS animal models have shown that many types of cilia are relatively unaffected (e.g. mouse renal tubular cell cilia, motile cilia of respiratory epithelial cells [60–64]), others are moderately affected (e.g. truncated sensory cilia in *C. elegans* [49], shortened olfactory epithelial cell cilia in mice [61], truncated Kupffer's vesicle cilia in zebrafish [91]) and others still are severely affected (e.g. complete absence of motile flagella on mouse spermatozoa [60, 62, 63] and aflagellar *Chlamydomonas* [25]). With the exception of sperm flagella, it can be concluded that BBS proteins are not generally required to form cilia in mammals. Instead, it would appear that in certain cases, in particular non-motile primary cilia, BBS protein function is necessary to build wholly intact and fully functional ciliary structures. This notion is supported by the fact that severely disrupting cilia function, as is the case with the IFT88/Polaris/*tg737* loss-of-function mouse, results in a lethal phenotype [102]. Accordingly, it would seem that partially affected BBS mutant ciliary structures retain enough function to ensure survival of the organism. The partial or complete loss of spermatozoan flagella in BBS mice [60, 62, 63] and humans [62] indicates that the molecular and genetic pathways underlying sperm flagella biosynthesis may not be identical to the IFT pathways that drive canonical cilia biogenesis. Although IFT almost certainly builds these specialized flagellar structures, subtle alterations to the pathways have likely defined more critical roles for BBS proteins in sperm flagellar biosynthesis. Alternatively, important non-IFT roles for the BBS proteins that are required for the differentiation of spermatozoa could be affected.

Concomitant with the variable morphological ciliary defects observed in animal models of BBS are numerous cilia-associated molecular abnormalities. These include mislocalisation of ciliary signaling proteins in mice [61–64] and IFT components in worms [49, 80], absence of stable microtubules in mammalian olfactory epithelial cell cilia [61], uncoordinated kinesin-2 motors in worms [80] and centrosomal/basal body abnormalities such as defects in microtubule organisation in human tissue culture cells [38, 56]. Taken together, all of the available data clearly indicate that BBS protein function is required for the normal structure and function of cilia.

BBS proteins play important roles in PCP pathways

The discovery that loss of *BBS6* and *BBS10* function is linked to PCP anomalies, including neural tube defects and cochlear stereociliary bundle abnormalities [18, 64], provides an intriguing explanation for some of the developmental phenotypes of BBS. These findings are especially interesting in light of some very recent observations demonstrating the association between cilia function and wnt signaling. First, it was shown that inversin, a basal body/ciliary protein associated with cystic kidneys and retinitis pigmentosa [99, 103], lies at a critical junction of wnt signaling, where it appears to act as a molecular switch between the canonical and non-canonical branches of this signaling cascade [75]. In the current model, it is proposed that fluid flow-induced bending of primary cilia in the developing renal tubules triggers non-canonical Wnt signaling (via inversin), leading ultimately to changes in gene expression and the control of apical-basolateral polarity in renal tubular epithelial cells [75, 104]. Second, in *Xenopus* embryos, it was shown that PCP components, Dishevelled and Inturned, are required for cilia biogenesis and Hedgehog signaling [105]. Indeed, these findings serve to consolidate previous studies, which found that ciliogenic intraflagellar transport pathway components are required for normal Hedgehog signaling in vertebrates [106–110]. Finally, the observation that key components of PCP, namely Inversin, Vangl2, Dishevelled and Inturned, all localise at or near basal bodies and/or cilia [64, 105, 111] provides further support for the notion that primary cilia-basal body-centrosomal components play central roles in conducting PCP signaling.

Overall, the above findings indicate that any molecular process that abrogates cilia structure and function (e.g. fluid flow sensing) has the potential to disrupt important developmental processes such as PCP. The challenge for future BBS research will therefore be to further define the specific molecular mechanisms by which BBS proteins function, especially their putative roles in intracellular transport, and how these functions impact upon cilia and basal-body/centrosome-dependent developmental signaling pathways.

A common role for BBS proteins in microtubule-based intracellular transport

In summary, BBS protein function is associated with (i) PCP in vertebrates, (ii) intraflagellar transport and coordination of kinesin-2 motors in worms, (iii) retrograde organelle trafficking in zebrafish and (iv) microtubule organisation and anchoring in mammalian cells. As described above, all of these observations can be explained by a role for BBS proteins in microtubule-based intracellular transport. A direct role in molecular transport pathways has been demonstrated in *C. elegans* [49, 80] and zebrafish [91]. In addition, the centrosomal abnormalities observed in the mammalian BBS tissue culture models (e.g. aberrant microtubule anchoring, cytokinesis defects, multiple centrosomes [38, 56]) are proposed to arise from mistrafficking of important centrosomal/basal body proteins such as PCM1 [56]. Furthermore, intracel-

lular trafficking defects would explain the inability of photoreceptor pigments (e.g. rhodopsin) and olfactory epithelial cell ciliary proteins (e.g. AC III) to localise at ciliary structures in vertebrate models of BBS. Finally, the ciliary localisation of developmental signaling components such as Smoothened (hedgehog signaling) and possibly Vangl2 (non-canonical wnt signaling) may be a requirement for their function [107]. Accordingly, a general role in intracellular transport for BBS proteins would ensure the proper ciliary localisation of various wnt and hedgehog signaling components, and therefore, normal embryonic development. The challenge is now to understand in precise molecular terms how the various BBS proteins help to modulate intracellular transport processes, and how disruption of these functions leads to the observed pleiotropic phenotypes of this multigenic syndrome [112, 113]. Such insights will undoubtedly help to shed light not only on BBS but also on the molecular basis of important developmental processes in vertebrates and the possible pathomechanisms of a multitude of different disorders.

Acknowledgments. M.R.L. acknowledges support from the Heart and Stroke Foundation of B.C. & Yukon and scholar awards from the Canadian Institutes of Health Research (CIHR) and Michael Smith Foundation for Health Research (MSFHR). O.E.B. received fellowships from CIHR and MSFHR.

1 Laurence, J. Z. and Moon, R. C. (1866) Four cases of retinitis pigmentosa occurring in the same family and accompanied by general imperfection of development. Opthal. Rev. 2, 32–41.
2 Bardet, G. (1920) Sur un syndrome d'obesité congénitale avec polydactylie et rétinite pigmentaire (contribution a l'étude des formes cliniques de l'obesité hypophysaire). Thèse de Paris (Le Grand) 470, 107.
3 Biedl, A. (1922) Ein Geschwister mit adiposogenitaler Dystrophie. Dtsch. Med. Wochenschr. 48, 1630.
4 Beales, P. L., Eleioglu, N., Woolf, A. S., Parker, D. and Flinter, F. A. (1999) New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey. J. Med. Genet. 36, 437–446.
5 Moore, S. J., Green, J. S., Fan, Y., Bhogal, A. K., Dicks, E., Fernandez, B. A., Murphy, C., Cramer, B. C., Dean, J. C., Beales, P. L. (2005) Clinical and genetic epidemiology of Bardet-Biedl syndrome in Newfoundland: a 22-year prospective, population-based, cohort study. Am. J. Med. Genet. 132A, 352–360.
6 Green, J. S., Parfrey, P. S., Harnett, J. D., Farid, N. R., Cramer, B. C., Johnson, G., Heath, O., McManamon, P. J., O'Leary, E. and Pryse-Phillips, W. (1989) The cardinal manifestations of Bardet-Biedl syndrome, a form of Lawrence-Moon-Bardet-Biedl syndrome. New Engl. J. Med. 321, 1002–1009.
7 Katsanis, N., Lupski, J. R. and Beales, P. L. (2001) Exploring the molecular basis of Bardet-Biedl syndrome. Hum. Mol. Genet. 10, 2293–2299.
8 Karmous-Benailly, H., Martinovic, J., Gubler, M.-C., Sirot, Y., Clech, L., Ozilou, C., Auge, J., Brahimi, N., Etchevers, H., Detrait, E. et al. (2005) Antenatal presentation of Bardet-Biedl syndrome may mimic Meckel syndrome. Am. J. Hum. Genet. 76, 493–504.
9 Hampshire, D. J., Ayub, M., Springell, K., Roberts, E., Jafri, H., Rashid, Y., Bond, J., Riley, J. H. and Woods, C. G. (2006) MORM syndrome (mental retardation, truncal obesity, retinal dystrophy and micropenis), a new autosomal recessive disorder, links to 9q34. Eur. J. Hum. Genet. 2006 14(5), 543–548.
10 Klein, D. and Ammann, F. (1969) The syndrome of Laurence-Moon-Bardet-Biedl and allied diseases in Switzerland. Clinical, genetic and epidemiological studies. J. Neurol. Sci. 9, 479–513.
11 Croft, J. B., Morrell, D., Chase, C. L. and Swift, M. (1995) Obesity in heterozygous carriers of the gene for the Bardet-Biedl syndrome. Am. J. Med. Genet. 55, 12–15.
12 Beales, P. L., Warner, A. M., Hitman, G. A., Thakker, R. and Flinter, F. A. (1997) Bardet-Biedl syndrome: a molecular and phenotypic study of 18 families. J. Med. Genet. 34, 92–98.
13 Farag, T. I. and Teebi, A. S. (1989) High incidence of Bardet-Biedl syndrome among the Bedouin. Clin. Genet. 36, 463–464.
14 Katsanis, N., Beales, P. L., Woods, M. O., Lewis, R. A., Green, J. S., Parfrey, P. S., Ansley, S. J., Davidson, W. S. and Lupski, J. R. (2000) Mutations in MKKS cause obesity, retinal dystrophy and renal malformations associated with Bardet-Biedl syndrome. Nat. Genet. 26, 67–70.
15 Slavotinek, A. M., Stone, E. M., Mykytyn, K., Heckenlively, J. R., Green, J. S., Heon, E., Musarella, M. A., Parfrey, P. S., Sheffield, V. C. and Biesecker, L. G. (2000) Mutations in MKKS cause Bardet-Biedl syndrome. Nat. Genet. 26, 15–16.
16 Nishimura, D. Y., Searby, C. C., Carmi, R., Elbedour, K., Van Maldergem, L., Fulton, A. B., Lam, B. L., Powell, B. R., Swiderski, R. E., Bugge, K. E. et al. (2001) Positional cloning of a novel gene on chromosome 16q causing Bardet-Biedl syndrome (BBS2). Hum. Mol. Genet. 10, 865–874.
17 Mykytyn, K., Braun, T., Carmi, R., Haider, N. B., Searby, C. C., Shastri, M. Beck, G., Wright, A. F., Iannaccone, A., Elbedour, K. et al. (2001) Identification of the gene that, when mutated, causes the human obesity syndrome BBS4. Nat. Genet. 28, 188–191.
18 Stoetzel, C., Laurier, V., Davis, E. E., Muller, J., Rix, S., Badano, J. L., Leitch, C. C., Salem, N., Chouery, E., Corbani, S. et al. (2006) *BBS10* encodes a vertebrate-specific chaperonin-like protein and is a major BBS locus. Nat. Genet. 38, 521–524.
19 Chiang, A. P., Beck, J. S., Yen, H. J., Tayeh, M. K., Scheetz, T. E., Swiderski, R. E., Nishimura, D. Y., Braun, T. A., Kim, K. Y., Huang, J. et al. (2006) Homozygosity mapping with SNP arrays identifies TRIM32, an E3 ubiquitin ligase, as a Bardet-Biedl syndrome gene (*BBS11*). Proc. Natl. Acad. Sci. USA 103, 6287–6292.
20 Mykytyn, K., Nishimura, D. Y., Searby, C. C., Shastri, M., Yen, H. J., Beck, J. S., Braun, T., Streb, L. M., Cornier, A. S., Cox, G. F. et al. (2002) Identification of the gene (BBS1) most commonly involved in Bardet-Biedl syndrome, a complex human obesity syndrome. Nat. Genet. 31, 435–438.
21 Badano, J. L., Ansley, S. J., Leitch, C. C., Lewis, R. A., Lupski, J. R. and Katsanis, N. (2003) Identification of a novel Bardet-Biedl syndrome protein, BBS7, that shares structural features with BBS1 and BBS2. Am. J. Hum. Genet. 72, 650–658.
22 Ansley, S. J., Badano, J. L., Blacque, O. E., Hill, J., Hoskins, B. E., Leitch, C. C., Kim, J. C., Ross, A. J., Eichers, E. R., Teslovich, T. M. et al. (2003) Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. Nature 425, 628–633.
23 Chiang, A. P., Nishimura, D., Searby, C., Elbedour, K., Carmi, R., Ferguson, A. L., Secrist, J., Braun, T., Casavant, T., Stone, E. M. and Sheffield, V. C. (2004) Comparative genomic analysis identifies an ADP-ribosylation factor-like gene as the cause of Bardet-Biedl syndrome (BBS3). Am. J. Hum. Genet. 75, 475–484.
24 Fan, Y., Esmail, M. A., Ansley, S. J., Blacque, O. E., Boroevich, K., Ross, A. J., Moore, S. J., Badano, J. L., May-Simera, H., Compton, D. S. et al. (2004) Mutations in a member of the

Ras superfamily of small GTP-binding proteins causes Bardet-Biedl syndrome. Nat. Genet. 36, 989–993.
25 Li, J. B., Gerdes, J. M., Haycraft, C. J., Fan, Y., Teslovich, T. M., May-Simera, H., Li, H., Blacque, O. E., Li, L., Leitch, C. C. et al. (2004) Comparative genomics identifies a flagellar and basal body proteome that includes the *BBS5* human disease gene. Cell 117, 541–552.
26 Nishimura, D. Y., Swiderski, R. E., Searby, C. C., Berg, E. M., Ferguson, A. L., Hennekam, R., Merin, S., Weleber, R. G., Biesecker, L. G., Stone, E. M. and Sheffield, V. C. (2005) Comparative genomics and gene expression analysis identifies *BBS9*, a new Bardet-Biedl syndrome gene. Am. J. Hum. Genet. 77, 1021–1033.
27 Beales, P.L., Badano, J. L., Ross, A. J., Ansley, S. J., Hoskins, B. E., Kirsten, B., Mein, C. A., Froguel, P., Scambler, P. J., Lewis, R. A., Lupski, J. R. and Katsanis, N. (2003) Genetic interaction of *BBS1* mutations with alleles at other BBS loci can result in non-Mendelian Bardet-Biedl syndrome. Am. J. Hum. Genet. 72, 1187–1199.
28 Mykytyn, K., Nishimura, D. Y., Searby, C. C., Beck, G., Bugge, K., Haines, H. L., Cornier, A. S., Cox, G. F., Fulton, A. B., Carmi, R. et al. (2003) Evaluation of complex inheritance involving the most common Bardet-Biedl syndrome locus (*BBS1*). Am. J. Hum. Genet. 72, 429–437.
29 Hichri, H., Stoetzel, C., Laurier, V., Caron, S., Sigaudy, S., Sarda, P., Hamel C, Martin-Coignard, D., Gilles, M., Leheup, B. et al. (2005) Testing for triallelism: analysis of six BBS genes in a Bardet-Biedl syndrome cohort. Eur. J. Hum. Genet. 13, 607–616.
30 Beales, P. L., Katsanis, N., Lewis, R. A., Elcioglu, N., Raza, J., Woods, M. O., Green, J. S., Parfrey, P. S., Davidson, W. S. and Lupski, J. R. (2001) Genetic and mutational analyses of a large multiethnic Bardet-Biedl cohort reveal a minor involvement of *BBS6* and delineate the critical intervals of other loci. Am. J. Hum. Genet. 68, 606–616.
31 Katsanis, N., Eichers, E. R., Ansley, S. J., Lewis, R. A., Kayserili, H., Hoskins, B. E., Scambler, P. J., Beales, P. L. and Lupski, J. R. (2002) *BBS4* is a minor contributor to Bardet-Biedl syndrome and may also participate in triallelic inheritance. Am. J. Hum. Genet. 71, 22–29.
32 Katsanis, N. (2004) The oligogenic properties of Bardet-Biedl syndrome. Hum. Mol. Genet. 13, R65–R71.
33 Stoetzel, C., Laurier, V., Faivre, L., Megarbane, A., Perrin-Schmitt, F., Verloes, A., Bonneau, D., Mandel, J. L., Cossee, M. and Dollfus, H. (2006) BBS8 is rarely mutated in a cohort of 128 Bardet-Biedl syndrome families. J. Hum. Genet. 51, 81–84.
34 Fan, Y., Rahman, P., Peddle, L., Hefferton, D., Gladney, N., Moore, S. J., Green, J. S., Parfrey, P. S. and Davidson WS. (2004) Bardet-Biedl syndrome 1 genotype and obesity in the Newfoundland population. Int. J. Obes. Relat. Metab. Disord. 28, 680–684.
35 Andersen, K. l., Echwald, S. M., Larsen, L. H., Hamid, Y. H., Glumar, C., Jorgensen, T., Borch-Johnsen, K., Andersen, T., Sorensen, T. I., Hansen, T. and Pedersen, O. (2005) Variation of the McKusick-Kaufman gene and large scale studies of relationships with common forms of obesity. J. Clin. Endocrinol. Metab. 90, 225–230.
36 Jawad, Z. and Paoli, M. (2002) Novel sequences propel familiar folds. Structure 10, 447–454.
37 Blatch, G. L. and Lassle, M. (1999) The tetratricopeptide repeat: a structural motif mediating protein-protein interactions. Bioessays 21, 932–939.
38 Kim, J. C., Ou, Y. Y., Esmail, M. A., Leitch, C. C., Fiedrich, E., Beales, P. L., Archibald, J. M., Katsanis, N., Rattner, J. B. and Leroux, M. R. (2005) MKKS/BBS6, a divergent chaperonin-like protein linked to the obesity disorder Bardet-Biedl syndrome, is a novel centrosomal component required for cytokinesis. J. Cell Sci. 118, 1007–1020.
39 Katsanis, N., Ansley, S. J., Badano, J. L., Eichers, E. R., Lewis, R. A., Hoskins, B. E., Scambler, P. J., Davidson, W. S., Beales, P. L. and Lupski, J. R. (2001) Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. Science 293, 2256–2259.
40 Badano, J. L., Kim, J. C., Hoskins, B. E., Lewis, R. A., Ansley, S. J., Cutler, D. J., Castellan, C., Beales, P. L., Leroux, M. R. and Katsanis, N. (2003) Heterozygous mutations in *BBS1*, *BBS2* and *BBS6* have a potential epistatic effect on Bardet-Biedl patients with two mutations at a second locus. Hum. Mol. Genet. 12, 1651–1659.
41 Slavotinek, A. M., Searby, C., Al-Gazali, L., Hennekam, R. C. M., Schrander-Stumpel, C., Orcana-Losa, M., Pardo-Reoyo, S., Cantani, A., Kumar, D., Capellini, Q. et al. (2002) Mutation analysis of the *MKKS* gene in McKusick-Kaufman syndrome and selected Bardet-Biedl syndrome patients. Hum. Genet. 110, 561–567.
42 Fauser, S., Muntz, M. and Besch, D. (2003) Further support for digenic inheritance in Bardet-Biedl syndrome. J. Med. Genet. 40, e104.
43 Badano, J. L., Leitch, C. C., Ansley, S. J., May-Simera, H., Lawson, S., Lewis, R. A., Beales, P. L., Dietz, H. C., Fisher, S. and Katsanis, N. (2006) Dissection of epistasis in oligogenic Bardet-Biedl syndrome. Nature 439, 326–330.
44 Nakane, T. and Biesecker, L. G. (2005) No evidence for triallelic inheritance of *MKKS*/*BBS* loci in Amish McKusick-Kaufman syndrome. Am. J. Med. Genet. 138A, 32–34.
45 Dutcher, S. K. (2003) Elucidation of basal body and centriole functions in *Chlamydomonas reinhardtii*. Traffic 4, 443–451.
46 Kubo, A., Sasaki, H., Yuba-Kubo, A., Tsukita, S. and Shiina, N. (1999) Centriolar satellites: molecular characterization, ATP-dependent movement toward centrioles and possible involvement in ciliogenesis. J. Cell Biol. 147, 969–980.
47 Rosenbaum, J. L. and Witman, G. B. (2002) Intraflagellar transport. Nat. Rev. Mol. Cell Biol. 3, 813–825.
48 Avidor-Reiss, T., Maer, A. M., Koundakjian, E., Polyanovsky, A., Keil, T., Subramaniam, S. and Zuker, C. S. (2004) Decoding cilia function: defining specialized genes required for compartmentalized cilia biogenesis. Cell 117, 527–539.
49 Blacque, O. E., Reardon, M. J., Li, C., McCarthy, J., Mahjoub, M. R., Ansley, S. J., Badano, J. L., Mah, A. K., Beales, P. L., Davidson, W. S et al. (2004) Loss of *C. elegans* BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. Genes Dev. 18, 1630–1642.
50 Efimenko, E., Bubb, K., Mak, H. Y., Holzman, T., Leroux, M. R., Ruvkun, G., Thomas, J. H. and Swoboda, P. (2005) Analysis of *xbx* genes in *C. elegans*. Development 132, 1923–1934.
51 Scholey, J. M. (2003) Intraflagellar transport. Annu. Rev. Cell Dev. Biol. 19, 423–443.
52 Otto, E. A., Loeys, B., Khanna, H., Hellemans, J., Sudbrak, R., Fan, S., Muerb, U., O'Toole, J. F., Helou, J., Attanasio, M. et al. (2005) Nephrocystin-5, a ciliary IQ domain protein, is mutated in Senior-Loken syndrome and interacts with RPGR and calmodulin. Nat. Genet. 37, 282–288.
53 Kyttala, M., Tallila, J., Salonen, R., Kopra, O., Kohlschmidt, N., Paavola-Sakki, P., Peltonen, L. and Kestila, M. (2006) MKS1, encoding a component of the flagellar apparatus basal body proteome, is mutated in Meckel syndrome. Nat. Genet. 38, 155–157.
54 Smith, U. M., Consugar, M., Tee, L. J., McKee, B. M., Maina, E. N., Whelan, S., Morgan, N. V., Goranson, E., Gissen, P., Lilliquist, S. et al. (2006) The transmembrane protein meckelin (MKS3) is mutated in Meckel-Gruber syndrome and the wpk rat. Nat. Genet. 38, 191–196.
55 Davenport, J. R. and Yoder, B. K. (2005) An incredible decade for the primary cilium: a look at a once forgotten organelle. Am. J. Physiol. Renal. Physiol. 289, F1159–F1169.

56 Kim, J. C., Badano, J. L., Sibold, S., Email, M. A., Hill, J., Hoskins, B. E., Leitch, C. C., Venner, K., Ansley, S. J., Ross, A. J. et al. (2004) The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression. Nat. Genet. 36, 462–470.

57 Nonaka, S., Tanaka, Y., Okada, Y., Takeda, S., Harada, A., Kanai, Y., Kido, M. and Hirokawa, N. (1998) Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell 95, 829–837.

58 McGrath, J., Somlo, S., Makova, S., Tian, X. and Brueckner, M. (2003) Two populations of node monocilia initiate left-right asymmetry in the mouse. Cell 114, 61–73.

59 Takada, T., Iida, K., Sasaki, H., Taira, M. and Kimura, H. (2005) Expression of ADP-ribosylation factor (ARF)-like protein 6 during mouse embryonic development. Int. J. Dev. Biol. 49, 891–894.

60 Mykytyn, K., Mullins, R. F., Andrews, M., Chiang, A. P., Swiderski, R. E., Yang, B., Braun, T., Casavant, T., Stone, E. M. and Sheffield VC. (2004) Bardet-Biedl syndrome type 4 (BBS4)- null mice implicate *Bbs4* in flagella formation but not global cilia assembly. Proc. Natl. Acad. Sci. USA 101, 8664–8669.

61 Kulaga, H. M., Leitch, C. C., Eichers, E. R., Badano, J., Leseman, A., Hoskins, B. E., Lupski, J. R., Beales, P. L., Reed, R. R. and Katsanis, N. (2004) Loss of BBS proteins causes anosmia in humans and defects in olfactory cilia structure in the mouse. Nat. Genet. 36, 994–998.

62 Nishimura, D. Y., Fath, M., Mullins, R. F., Searby, C., Andrews, M., Davis, R., Andorf, J. L., Mykytyn, K., Swiderski, R. E., Yang, B., Carmi, R., Stone, E. M. and Sheffield, V. C. (2004) *Bbs2*-null mice have neurosensory deficits, a defect in social dominance, and retinopathy associated with mislocalization of rhodoposin. Proc. Natl. Acad. Sci. USA 101, 16588–16593.

63 Fath, M. A., Mullins, R. F., Searby, C., Nishimura, D. Y., Wei, J., Rahmouni, K., Davis, R. E., Tayeh, M. K., Andrews, M., Yang, B. et al. (2005) *MKKS*-null mice have a phenotype resembling Bardet-Biedl syndrome. Hum. Mol. Genet. 14, 1109–1118.

64 Ross, A. J., May-Simera, H., Eichers, E. R., Kai, M., Hill, J., Jagger, D. J., Leitch, C. C., Chapple, J. P., Munro, P. M., Fisher, S. et al. (2005) Disruption of Bardet-Biedl syndrome ciliary proteins perturbs planar cell polarity in vertebrates. Nat. Genet. 37, 1135–1140.

65 Friedman, J. M. and Halaas, J. L. (1998) Leptin and the regulation of body weight in mammals. Nature 395, 763–770.

66 Iannaccone, A., Mykytyn, K., Persico, A. M., Searby, C. C., Baldi, A., Jablonski, M. M. and Sheffield, V. C. (2005) Clinical evidence of decreased olfaction in Bardet-Biedl syndrome caused by a deletion in the *BBS4* gene. Am. J. Med. Genet. 132A, 343–346.

67 Barnett, S., Reilly, S., Carr, L., Ojo, I., Beales, P. L. and Charman, T. (2002) Behavioural phenotype of Bardet-Biedl syndrome. J. Med. Genet. 39, e76.

68 Grace, C., Beales, P., Summerbell, C., Jebb, S. A., Wright, A., Parker, D. and Kopelman, P. (2003) Energy metabolism in Bardet-Biedl syndrome. Int. J. Obesity 27, 1319–1324.

69 Young, T. L., Woods, M. O., Parfrey, P. S., Green, J. S., O'Leary, E., Hefferton, D. and Davidson, W. S. (1998) Canadian Bardet-Biedl syndrome family reduces the critical region of BBS3 (3p) and presents with a variable phenotype. Am. J. Med. Genet. 78, 461–467.

70 Lorda-Sanchez, I., Ayuso, C. and Ibanez, A. (2000) Situs inversus and hirschsprung disease: two uncommon manifestations in Bardet-Biedl syndrome. Am. J. Med. Genet. 90, 80–81.

71 Pazour, G. J., Baker, S. A., Dean, J. A., Cole, D. G., Dickert, B. L., Rosenbaum, J. L., Witman, G. B. and Besharse, J. C. (2002) The intraflagellar transport protein, IFT88, is essential for vertebrate photoreceptor assembly and maintenance. J. Cell Biol. 157, 103–114.

72 Montcouquiol, M., Rachel, R. A., Lanford, P. J., Copeland, N. G., Jenkins, N. A. and Kelley, M. W. (2003) Identification of *Vangl2* and *Scrb1* as planar polarity genes in mammals. Nature 423, 173–177.

73 Strutt, D. (2003) Frizzled signalling and cell polarisation in *Drosophila* and vertebrates. Development 130, 4501–4513.

74 Keller, R. (2002) Shaping the vertebrate body plan by polarized embryonic cell movements. Science 298, 1950–1954.

75 Simons, M., Gloy, J., Ganner, A., Bullerkotte, A., Bashkurov, M., Krönig, C., Schermer, B., Benzing, T., Cabello, O. A., Jenny, A. et al. (2005) Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. Nat. Genet. 37, 537–543.

76 Dammermann, A. and Merdes, A. (2002) Assembly of centrosomal proteins and microtubule organization depends on PCM-1. J. Cell Biol. 159, 255–266.

77 Vale, R. D. (2003) The molecular motor toolbox for intracellular transport. Cell 112, 467–480.

78 Lee, S., Walker, C. L., Karten, B., Kuny, S. L., Tennese, A. A., O'Neill, M. A. and Wevrick, R. (2005) Essential role for the Prader-Willi syndrome protein nectin in axonal outgrowth. Hum. Mol. Genet. 14, 627–637.

79 Perkins, L. A., Hedgecock, E. M., Thomson, J. N. and Culotti, J. G. (1986) Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev. Biol. 117, 456–487.

80 Ou, G., Blacque, O. E., Snow, J. J., Leroux, M. R. and Scholey, J. M. (2005). Functional coordination of intraflagellar transport motors. Nature 436, 583–587.

81 Kozminski, K. G., Johnson, K. A., Forscher, P. and Rosenbaum, J. L. (1993) A motility in the eukaryotic flagellum unrelated to flagellar beating. Proc. Natl. Acad. Sci. USA 90, 5519–5523.

82 Piperno, G. and Mead, K. (1997) Transport of a novel complex in the cytoplasmic matrix of *Chlamydomonas* flagella. Proc. Natl. Acad. Sci. USA 94, 4457–4462.

83 Cole, D. G., Diener, D. R., Himelblau, A. L., Beech, P. L., Fuster, J. C. and Rosenbaum, J. L. (1998) Chlamydomonas kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in *Caenorhabditis elegans* sensory neurons. J. Cell. Biol. 141, 993–1008.

84 Snow, J. J., Ou, G., Gunnarson, A. L., Walker, M. R., Zhou, H. M., Brust-Mascher, I. and Scholey, J. M. (2004). Two anterograde intraflagellar transport motors cooperate to build sensory cilia on, *C. elegans* neurons. Nat. Cell Biol. 6, 1109–1113.

85 Reese, T. S. (1965). Olfactory cilia in the frog. J. Cell Biol. 25, 209–230.

86 Webber, W. A. and Lee, J. (1975) Fine structure of mammalian renal cilia. Anat. Rec. 182, 339–343.

87 Mesland, D. A., Hoffman, J. L., Caligor, E. and Goodenough, U. W. (1980). Flagellar tip activation stimulated by membrane adhesions in *Chlamydomonas* gametes. J. Cell Biol. 84, 599–617.

88 Hidaka, K., Ashizawa, N., Endoh, H., Watanabe, M. and Fukumoto, S. (1995) Fine structure of the cilia in the pancreatic duct of WBN/Kob rat. Int. J. Pancreatol. 18, 207–213.

89 Orozco, J. T., Wedaman, K. P., Signor, D., Brown, H., Rose, L. and Scholey, J. M. (1999). Movement of motor and cargo along cilia. Nature 398, 674.

90 Signor, D., Wedaman, K. P., Orozco, J. T., Dwyer, N. D., Bargmann, C. I., Rose, L. S. and Scholey, J. M. (1999a). Role of a class DHC1b dynein in retrograde transport of IFT motors and IFT raft particles along cilia, but not dendrites, in chemosensory neurons of living *Caenorhabditis elegans*. J. Cell Biol. 147, 519–530.

91 Yen, H. J., Tayeh, M. K., Mullins, R. F., Stone, E. M., Sheffield, V. C. and Slusarski, D.C. (2006) Bardet-Biedl syndrome genes are important in retrograde intracellular trafficking and Kupffer's vesicle cilia function. Hum. Mol. Genet. 15, 667–677.

92 Essner, J. J., Amack, J. D., Nyholm, M. K., Harris, E. B. and Yost, H. J. (2005) Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut. Development 132, 1247–1260.

93 Hearn, T., Spalluto, C., Phillips, V. J., Renforth, G. L., Copin, N., Hanley, N. A. and Wilson, D. I. (2005) Subcellular localization of ALMS1 supports involvement of centrosome and basal body dysfunction in the pathogenesis of obesity, insulin resistance, and type 2 diabetes. Diabetes 54, 1581–1587.

94 Mak, H. Y., Nelson, L. S., Basson, M., Johnson, C. D. and Ruvkun, G. (2006) Polygenic control of *Caenorhabditis elegans* fat storage. Nat. Genet. 38, 363–368.

95 Noben-Trauth, K., Naggert, J. K., North, M. A. and Nishina, P. M. (1996) A candidate gene for the mouse mutation tubby. Nature 380, 534–538.

96 Kleyn, P. W., Fan, W., Kovats, S. G., Lee, J. J., Pulido, J. C., Wu, Y., Berkemeier, L. R., Misumi, D. J., Holmgren, L., Charlat, O. et al. (1996) Identification and characterization of the mouse obesity gene tubby: a member of a novel gene family. Cell 85, 281–290.

97 Brailov, I., Bancila, M., Brisorgueil, M. J., Miquel, M. C., Hamon, M. and Verge, D. (2000) Localization of 5-HT(6) receptors at the plasma membrane of neuronal cilia in the rat brain. Brain Res. 872, 271–275.

98 Vickers, S. P. and Dourish, C. T. (2004) Serotonin receptor ligands and the treatment of obesity. Curr. Opin. Investig. Drugs 5, 377–388.

99 Otto, E. A., Schermer, B., Obara, T., O'Toole, J. F., Hiller, K. S., Mueller, A. M., Ruf, R. G., Hoefele, J., Beekmann, F., Landau, D. et al. (2003) Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. Nat. Genet. 34, 413–420.

100 Parisi, M. A., Bennett, C. L., Eckert, M. L., Dobyns, W. B., Gleeson, J. G., Shaw, D. W., McDonald, R., Eddy, A., Chance, P. F. and Glass IA. (2004) The *NPHP1* gene deletion associated with juvenile nephronophthisis is present in a subset of individuals with Joubert syndrome. Am. J. Hum. Genet. 75, 82–91.

101 Banizs, B., Pike, M. M., Millican, C. L., Ferguson, W. B., Komlosi, P., Sheetz, J., Bell, P. D., Schwiebert, E. M. and Yoder, B. K. (2005) Dysfunctional cilia lead to altered ependyma and choroid plexus function, and result in the formation of hydrocephalus. Development 132, 5329–5339.

102 Pazour, G. J., Dickert, B. L., Vucica, Y., Seeley, E. S., Rosenbaum, J. L., Witman, G. B. and Cole, D. G. (2000) *Chlamydomonas* IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. J. Cell Biol. 151, 709–718.

103 O'toole, J. F., Otto, E. A., Frishberg, Y. and Hildebrandt, F. (2006) Retinitis pigmentosa and renal failure in a patient with mutations in INVS. Nephrol. Dial. Transplant. Mar 7; [Epub ahead of print] PMID: 16522655.

104 Germino, G. G. (2005) Linking cilia to Wnts. Nat. Genet. 37, 455–457.

105 Park, T. J., Haigo, S. L. and Wallingford, J. B. (2006) Ciliogenesis defects in embryos lacking inturned or fuzzy function are associated with failure of planar cell polarity and Hedgehog signaling. Nat. Genet. 38, 303–311.

106 Huangfu, D., Liu, A., Rakeman, A. S., Murcia, N. S., Niswander, L. and Anderson, K. V. (2003) Hedgehog signalling in the mouse requires intraflagellar transport proteins. Nature 426, 83–87.

107 Corbit, K. C., Aanstad, P., Singla, V., Norman, A. R., Stainier, D. Y. and Reiter JF. (2005) Vertebrate Smoothened functions at the primary cilium. Nature 437, 1018–1021.

108 Haycraft, C. J., Banizs, B., Aydin-Son, Y., Zhang, Q., Michaud, E. J. and Yoder, B. K. (2005) Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. PLoS Genet. 1: e53.

109 May, S. R., Ashique, A. M., Karlen, M., Wang, B., Shen, Y., Zarbalis, K., Reiter, J., Ericson, J. and Peterson, A. S. (2005) Loss of the retrograde motor for IFT disrupts localization of Smo to cilia and prevents the expression of both activator and repressor functions of Gli. Dev. Biol. 287, 378–389.

110 Liu, A., Wang, B. and Niswander, L. A. (2005) Mouse intraflagellar transport proteins regulate both the activator and repressor functions of Gli transcription factors. Development 132, 3103–3111.

111 Morgan, D., Eley, L., Sayer, J., Strachan, T., Yates, L. M., Craighead, A. S. and Goodship, J. A. (2002) Expression analyses and interaction with the anaphase promoting complex protein Apc2 suggest a role for inversin in primary cilia and involvement in the cell cycle. Hum. Mol. Genet. 11, 3345–3350.

112 Gerdes, J. M. and Katsanis, N. (2005) Microtubule transport defects in neurological and ciliary disease. Cell Mol. Life Sci. 62, 1556–1570.

113 Beales, P. L. (2005) Lifting the lid on Pandora's box: the Bardet-Biedl syndrome. Curr. Opin. Genet. Dev. 15, 315–323.