

Chapter 6

Neuronal Cilia and Obesity

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Abstract Obesity is an emerging public health concern that has numerous secondary health consequences, including heart disease, high blood pressure, diabetes mellitus, osteoarthritis, and overall reduced quality of life. Historically, obesity has been viewed as increased body fat caused by overconsumption of food, combined with the sedentary lifestyle of modern society. Simply put, energy input exceeds energy output, creating an excess in fat mass. This viewpoint largely focuses on environmental and social factors in the obesity epidemic. However, it fails to take into account a growing body of evidence from several monogenetic human obesity

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disorders and mutant mouse and rat obesity models that indicate a profound role for genetic factors. Although most of these monogenetic human conditions are rare, it is clear that the study of their molecular and cellular etiology will offer insights into the mechanisms that regulate appetite and satiety. The objectives of this review are to discuss how mutations in genes required for the formation or function of the cilium result in obesity in human and mouse models and how the cilium may function to regulate appetite and satiation responses.

Keywords Obesity • Ciliopathy • Bardet-Biedl syndrome • Alström syndrome • Neuronal cilia • Leptin • Intraflagellar transport • Mouse models • Hedgehog • Melanin concentrating hormone • Hyperphagia • Food anticipatory behavior

6.1 Ciliopathies Associated with Obesity

The cilium is a small microtubule based cellular appendage found on most mammalian cells where it plays a crucial role as a complex sensory and signaling center (for a review on cilia signaling see Berbari et al. 2009). There is now an emerging class of genetic diseases coined ciliopathies that is associated with dysfunction of the cilium. Interestingly, the clinical features of ciliopathies range from renal cysts in humans with polycystic kidney disease (PKD1, OMIM #601313) to the cystic kidneys, skeletal anomalies, neurodevelopmental defects, heart malformations and perinatal lethality associated with Meckel-Gruber Syndrome (MKS1, OMIM # 249000) (for an in-depth review on ciliopathies see Sharma et al. 2008). This broad scope of clinical features has been attributed to both the ubiquitous nature of the cilium and the types of mutations affecting its function. PKD, for example, is associated with mutations in signaling proteins that appear to have crucial roles in renal homeostasis, while the mutations in MKS are often associated with proteins found at the base of the cilium, where they are thought to play more general roles in cilia structural integrity and the regulation of cilia protein composition. Until relatively recently much of our knowledge of cilia has come from studies in model systems focusing on the process of building and maintaining the cilium, known as Intraflagellar Transport (IFT) (for a review on IFT machinery see Pedersen and Rosenbaum 2008). While the list of ciliopathies continues to grow at an incredible pace, our current understanding of how dysfunction of the cilium leads to some of the clinically observed phenotypes remains elusive. One of the clinical features where this is most evident is the hyperphagia-associated obesity that occurs in two ciliopathies, Alström Syndrome (ALMS, OMIM #203800) and Bardet-Biedl Syndrome (BBS, OMIM #209900). The proteins affected in both of these disorders are associated with the cilium and are required for normal cilia function or formation.

Bardet-Biedl Syndrome (BBS) is a group of rare genetically heterogeneous disorders resulting in an array of seemingly unrelated symptoms and progressive degenerative defects. A triad of symptoms including obesity, retinitis pigmentosa/retinal degeneration, and polydactyly remains the hallmark for diagnosing the disease, as was the case when it was independently classified by Georges Bardet and Arthur

Biedl in the early twentieth century (Bardet 1920; Biedl 1922). Subsequent analysis has revealed that hypogonadism, renal dysfunction, and mental retardation are also highly prevalent among BBS patients. Nearly half of BBS patients are completely or partially anosmic and have deficits in thermosensation (Kulaga et al. 2004; Tan et al. 2007). Also of consequence are the secondary features of BBS, including diabetes mellitus, hypertension, and heart disease, which develop likely as a result of the obesity. Less commonly, BBS patients can have *situs inversus*, a defect often caused by dysfunctional cilia on the embryonic gastrulation stage structure called the node (Lorda-Sanchez et al. 2000; Deffert et al. 2007). Among North American and European populations, BBS is relatively uncommon with an estimated occurrence of 1 in 120,000 live births, while in Middle Eastern populations it has been reported to be as high as 1 in 13,500 (Klein and Ammann 1969; Croft et al. 1995; Beales et al. 1997). Although it is possible to detect the condition during gestation, patients are typically diagnosed when both obesity and retinal degeneration are apparent and polydactyly or mental deficits have been observed.

Confusion in diagnosing BBS exists, as a similar condition, Laurence-Moon Syndrome, is also a genetically-inherited disease in which patients present with retinitis pigmentosa and mental disability. This observation subsequently caused Solis-Cohens and Weiss to conclude that both diseases are synonymous and thus both disorders have been referred to as Laurence-Moon-Bardet-Biedl Syndrome. However, there is debate about the synonymous classification as the patients reported by Laurence and Moon displayed paraplegia of the lower extremities that is not typically associated with BBS. Furthermore, polydactyly and obesity are not generally traits of Laurence-Moon syndrome, while they are clinical hallmarks of BBS. Given that polydactyly is not fully penetrant among BBS patients, and that the obesity experienced by most BBS patients can be attenuated or eliminated with diet and exercise, the lack of these symptoms does not necessarily mean the two disorders are not allelic variations (Beales et al. 1999; Ghadami et al. 2000).

BBS has extensive genetic heterogeneity with 16 known loci (*BBS1-BBS16*, Table 6.1). Mutations in *BBS1-10* are responsible for approximately 70% of known BBS cases, and even the identification of *BBS11-16* only added fractional amounts to that number; thus it remains almost certain that other *BBS* genes have yet to be found (Chen et al. 2011; Stoetzel et al. 2006; Leitch et al. 2008; Chiang et al. 2006). Conversely, the genetic basis of Laurence-Moon is currently unknown, so it remains to be seen if the symptoms of Laurence-Moon are caused by mutations in *BBS* genes.

The mode of *BBS* inheritance is also complicated. Although originally believed to be a typical recessive Mendelian disorder, analysis by Katsanis et al. demonstrated this may not always be the case (Katsanis et al. 2001). In their study, the authors reported families where both affected and unaffected individuals could carry homozygous mutant alleles of *BBS2*. However, the affected individuals were also heterozygous for a mutation in a second *BBS* gene, *BBS6*. This phenomenon, which the authors named 'trialelic inheritance', demonstrates that manifestations of *BBS* phenotypes sometimes rely on mutations at a second locus and thus *BBS* may result from the overall genetic mutational load in a patient. However, analysis of the triallelic inheritance hypothesis in several other *BBS* cohorts did not reveal evidence of triallelism suggesting this may be an exception (Mykytyn et al. 2003; Abu-Safieh et al. 2012;

Table 6.1 Bardet-Biedl syndrome genes

BBS	Gene	Human locus	Motifs/domains	References
BBS1	BBS1	11q13.2	Beta propeller ^a	Mykytyn et al. (2002)
BBS2	BBS2	16q12.2	Beta propeller ^a	Nishimura et al. (2001)
BBS3	ARL6	3q11.2	ADP ribosylation factor-like 6	Chiang et al. (2004)
BBS4	BBS4	15q24.1	TPR repeats ^a	Mykytyn et al. (2001)
BBS5	BBS5	2q31.1	Pleckstrin homology ^a	Kulaga et al. (2004)
BBS6	MKKS	20p12.2	Chaperone-like ^b	Slavotinek et al. (2000)
BBS7	BBS7	4q27	Beta propeller ^a	Badano et al. (2003)
BBS8	TTC8	14q31.3	TPR repeats ^a	Ansley et al. (2003)
BBS9	PTHB1	7p14.3	Beta propeller ^a	Nishimura et al. (2005)
BBS10	BBS10	12q21.2	Chaperone-like ^b	Stoetzel et al. (2006)
BBS11	TRIM32	9q33.1	E3 Ubiquitin Ligase	Chiang et al. (2006)
BBS12	BBS12	4q27	Chaperone-like ^b	Stoetzel et al. (2007)
BBS13	MKS1	17q22	B9/C2	Leitch et al. (2008)
BBS14	CEP290	12q21.32	Coiled-coil	Leitch et al. (2008)
BBS15	WDPCP	2p15	Coiled-coil, WD40	Kim et al. (2010)
BBS16	SDCCAG8	1q43	Globular, coiled-coil	Schaefer et al. (2011)

Known *BBS* genes are listed with their location in the human genome as well as their known protein motifs and domains. Members of the BBSome are indicated with an^a and BBS chaperone proteins are indicated with an^b

^aIndicates a member of the BBSome protein complex (Nachury et al. 2007)

^bIndicates a member of a BBS chaperone protein complex (Seo et al. 2010)

Smaoui et al. 2006; Laurier et al. 2006). Information from these cohorts suggests that BBS is an autosomal recessive inherited disease. But these data cannot rule out possible contributions from unidentified *BBS* alleles and it remains possible that BBS can manifest through both an autosomal recessive fashion and triallelic inheritance.

The great degree of variability in both inheritance and symptoms presented by BBS patients leads to the question of whether there exists any correlation between the genes and mutations involved and the way the disease manifests itself. For example, while obesity and renal abnormalities are frequent, the degree of mental retardation or learning disabilities varies greatly, with patients from some families showing little or no mental deficits (Riise et al. 1997). Thus, the relationship between BBS mutations and the expressivity of traits has been of great interest, but largely remains inconclusive with limited genotype-phenotype correlation.

Birth weight in BBS patients is usually normal, with obesity developing during childhood continuing into adulthood (Beales et al. 1999). This observation indicates that the obese phenotype may not be a direct consequence of defects in development, but rather due to errors in energy metabolism or appetite regulation. Evidence for this hypothesis is found in the fact that when compared to BMI matched controls, BBS patients did not possess significant differences in body fat or resting metabolic rate (RMR) (Grace et al. 2003). Although this information indicates that body fat and RMR is the same, subsequent work demonstrated that circulating

leptin and triglyceride levels were significantly higher in BBS patients compared to other BMI matched obese individuals, despite the fact that glucose tolerance and insulin resistance was comparable between the two groups (Feuillan et al. 2011). Leptin has a known role in suppressing appetite, and this finding supports the possibility that BBS patients have a higher degree of leptin resistance. Recent work in animal models have strongly implicated primary cilia as being necessary for regulating appetite, and that cilia regulated signaling can be disrupted in mouse models of BBS (Davenport et al. 2007; Weatherbee et al. 2009). However, the molecular mechanism causing this disease in BBS patients remains uncertain.

The other human ciliopathy associated with obesity is Alström syndrome (ALMS), which was first classified in 1959 (for a review of ALMS see Girard and Petrovsky 2011). Human ALMS patients manifest with several symptoms including obesity, retinitis pigmentosa, and hearing loss with a tendency towards shorter stature, and a disruption in the growth hormone/Insulin-like growth factor 1 signaling axis. They also exhibit phenotypes likely related to their obesity that include diabetes mellitus and elevated leptin levels when compared to unaffected individuals (Maffei et al. 2007). ALMS is a rare autosomal recessive disorder with an occurrence at less than 1 in 100,000 and is caused by mutations in the gene *ALMS1* (Collin et al. 2002; Hearn et al. 2002). To date, 81 different disease causing mutations in *ALMS1* have been reported (Joy et al. 2007; Marshall et al. 2007). The exact function of *ALMS1* remains unknown, but clues to its possible cellular function were uncovered when it was found that *ALMS1* is widely expressed and localizes to the centrosome and the basal body of the primary cilium in cultured human function cells (Hearn et al. 2005; Knorz et al. 2010). Interestingly, dermal fibroblasts derived from an ALMS patient had normal basal body localization and primary cilia assembly suggesting that *ALMS1* might be involved in ciliary related signaling pathways, but not in establishing cilia architecture itself (Hearn et al. 2005). In contrast, knockdown of *Alms1* by siRNA in mouse inner medullary collecting duct (mIMCD3) cells caused a stunted cilia phenotype, and also impaired their mechanical stimuli sensing abilities. This discrepancy in phenotype could be due to the nature of the mutation, which may not have caused a complete lack of protein function.

Although BBS and ALMS are relatively rare diseases, understanding how these proteins normally regulate satiation responses will provide important insights into molecular pathways that could be manipulated to control satiation and obesity.

6.2 Ciliopathy Mouse Models of Obesity

To better understand the causes of human obesity, genetically manipulated mouse models are continuously being developed with the ultimate goal of elucidating the molecular mechanisms driving the phenotype. Surprisingly, over the past decade the primary cilium has emerged as a key factor regulating satiation responses. The *Bbs* and *Alms1* mouse models along with mutations affecting the Intraflagellar transport

88 (*Ift88*) gene have established a strong link between defects in cilia mediated sensory or signaling activity and obesity (Table 6.2). In this section, we will review data derived from several of the obesity mouse models that have been associated with ciliary dysfunction and highlight the proposed function of the affected proteins.

6.2.1 *Bbs Mutant Mouse Models*

Seminal work in the BBS field by Nachury et al. has shown that BBS proteins 1, 2, 4, 5, 7, 8, and 9 form a ~450 kDa complex called the BBSome (Nachury et al. 2007). The BBSome is thought to function in transport of membrane along with specific transmembrane proteins to the cilium (Jin et al. 2010). Of these BBSome genes, mouse models of *Bbs1*, 2, 4, and 8 have been generated and characterized. The validity of utilizing mice to model human BBS was demonstrated when a knock-in allele of the *Bbs1* M390R mutation, one of the most common single human BBS disease alleles, was created and replicated many of the human symptoms of BBS, including retinal degeneration, male infertility, and obesity (Davis et al. 2007). Strikingly, these hallmark features of BBS are shared phenotypes among other mouse models of BBS such as *Bbs2* and *Bbs4* mutants (Mykytyn et al. 2004; Nishimura et al. 2004) (Fig. 6.1a). Neurological defects were also observed. For example, disruption of *Bbs1* or *Bbs4* caused cilia loss on the olfactory epithelium, and the same report demonstrated partial or total anosmia in a cohort of human BBS patients. These same studies found a common social dominance defect among *Bbs2* and *Bbs4* mutant mice, in which the mutants were more submissive to control mice. Although the olfactory and behavior phenotypes may not directly be related to the obesity seen in these mice, it does reflect the importance of the BBSome genes in the regulation of behavior.

As in BBS patients, obesity is not present in young *Bbs* mutant mice. In fact, most *Bbs* mutant mice are initially runted, and it has been proposed that this is possibly due to olfactory defects that make it difficult for pups to accomplish nipple searching and suckling (Eichers et al. 2006). However, mutants eventually developed hyperphagia and became obese. The obesity phenotype also correlated with hyperleptinemia in *Bbs1* M390R knock-in mice. More recently, *Bbs8*-null mice have been reported that also have defects in olfactory function, as has been shown in *Bbs1* and 4 mutant mice. When these *Bbs8* mutant mice were crossed to an olfactory receptor reporter line (M72^{TL}), severe defects in the targeting of olfactory sensory neurons became apparent, and individual axonal fibers seemed to wander, instead of terminating at a single glomerulus as in the control mice (Tadenev et al. 2011). The axonal targeting defects reported in the *Bbs8* mutant mice further confirm the importance of the BBSome in proper neuronal development and signaling.

Other genes involved in human BBS that do not encode direct BBSome components have been identified. These proteins share homology to chaperones, and there are indications that these too are necessary for normal activity of satiation pathways. These genes include *BBS6*, 10, and 12, and they encode proteins that may be

Table 6.2 Cilia associated mouse models of obesity

Gene	Allele (MGI)	Type of allele	Mouse phenotypes	References
<i>AC3</i>	<i>Adcy3^{miDrs}</i>	Knock-out	Obesity, anosmia	Wang et al. (2009) and Wong et al. (2000)
<i>Alms1</i>	<i>Alms1^{G0XH152/Bsg}</i>	Gene-trap	Obesity, retinopathy, male infertility, late onset hearing loss	Collin et al. (2005)
<i>Alms1</i>	<i>Alms1^{foz}</i>	Spontaneous	Obesity, male infertility, late onset hearing loss	Arsov et al. (2006)
<i>Bbs1</i>	<i>Bbs1^{miVes}</i>	Knock-in	Obesity, retinopathy, male infertility, ventriculomegaly	Davis et al. (2007)
<i>Bbs2</i>	<i>Bbs2^{miVes}</i>	Knock-out	Obesity, retinopathy, renal cysts, male infertility, anosmia, social submissiveness, ventriculomegaly	Nishimura et al. (2004)
^a <i>Bbs3/Arf6</i>	<i>Arf6^{tm2Ves}</i>	Knock-out	Increased fat mass, retinopathy, male infertility, hydrocephalus, elevated blood pressure	Zhang et al. (1994)
^b <i>Bbs4</i>	<i>Bbs4^{miVes}</i>	Knock-out	Obesity, retinopathy, male infertility, social submissiveness, ventriculomegaly	Mykytyn et al. (2004)
^b <i>Bbs4</i>	<i>Bbs4^{GrlNk}</i>	Gene-trap	Obesity (sex dependent penetrance and severity), retinopathy, social submissiveness, increased anxiety	Kulaga et al. (2004) and Eichers et al. (2006)
<i>Bbs6/Mkks</i>	<i>Mkks^{miVes}</i>	Knock-out	Obesity, retinopathy, male infertility, anosmia, elevated blood pressure, social submissiveness, ventriculomegaly	Fath et al. (2005)
^a <i>Bbs11/Trim32</i>	<i>Trim32^{G0(BGA355)/Bsg}</i>	Gene-trap	Increased body weight, muscular myopathy	Kudryashova et al. (2011)
<i>Ift88</i>	<i>Ift88^{miLlBly}</i>	Conditional knock-out	Obesity, renal cysts, hepatic cysts	Davenport et al. (2007)
<i>Kif3a</i>	<i>Kif3a^{miGsn}</i>	Conditional knock-out	Obesity, renal cysts, hepatic cysts	Davenport et al. (2007)
<i>Tub</i>	<i>Tub^{ub}</i>	Spontaneous	Obesity, retinopathy, late onset hearing loss	Coleman and Eicher (1990)
<i>Tub</i>	<i>Tub^{miRok}</i>	Knock-out	Obesity, retinopathy, late onset hearing loss	Stubdal et al. (2000)

Mutant mice with defects in known or potential cilia genes are shown alphabetically, along with the allele listing according to Mouse Genome Informatics (MGI <http://www.informatics.jax.org/>). Please note that the mouse phenotypes column includes some of the more prominent features, but for the sake of brevity, is not comprehensive

^a*Bbs3* mutant mice do not become obese, but do display increased fat mass. Likewise, *Bbs11* mutants have not been reported to be obese, but do display a significant and persistent increase in body weight starting at 2 months of age

^bTwo different *Bbs4*^{-/-} knockout mouse lines have been independently generated and different penetrance and severity of obesity have been reported for each

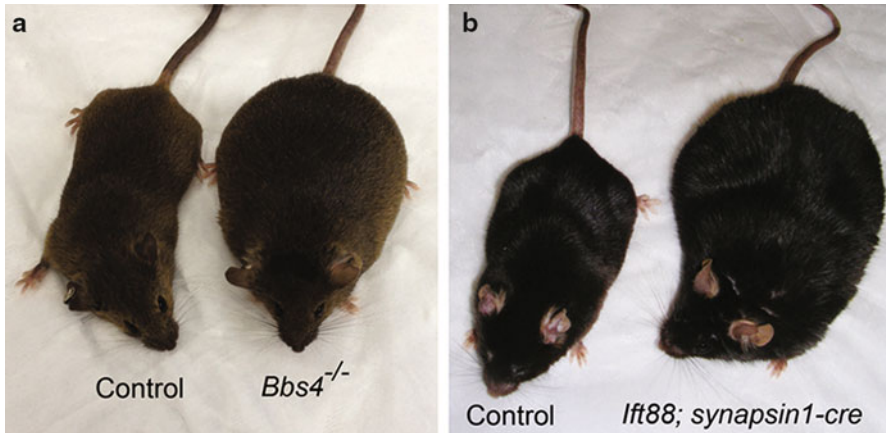


Fig. 6.1 Obese cilia mutant mouse models. (a) An obese *Bbs4*^{-/-} mutant (right) next to his wild-type littermate (left). (b) An obese conditional *lft88* mutant that has lost cilia throughout the central nervous system as the result of *synapsin1-cre* expression (right) next to a wild-type littermate (left)

necessary to stabilize the BBSome (Seo et al. 2010). Of these three, only a mouse mutant of *Bbs6* (previously referred to as *Mkks* for its involvement in McKusick-Kaufman syndrome) has been reported. As seen in the other BBSome mutant mice, *Bbs6* mutants display age dependent retinal degeneration and exhibit hyperphagic behavior leading to obesity with elevated leptin levels (Fath et al. 2005). Furthermore, male infertility was reported due to a failure in the formation of spermatozoa flagella, similar to the findings of *Bbs1*, 2, and 4 mutant mice.

Not all known *BBS* genes fall into the category of being a BBSome complex member or having chaperone-like properties. This includes the *BBS3* gene that encodes the small GTPase ARL6. Mouse *Bbs3* mutants exhibit both retinal degeneration and male infertility due to loss of sperm flagella (Zhang et al. 2011). In addition, severe hydrocephalus accompanied by altered beating of ependymal cilia was found, but no loss or obvious defects in primary cilia morphology were evident. Most strikingly however, was the apparent lack of an overt obesity phenotype in the *Bbs3* mutants. Likewise, leptin levels in the mutants were not statistically different than controls (Zhang et al. 2011). Although the *Bbs3* mutants do not have a significant increase in body weight, they do have an increase in the amount of gonadal and retroperitoneal fat. The reason that *Bbs3* mutant mice do not display an obesity phenotype is unknown. It was proposed this may be due to the early onset hydrocephalus; however, it should be noted that obesity along with hydrocephalus is seen in some of the other *Bbs* models. The lack of an obesity phenotype could also be related to different functions of the *Bbs* proteins and differential effects they have on protein trafficking. Melanin concentrating hormone receptor1 (*Mchr1*) is a ciliary localized G protein coupled receptor (GPCR) known to have orexigenic effects. Intriguingly, in obese models such as *Bbs2* and *Bbs4* mutants, *Mchr1* is not

present in the cilium while it does localize to in the cilia of cultured neurons from *Bbs3* mutants (Zhang et al. 2011). It is also important to note that *Bbs3* is neither a BBSome complex member nor a BBS chaperone protein, raising the possibility that *Bbs3* has functions independent of the other BBS proteins.

In some cases, previously identified genes are now being recognized as belonging to the BBS family. For example, mutations in the E3 ubiquitin ligase TRIM32 was identified in BBS patient (hence called *BBS11*) through the use of homozygosity mapping with SNP arrays (Chiang et al. 2006). *Trim32/Bbs11* mutant mice display muscular dystrophy and a decreased concentration of neurofilaments, as well as a reduction in myelinated motoraxon diameters (Kudryashova et al. 2009, 2011). A small increase in body weight was found in the *Bbs11* mice when compared to controls, but this was only a 10% difference at 8 weeks of age (Kudryashova et al. 2009). Much like *Bbs3*, *Bbs11* is neither a BBSome complex member nor a BBS chaperone protein, and thus may also have independent or unique functions from the rest of the BBSome. However, it remains possible that the muscular dystrophy and reduction in motor axon myelination are precluding the emergence of an obesity phenotype. In addition, mutations in *BBS11* can cause two distinct clinical disorders; BBS and limb-girdle muscular dystrophy type 2H (LGMD2H). BBS phenotypes were associated with N-terminal mutations (P130S) while LGMD2H appears to be caused by mutations in the C-terminal region (R394H, D487N, D588del, or T520TfsX13) that do not disrupt its ability to function in ubiquitination.

Studies of more recently identified BBS genes include *MKS1/BBS13* and *CEP290/BBS14*. However, current reports utilize *Bbs13* and *Bbs14* mutant mice that are either embryonically lethal and/or not true genetic nulls, thus making the potential role of *Bbs13* and 14 in obesity and appetite regulation ambiguous (Tadenev et al. 2011; Weatherbee et al. 2009; Lancaster et al. 2011). Regardless, the fact that not all *Bbs* mutant mice have the same phenotypes indicates complexity and diversity in the functions of the different *BBS* genes along with differential effects of the specific mutations on gene function.

6.2.2 Mouse Model of Alström Syndrome

The other ciliopathy with obesity as a symptom is Alström syndrome. In contrast to BBS, Alström syndrome appears to be caused by mutations in a single gene *ALMS1*. The *ALMS1* protein localizes to the basal body of ciliated cells, but the function of the protein is not certain (Hearn et al. 2005; Collin et al. 2005). Mouse models corresponding to Alström syndrome have also been reported and include a gene-trapped allele (*Alms1^{-/-}*) and a spontaneous mutant (*fat aussie, foz*) (Arsov et al. 2006; Collin et al. 2005). Mice lacking functional *Alms1* are born at a normal weight much like their human counterparts. However, hyperphagic behavior and obesity ensue that is accompanied by hyperinsulinemia and type II diabetes. The *Alms1^{-/-}* mutant mice also have enlarged livers with the accumulation of lipid deposits, and the pancreas

is hyperplastic. In addition to obesity, mice lacking *Alms1* display male infertility, as well as retinal and cochlear defects, all of which are reminiscent of cilia associated defects in human patients.

6.2.3 Obesity in the Intraflagellar Transport Mutants

Cilia formation and maintenance, and possibly its signaling activity, is dependent on the intraflagellar transport (IFT) system to mediate bidirectional transport of proteins between the base and tip of the cilium. Null alleles of the *Ift88* gene (originally referred to as *Tg737* in mouse) caused early embryonically lethality and even hypomorphic alleles caused death prior to adulthood with systemic effects (Moyer et al. 1994; Murcia et al. 2000; Lehman et al. 2008). The necessity of cilia for normal mammalian development has made analyzing possible roles of the cilium in satiation and obesity difficult. This problem was circumvented with the creation of conditional alleles of *Ift88* and the IFT motor *Kif3a* (Marszalek et al. 1999; Haycraft et al. 2007). Using a tamoxifen-inducible cre recombinase expressed from the actin promoter (CAGG-creERTM) (Davenport et al. 2007), cilia loss could be induced systemically in adult mice. This was found to cause hyperphagia within 3 weeks of inducing cilia loss and subsequently caused obesity. Furthermore, the obesity phenotype was prevented by maintaining adult conditional cilia mutant mice on a restricted diet, wherein they were provided the same daily amount of food as normal controls consumed. This observation indicates that the obesity phenotype is caused by the lack of a satiation response that leads to the overconsumption and not a general alteration in metabolic or locomotor activity.

The change in feeding behavior observed in the *Ift88* and *Kif3a* conditional mutant mice led to the possibility that cilia on neurons may be responsible for the obesity phenotype. To test this hypothesis, conditional *Ift88* and *Kif3a* mutant mice were crossed to synapsin1-cre mice to cause loss of cilia exclusively in neurons (Zhu et al. 2001). As with the systemically induced cilia mutants, neuronal specific cilia mutant mice became morbidly obese and strongly implicated a previously unappreciated role for neuronal cilia in regulating appetite (Fig. 6.1b).

The hypothalamus is a critically important signaling center of the brain known to regulate appetite. This action is done in large part by neurons that express either pro-opiomelanocortin (POMC) or agouti-related protein (AgRP) that release signaling factors ultimately suppressing or enhancing appetite, respectively (for a review see Mountjoy 2010). Importantly, hypothalamic neurons each possess a single primary cilium, although the function of the cilium on these neurons is largely unexplored. To address the role of neuronal cilia and appetite, *Ift88* and *Kif3a* conditional mutants were crossed to POMC-cre or AgRP-cre expressing mice, to conditionally ablate cilia on POMC or AgRP expressing neurons, respectively (Xu et al. 2005b). By 6 weeks of age, both male and female POMC cilia mutant mice weighed significantly more than control mice, and continued to become morbidly obese into adulthood. This was not evident in the mice lacking cilia on AgRP neurons (Berbari and Yoder, unpublished data). Another observation

that was reported in the POMC cilia mutant mice was an increase in the levels of leptin, fasting serum glucose, and insulin (Davenport et al. 2007). This was observed only in the obese state and not in mice kept lean by pair-feeding, indicating that these elevated levels were a secondary consequence of the obesity. This report was significant for providing some of the initial evidence indicating the importance of neuronal cilia in regulating obesity.

6.2.4 Other Obesity Mouse Models Associated with Ciliary Proteins

In addition to the *Bbs*, *Alms1*, and *Ift88* mouse models there are several other mutant mouse strains supporting a connection between cilia and obesity. One prime example is a mutation in the type III adenylyl cyclase (ACIII). ACIII localizes to the primary cilia throughout the adult rodent brain (Bishop et al. 2007) and loss of ACIII causes anosmia and obesity by 3 months of age. Interestingly, even when ACIII mutants do not weigh significantly more than wildtype siblings, they have an increased level of serum leptin (Wang et al. 2009). It is interesting to note that a recent Genome Wide Association Study revealed there is a SNP near ACIII that is associated with obesity in humans (Hebebrand et al. 2010; Nordman et al. 2008).

Another example may be the *tubby* mouse that was first identified at the Jackson Laboratory as a spontaneous mutant causing a maturity-onset obesity phenotype (Coleman and Eicher 1990) and subsequently regenerated by gene targeting (Ashrafi et al. 2003). The *tubby* mutants have progressive loss of hearing and vision (Ohlemiller et al. 1995), similarities that are also shared with the *Bbs* and *Alms1* mutant mice. The spontaneous *tubby* mouse possesses a single base pair mutation within a splice site of the gene *Tub* (named after the mutant mouse) resulting in the expression of an aberrant transcript. The functions of the *Tub* protein remain ambiguous, but it is found to be highly expressed in portions in the brain, including the arcuate nucleus of the hypothalamus (Kleyn et al. 1996). The *Tub* protein is dispensable in assembly of the cilia, and no defects in the cilia assembly process of intraflagellar transport (IFT) have been reported. Intriguingly, despite the fact that *Tub* has yet to be reported in mammalian primary cilia, a physical association between *Tub* and the IFT complex has been reported in an immortalized human cell line (Mukhopadhyay et al. 2010). Further evidence for a ciliary role of *Tub* can be found with the *C. elegans* homolog *tub-1* that undergoes transport along the ciliary axoneme (Mukhopadhyay et al. 2005). Like the *tubby* mouse, *C. elegans* with a deletion of *tub-1* show an increase in fat content, suggesting an evolutionarily conserved role of the gene in regulating fat storage. Other proteins in the *tubby* family of proteins have also been implicated as having ciliary roles. For example, *tubby*-like protein 3 (*Tulp3*) localizes to the primary cilia during mouse development, and is necessary for proper *Shh* signaling but its association with obesity has not yet been determined (Norman et al. 2009).

6.3 Potential Molecular Mechanisms of Ciliopathy Associated Obesity

The hyperphagia induced obesity is one of the more intriguing phenotypes of ciliopathies that remains to be fully explained. There are indeed several possibilities described in the literature as to how loss of the cilia could alter states of satiety and appetite. Here we review a few of the candidate molecular pathways that may play roles in obesity associated with cilia dysfunction. These possibilities include primary deficits in leptin signaling, altered G-protein coupled receptor (GPCR) signaling, and abnormal regulation of mTor and hedgehog signaling pathways (Table 6.3).

6.3.1 Cilia and the Leptin Signaling Axis

While the conditional allele of *Ift88* implicated a role for neuronal cilia in satiety and more specifically a role for POMC neuronal cilia, this study did not specify a molecular framework for the underlying hyperphagia phenotype (Davenport et al. 2007). More recent data has aimed to accomplish this by showing that *Bbs1*, a component of the BBSome, directly binds to the leptin receptor and that BBS proteins may have a role in leptin receptor trafficking (Fig. 6.2a) (Seo et al. 2009). The initial identification of the leptin gene encoding a small protein hormone in the spontaneous obese mouse mutant *ob/ob* was the source of much excitement (Zhang et al. 1994). Importantly, leptin suppresses feeding activity and it is secreted into serum at levels proportionate to the amount of adipose tissue, the hormone's primary source (Considine et al. 1996). Interestingly, these recent studies in *Bbs2*, *Bbs4*, and *Bbs6* mutant mice also revealed that they are hyper-leptinemic and importantly, they fail to reduce food intake in response to IP or ICV injection of leptin (Rahmouni et al. 2008). Thus, defects in leptin signaling were thought to contribute directly to the obesity phenotype in BBS.

The excitement surrounding leptin's initial discovery was attenuated when it was determined that nearly all obese mice and humans have markedly elevated levels of circulating leptin, yet do not have normal leptin-mediated repression of appetite (Considine et al. 1996; Maffei et al. 1995). This barrier phenomenon is known as *leptin resistance*, the mechanism of which remains an active area of research. Thus, in obesity research, one challenge is determining whether leptin resistance is a primary cause or a consequence of the obesity. One approach used to overcome this situation is to decrease the amount of adipose tissue, and consequently the levels of circulating leptin, through caloric restriction. Interestingly, when this was performed on the BBS mutant mouse models to maintain body weight and leptin levels as seen in controls, they were still resistant to leptin, suggesting leptin signaling defects are a primary cause of the phenotype (Seo et al. 2009). However, the study did not take into account a phenomenon called *food anticipatory behavior* wherein the mice alter their meal structure in response to the calorie restriction such that they consume

Table 6.3 Neuronal cilia signaling proteins that may contribute to obesity

Candidate molecule	Known receptors	Localization	Potential mechanism	References
Leptin	Lep-R	Cell membrane, 'peri'-ciliary membrane	Changes in leptin receptor localization	Seo et al. (2009)
Melanin concentrating hormone	Mchr1, ^a Mchr2	Ciliary membrane	Changes in Mchr1 localization	Barbari et al. (2008)
Dopamine	D ₁	Ciliary	Changes in Drd1 cilia localization	Domire et al. (2011)
Serotonin	5-HT ₆	Ciliary membrane in certain brain regions	Changes in ciliary receptor 5HT6 signaling	Brailov et al. (2000)
Adenylate cyclase III	Olfactory GPCRs	Cilia throughout the brain	Altered coupling downstream of ciliary GPCRs	Bishop et al. (2007)
Hedgehog	Patched	Ciliary membrane in regions of adult neurogenesis	Altered adult neurogenesis Altered hypothalamic development Non-canonical hedgehog signaling	N/A

Potential pathways behind the obesity phenotype are listed with the components that localize to the cilium or at the base of the cilium, as well as a current hypothesized mechanism.

^aMchr2 is common to primates, canines, and other carnivores, but is absent among rodents

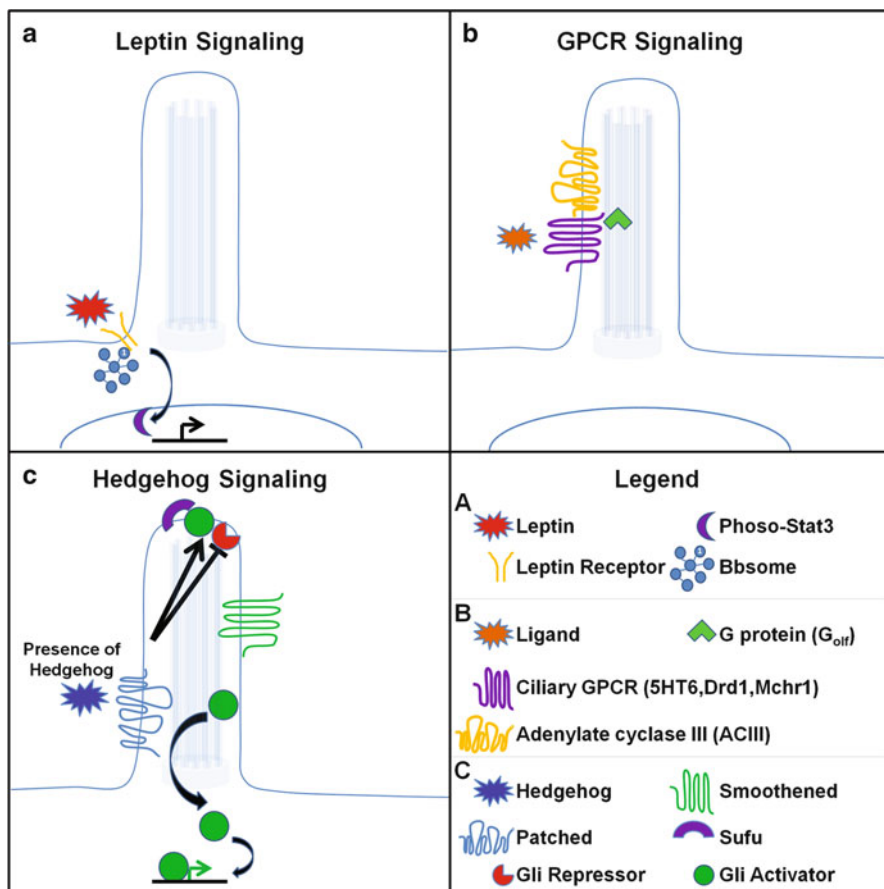


Fig. 6.2 Potential ciliary signaling pathways necessary for appetite regulation. (a) Depicts the leptin receptor interacting with Bbs1 of the BBSome near the base of the cilia where it has been proposed to be available for leptin activation and subsequent phospho-Stat3 induction and translocation to the nucleus. (b) A depiction of cilia specific GPCRs such as Mchr1, Sstr3, Drd1, and 5HT6, and their potential effector ACIII and G proteins such as $G_{s/q/i/o}$. (c) The Hedgehog signaling pathway, with patched repressing smoothened translocation into the cilium until ligand stimulation, upon which Gli transcription factors are processed from the inhibitor to the activator forms followed by subsequent translocation to the nucleus

nearly all of their calories within the first few hours of food access (for a review on anticipatory feeding behavior and methods see Mistlberger 2009). Interestingly, during this entrained period the mice consume nearly the same amount of food as they were given during calorie restriction, even when they are given *ad libitum* amounts of food. This entrained behavior persists for over a week and during this period the mice do not reduce food intake in response to leptin and thus appear leptin resistant (Berbari and Yoder unpublished). This feeding behavior resulting from the anticipation of food is in large part thought to be the result of a feeding

clock, somewhat analogous to but distinct from the circadian clock (Mistlberger 2009). It remains to be seen whether either *Bbs* or *Ift* conditional mice would respond to leptin when both body weight and anticipatory feeding behavior are experimentally controlled. This would require testing animals for leptin sensitivity after deterioration of the food anticipatory behavior.

The BBS studies utilized congenital mutants and reported a loss of approximately 20% of the leptin responsive pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus (Seo et al. 2009). Thus, the authors propose that improper leptin receptor trafficking in POMC neurons of the arcuate nucleus leads to their inability to sense leptin and thus mediate its anorectic effects. While several possibilities exist for the loss of 20% of POMC neurons in *Bbs* mice, it is of note that alteration in the *Foxo1/Insulin* signaling pathway have resulted in similar changes in adult POMC neuronal populations (Plum et al. 2012). Loss of POMC neurons in *Bbs* models could be the result of neurodevelopmental changes or possibly maintenance of POMC neuron population through altered adult neurogenesis that lead to the hyperphagic phenotype. Similarly, the obesity phenotype observed in the hyperphagic *Ift88* conditional POMC-cre model could be a hypothalamic developmental phenotype. However, the rapid onset of obesity upon ubiquitous loss of cilia induced by the actin promoter driving cre line (CaGG-CreER) in adult mice suggests that cilia play a direct role in appetite and satiety. What is needed is an investigation using inducible cilia mutants in POMC neurons or other neuronal populations implicated in feeding behavior in order to elucidate the role of primary cilia signaling in appetite and satiety.

6.3.2 Cilia and Melanin-Concentrating Hormone Pathway

While both genetic models and biochemical approaches have informed our current understanding of BBS the precise mechanism behind BBS-associated obesity is unknown. Many of the BBS proteins form a protein complex (BBSome) that is involved in proper cilia membrane formation (Nachury et al. 2007). Indeed there is evidence that the BBSome may be a membrane vesicle coat complex that is critical for establishing and maintaining the cilia membrane's signaling capabilities by directing specific receptors to this compartment (Jin et al. 2010). Furthermore, *Bbs* mutant mouse models appear to improperly localize several cilia-specific GPCRs, and most relevant with regard to obesity is the melanin-concentrating hormone receptor 1 (Mchr1) (Berbari et al. 2008). Mchr1 couples through G α i to reduce cAMP and decreases the frequency of spontaneous action potentials in hypothalamus (Gao and van den Pol 2001, 2002). Mch injections induce feeding behavior while Mchr1 mutant mice are resistant to diet induced obesity (Gomori et al. 2003; Chen et al. 2002). Intriguingly, Mchr1 fails to localize normally in neuronal cilia of *Bbs2*, *Bbs3*, and *Bbs4* mutant mice (Berbari et al. 2008; Zhang et al. 2011). Thus in both *Ift88* and *Bbs* obese mutants, Mchr1 fails to be properly localized creating a defect in Mchr1 signaling, possibly leading to the hyperphagic behavior in these models. Both

pharmacological and genetic agonism of the Mchr1 pathway are associated with hyperphagia while antagonism is associated with anorectic behavior, as such antagonism of this receptor has been of interest to the pharmaceutical industry (Qu et al. 1996; Borowsky et al. 2002; Ludwig et al. 2001; Shimada et al. 1998; Chen et al. 2002). However, assuming that the obese phenotype behind both *Bbs* and *lft* models is driven by a similar molecular pathway, then one would have to propose that in the absence of the cilium or the ability to reach the cilium that the Mchr1 pathway is ectopically activated or not efficiently desensitized after activation.

While there is circumstantial evidence for Mchr1 cilia mis-localization driving hyperphagia in cilia mutant mouse models, it is interesting to note that there is an emerging list of GPCRs that preferentially localize to neuronal cilia in different regions of the brain (Fig. 6.2b). Some of these neuronal cilia specific GPCRs include somatostatin receptor 3 (Sstr3), serotonin receptor 6 (5HT6), and dopamine receptor 1 (Drd1) (Handel et al. 1999; Schulz et al. 2000; Hamon et al. 1999; Brailov et al. 2000; Marley and von Zastrow 2010; Domire et al. 2011). While the significance of localizing these receptors within the ciliary compartment remain unknown, it is enticing to speculate that perhaps they may play a role in appetite and satiety, especially when one considers that the somatostatin, serotonin, and dopaminergic systems have all been implicated in either reward or feeding behaviors directly (Vijayan and McCann 1977; Aponte et al. 1984; Pollock and Rowland 1981; Salamone et al. 1990).

6.3.3 Cilia and the Mammalian Target of Rapamycin (mTOR) Pathway

Another pathway that may be involved in neuronal cilia regulation of satiety is the mammalian target of rapamycin (mTOR) pathway. mTOR signaling is complex and involves many factors (for an in depth review of mTOR and disease see Dazert and Hall 2011). mTOR is a serine/threonine protein kinase which as its name implies can be inhibited by the antifungal rapamycin. It has been established as a regulator/coordinator of cellular metabolic activity that responds to both the energy and stress levels experienced by the cell. It carries out these regulatory roles by participating in two protein complexes, the rapamycin-sensitive mTOR Complex 1 and the rapamycin-insensitive mTORC2. In general mTORC1 regulates translational control and mTORC2 is involved in cytoskeleton organization. While the functions of mTOR and its interactors have been determined in considerable detail at the genetic and cellular levels, the effects of mTOR signaling on the organismal level continue to emerge. Interestingly, there are several reports associating the cilium or its signaling proteins with overactivation of mTOR activity or in changes in the cytoskeleton and cell size (Sharma et al. 2011; Bell et al. 2011; Boehlke et al. 2010). In addition, rapamycin is able to partially rescue renal cystic disease in mouse models of PKD, further supporting a connection between cilia and mTOR (Shillingford et al. 2006, 2010). While the *in vivo* relevance of cilia and mTOR signaling with regards to the

obesity phenotype in cilia mutants remains to be determined, it is of note that mTOR signaling within the hypothalamus has been associated with obesity in other animal models (Cota et al. 2006). It will be interesting to determine if mTOR signaling activity within adult neurons is regulated through neuronal cilia and influences feeding behavior.

6.3.4 *Hedgehog Signaling and the Cilium*

The final pathway that we will discuss with regard to neuronal cilia and obesity is the hedgehog (Hh) pathway. Hh and its role in cilia and neuronal development is reviewed in Chap. 2 of this work by Mariani and Caspary. Several groups have demonstrated that canonical hedgehog signaling in mammalian cells utilizes the ciliary compartment. This is best demonstrated by the transient localization of several of the pathway components to the cilium and altered pathway activity when the cilium has been disrupted (reviewed in detail by Goetz and Anderson 2010). With regard to hedgehog signaling and neuronal cilia, there is a consensus emerging that primary cilia within the adult central nervous system sense hedgehog ligand and mediate the process of adult neurogenesis (Breunig et al. 2008; Han et al. 2008). Furthermore when neuronal cilia-mediated hedgehog signaling is altered in a gain of function fashion it can result in medulloblastoma and when it is disrupted in a loss of function fashion in the developing brain it is associated with a range of neurodevelopmental phenotypes (Chizhikov et al. 2007; Han et al. 2009).

If altered hedgehog signaling and cilia mutations have such profound effects on the adult and developing nervous system, how may they account for the hyperphagia associated obesity in adults? In both *Ift* conditional and *Bbs* models the possibility that altered hedgehog signaling in the developing hypothalamus can lead to obesity in adulthood has yet to be thoroughly investigated. For example, POMC-cre conditional *Ift88* mutant models appear normal other than the onset of hyperphagia and obesity, but the potential for a developmental phenotype remains. This becomes important when the expression pattern of POMC is taken into account. POMC is known to be expressed in places outside of the arcuate nucleus, of the hypothalamus such as the nucleus tract solitarius of the hindbrain, and the anterior and intermediate lobe of the pituitary in neonatal animals (Xu et al. 2005a). This becomes particularly important when the crucial role of hedgehog not only in the developing neural tube but also in the developing hypothalamus is taken into account (Szabo et al. 2009; Alvarez-Bolado et al. 2012). To address these potentials both *Ift* and *Bbs* conditional models need to be tested with inducible POMC-cre deletion. These experiments would also be useful in assessing whether different molecular mechanisms may be involved in driving obesity in *Bbs* and *Ift* mouse models.

The possibility remains that hedgehog signaling, which is required for adult neurogenesis is disrupted, thus contributing to hyperphagia. There are also reports of adult neurogenesis within the hypothalamus (Kokoeva et al. 2005; Xu et al.

2005c; Pierce and Xu 2010; Lee et al. 2012). Perhaps this process is compromised in CAGG-creER;*Ift88* conditional mutants. However, hyperphagic behavior is observed within 3 weeks of cilia loss in these mice and thus it may not have sufficient time to be a result of altered adult neurogenesis.

Finally a third potential for a non-canonical form of hedgehog signaling exists in the adult hypothalamus that requires neuronal cilia. The relevance of a non-canonical Hh pathway emerges when the expression pattern of certain pathway components in the adult brain is analyzed. For example, the Hh receptor, Patched, is expressed in regions of the brain that do not co-express the Hh effector Smoothed (Traiffort et al. 1998, 1999). This incongruence in pathway component expression pattern is especially true with regard to the hypothalamus (for a review of hedgehog in the adult brain see Traiffort et al. 2010). Furthermore it has been shown that hedgehog can directly alter neuronal activity (Bezard et al. 2003; Pascual et al. 2005). In developing spinal neurons, Hh stimulation causes a transient increase in Ca^{2+} activity that was dependent on Smo and $G\alpha i$ (Belgacem and Borodinsky 2011). Since hedgehog pathway components such as Patched are expressed in the adult hypothalamus, it is feasible that cilia may alter satiation responses through regulation of neuronal firing activity (Traiffort et al. 1998, 1999). Exploring whether loss of cilia alters this increase in Ca^{2+} in response to Hh in POMC neurons could prove to be a very fruitful avenue of investigation to connect cilia dysfunction to abnormal satiation.

6.4 Non-Mammalian Ciliopathy Models of BBS

Although more distantly related to human beings than mice, non-mammalian models have proven to be invaluable to the study of the role of human cilia and their relation to disease. This is particularly evident in the study of the assembly and maintenance of the cilium through IFT, and how disruption of this event can lead to certain phenotypes. This process, referred to as intraflagellar transport (IFT), was largely characterized biochemically using the small green algae, *Chlamydomonas*, and genetically using *C. elegans* (for an in depth review see Pedersen and Rosenbaum 2008). In this section we focus solely on the genes and proteins known to be associated with the obesity phenotype observed in ciliopathies, as such it will largely focus on the functional roles of the BBS genes in both *Chlamydomonas reinhardtii* and *Caenorhabditis elegans*, two of the most well studied non-mammalian organisms in regards to cilia/flagella biology

While *Chlamydomonas* has served as good model for biochemical purification of flagellar and IFT components it has also proven useful for comparative genomics studies in discovering new ciliopathy genes, such as *BBS5* (Kulaga et al. 2004). Through the use of this simple model, elegant studies have begun to shed new light on how the BBS proteins may function as modulators of ciliary signaling and even serve as structural components of the transition zone (Lechtreck et al. 2009; Craige et al. 2010).

Much of what we know about the molecular motors that mediate cilia formation and maintenance has come from studies visualizing IFT movement in the cilia of *C. elegans*. In *C. elegans* cilia of the sensory neurons it has been demonstrated that both BBS7 and BBS8 serve as adaptors to the IFT complexes and their cargoes (Blacque et al. 2004), however, whether they play similar roles in mammalian systems has not been determined. Interestingly, it has been shown that *C. elegans* ciliary morphology can change dependent on cilia-mediated signaling and that the phenotypes of *bbs* mutant worms can be ameliorated by altering the downstream second messengers (Tan et al. 2007; Mukhopadhyay et al. 2008; Mok et al. 2011). Recent work has also suggested that altered neuroendocrine signaling and exocytosis of factors such as insulin drives many of the phenotypes observed in *bbs* mutant worms (Lee et al. 2011). Interestingly, another study points to more general roles for *bbs* proteins in cilia membrane homeostasis (Kaplan et al. 2012). Although the invertebrates lack many of the organ systems present in mammals, it is clear that both *C. elegans* and *Chlamydomonas* models offer advantages in both cost, time and in some instances genetic tractability. These models will continue to provide insights into the fundamental processes that are mediated by the ciliopathy proteins and the cilium and thus further serve to inform our understanding of complex phenotypes such as feeding behavior and the regulation of appetite and satiety.

6.5 Conclusion

In summary, remarkable progress has been made in the past 20 years demonstrating the clinic importance of the cilium in many tissues and developmental processes. Despite this progress, there remain several key questions that must be addressed before we can understand the molecular and cellular mechanisms responsible. Hopefully, as research on the rare ciliopathies advances we will gain an understanding of fundamental processes such as satiety and appetite that we can then apply to direct therapeutic strategies for an exceedingly common clinical feature such as obesity.

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