

Charles University

Faculty of Science

Study programme: Biology

Branch of study: Biology



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Searching for the common function of the BBSome across the
evolution and development

Hledání jednotné funkce BBSomu napříč evolucí a vývojem

Bachelor's thesis

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Prague, 2022

Poděkování:

Ráda bych poděkovala mému školiteli Mgr. Ondřeji Štěpánkovi, Ph.D. za trpělivost, ochotu a hlavně za odborné rady, které mi poskytl při psaní této bakalářské práce. Dále bych chtěla vyjádřit poděkování své rodině za neutuchající podporu. V neposlední řadě také děkuji i všem kolegům z laboratoře za cenné zkušenosti, které se mnou ochotně sdílejí.

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 02.05.2022

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Abstract

The BBSome is a protein complex whose function is associated with ciliary trafficking. It has been found that the BBSome is evolutionarily conserved among ciliated organisms. The disruption of the BBSome leads to cilia dysfunctions and affects many signalling pathways. In humans, genetic defects in the BBSome are the cause of Bardet-Biedl Syndrome, which is a pleiotropic disease. The BBSome is studied separately in various model organisms and cell lines to understand the molecular functions of the BBSome. These studies have not been compared to see if there is a common BBSome function, which could be why the BBSome is evolutionarily conserved among ciliated organisms. In this bachelor's thesis, the knowledge about the BBSome was summarized and compared to identify a putative common function of the BBSome among various model organisms or cell types. It seems that there has not been found any BBSome function that could be identified as the common function because the BBSome has many specialized functions in different organisms and tissues. The only distant similarity is BBSome-dependent ciliary retrograde transport, which has been described in most of the studied model organisms and cell types.

Keywords:

BBSome, Bardet-Biedl Syndrome, function, evolution, cilia, model organisms

Abstrakt

BBSom je proteinový komplex, jehož funkce je spojena s ciliárním transportem. Bylo zjištěno, že BBSom je evolučně konzervován napříč organismy tvořícími cilie. Narušení BBSomu vede k ciliárním dysfunkcím a ovlivňuje i mnoho signálních drah. U člověka jsou genetické defekty BBSomu příčinou pleiotropického onemocnění, a to Bardet-Biedlova syndromu. BBSom je studován jednotlivě v mnoha modelových organismech a buněčných liniích za účelem pochopení molekulárních funkcí BBSomu. Tyto studie nebyly zatím porovnány, aby se zjistilo, zda existuje jednotná funkce BBSomu, která by mohla být důvodem jeho evoluční konzervovanosti mezi ciliárními organismy. V této bakalářské práci byly znalosti týkající se BBSomu shrnuty a porovnány za účelem identifikace domnělé jednotné funkce BBSomu mezi různými modelovými organismy a buněčnými typy. Zdá se, že nebyla nalezena funkce BBSomu, která by se dala označit za jednotnou funkci, důvodem je zřejmě mnoho specializovaných funkcí BBSomu v různých organismech a tkáních. Jediná vzdálená podobnost je BBSom dependentní retrogradní ciliární transport, který byl popsán ve většině studovaných modelových organismů a buněčných typů.

Klíčová slova:

BBSom, Bardet-Biedlov syndrom, funkce, evoluce, cilie, modelové organismy

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List of abbreviations

AgRP	Agouti-related peptide
AMPK	AMP-dependent protein kinase
AQP2	Aquaporin 2
AVRP2	Vasopressin receptor 2
BBS	Bardet-Biedl Syndrome
D1	Dopamine receptor 1
ESAG2	Expression-site associated gene 2
GPCRs	G-protein coupled receptors
GPR161	G-protein coupled receptor 161
HCD	Human collecting duct
HEK-293	Human embryonic kidney 293
HK-2	Human kidney 2
(HT) _{2c} R	Serotonin 5-hydroxytryptamine receptor
IFT	Intraflagellar transport
IFT-A	Intraflagellar transport protein A
IFT-B	Intraflagellar transport protein B
IMCD3	Inner medullary collecting duct 3
IR	Insulin receptor
ISG	Invariant surface glycoprotein family
LECA	Last eukaryotic common ancestor
LOF	Loss-of-function
LRb	Leptin receptor b
MBAP	Membrane bound acidic phosphatase
MEF	Mouse embryonic fibroblast

MCH	Melanin-concentrating hormone
MCHR1	Melanin-concentrating hormone receptor 1
NICD	Notch intracellular domain
NPY	Neuropeptide Y
NPY2R	Neuropeptide Y receptor type 2
ODR-10	Olfactory serpentine receptor class r-10
OSM-9	Osmotic avoidance family member 9
PC-1	Polycystin-1
PC-2	Polycystin-2
PKD-2	<i>C. elegans</i> orthologue of human polycystin-2
PLD	Phospholipase D
POMC	Proopiomelanocortin
PTCH	Patched
RPE	Retinal pigment epithelium
SHH	Sonic hedgehog
SMO	Smoothened
SSTR3	Somatostatin receptor 3
SuFu	Suppressor of Fused
STPK	Serin/ Threonine protein kinase
TRPV1	Transient receptor potential cation channel subfamily V member 1
TRPV4	Transient receptor potential cation channel subfamily V member 4
VSG	Glycosylphosphatidylinositol-anchored variant surface glycoprotein

1. Introduction

Bardet-Biedl Syndrome (BBS) is a rare genetic pleiotropic human disease, which was first described by Laurence and Moon in 1866. Later, it was proposed that BBS is associated with ciliary dysfunctions and BBS proteins (Ansley et al., 2003). Furthermore, eight of these BBS proteins form a complex called the BBSome (Loktev et al., 2008; Nachury et al., 2007). The BBSome is evolutionary conserved among ciliated organisms and its function is associated with the cilium (Nachury et al., 2007).

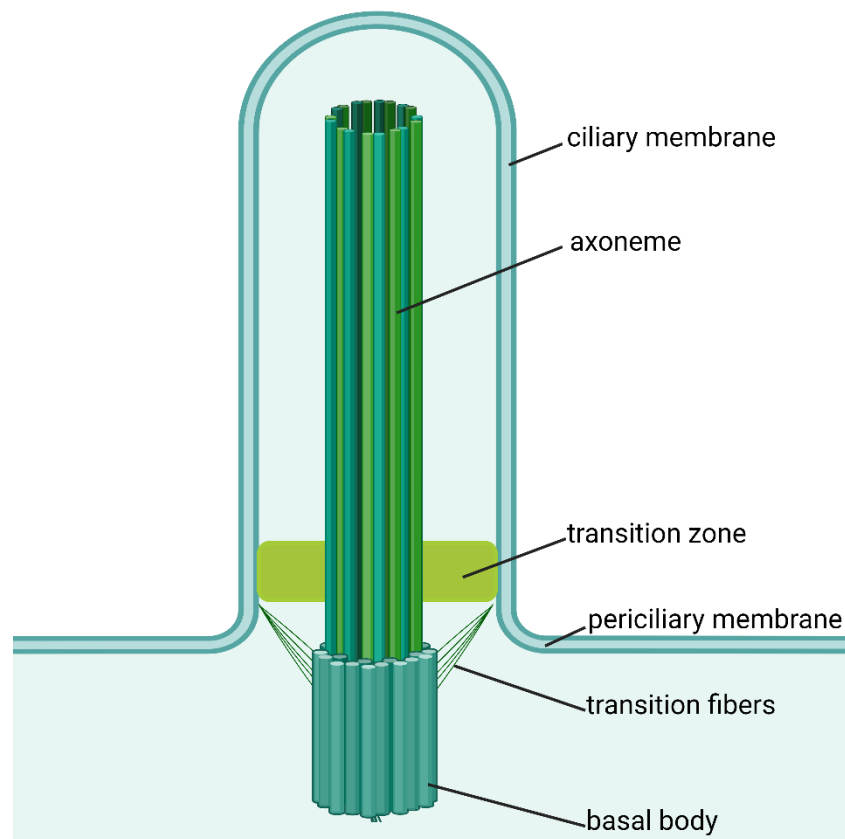


Figure 1 The structure of the cilium. In general, the surface of the cilium is formed by a ciliary membrane, which turns into the periciliary membrane at the base. Moreover, the central part of the cilium is formed by an axoneme, which consists of microtubules, and it is anchored in the basal body. In addition, the proximal region of the cilium, called the ciliary gate, is formed by a transition zone and transition fibers (modified from Ainsworth, 2007; Szymanska and Johnson, 2012).¹

Two types of cilia are described: motile (flagella) and non-motile (primary). Motile cilia provide motility, but the primary cilium works as a cell antenna that is involved in cell signalling (reviewed in Satir and Christensen, 2008; Silflow and Lefebvre, 2001). These two types of cilia differ in the axonemal structure because the axoneme is formed from 9+2 microtubules in motile cilia and 9+0

¹ All figures in this bachelor's thesis were created by the author using BioRender. <https://biorender.com/>

microtubules in primary cilia. The BBSome was found in both types of cilia: flagella and primary cilia, depending on the organism (van Dam et al., 2013).

Studies on various model organisms were performed to understand the molecular function of the BBSome. *Chlamydomonas reinhardtii*, *Trypanosoma brucei*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Danio rerio*, *Mus musculus*, or *Homo sapiens* are the main model organisms, which are used for the BBSome study. *Chlamydomonas reinhardtii* (Class: Chlorophyceae) is a single-cell green alga. *C. reinhardtii* is used to study ciliopathies, due to similar cilia structures and ciliary proteins to mammals (reviewed in Sasso et al., 2018). *Trypanosoma brucei* (Class: Kinetoplastea) is a parasite that is transmitted by Tsetse fly and causes sleeping illness or African trypanosomiasis. *Caenorhabditis elegans* (Class: Chromadoera) is a hermaphrodite/male nematode, used as a model organism due to conserved cellular and molecular pathways between *C. elegans* and *H. sapiens* (reviewed in Kaletta and Hengartner, 2006). *Drosophila melanogaster* (Class: Insecta) is a gonochoristic fly, where basal bodies and signal transduction mechanism mediated by cilia are studied (reviewed in Jana et al., 2016; Vincensini et al., 2011). *Danio rerio* (Class: Actinopterygii) is a freshwater fish, which is used mainly in developmental studies. Finally, clinical manifestations of cilia dysfunctions are studied in *Mus musculus* and *Homo sapiens* (Class: Mammalia) (reviewed in Vincensini et al., 2011). Even though the BBSome is present in all of these organisms, the BBSome differs between model organisms in the number of protein subunits and the percent identity (similarity), which is related to human (Table 1).

Protein	Percent identity of the BBSome subunits with their human orthologues					
	<i>C. reinhardtii</i>	<i>T. brucei</i>	<i>D. melanogaster</i>	<i>C. elegans</i>	<i>D. rerio</i>	<i>M. musculus</i>
ARL6/BBS3	46	47	56	44	90	95
BBS1	40	31	30	28	68	93
BBS2	46	32	not found	33	74	90
BBS4	51	40	35	38	77	89
BBS5	53	40	45	45	91	98
BBS7	33	21	not found	34	76	92
BBS8	51	37	33	41	76	95
BBS9	51	29	28	23	63	84
BBIP	not found	not found	42	53	77	89

Table 1 Comparison of the percent identity of the BBSome subunits orthologues with human proteins. This comparison table was created by the author using BLAST² and TriTrypDB³. The protein of the BBSome in each model organism was compared to the corresponding protein of the human BBSome. The ARL6/BBS3 protein is not a subunit of the BBSome, but it is an important GTPase, which interacts with the BBSome (Jin et al., 2010). Therefore, ARL6 was added to the comparison table. In

² <https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>

³ <https://tritrypdb.org/tritrypdb/app/>

summary, *ARL6* and *BBS5* proteins have the largest homology among model organisms. The lowest homology was found for *BBS1* and *BBS9* proteins.

Overall, the BBSome is studied in many ciliated organisms, where BBSome subunits are evolutionarily conserved (Table 1). However, the results from particular model organisms have not been compared. Therefore, it is unclear whether the BBSome has a common function in the most ciliated organisms, as suggested by its high level of conservation, and/or whether the BBSome function was repurposed during evolution to establish unique functions in different organisms.

This bachelor's thesis aims to assess whether the putative common function of the BBSome between model organisms can be identified. In this study, I will compare published studies on the BBSome in various model organisms. Knowledge about the BBSome and its specific functions in these model organisms will be summarized. Finally, these summarized studies will be compared to identify the putative ancestor or common function of the BBSome in these species.

2. BBSome in *Chlamydomonas reinhardtii*

In *Chlamydomonas reinhardtii*, the BBSome consists of BBS1, -2, -4, -5, -7, -8, -9 (van Dam et al., 2013). It means that all human subunits except BBIP are conserved in *C. reinhardtii* (Lechtreck et al., 2009). There is 40-53 % identity between the BBSome of *C. reinhardtii* and *H. sapiens* BBSome, except for the BBS7 protein, there is only 33 % identity (Table 1). It was observed that proteins BBS1, -4, -5, -7, -8 enter two *C. reinhardtii* flagella (Lechtreck et al., 2009).

Three BBSome mutants of *C. reinhardtii* carrying mutations in BBS1, BBS4, or BBS7 are not capable of phototaxis (Lechtreck et al., 2009; Pazour, 1995). These mutations are classified as loss-of-function (LOF) mutations. The length and ultrastructure of flagella are not affected in these mutants (Lechtreck et al., 2009). The BBS4 mutant was studied in greater detail. It was shown that their axonemes cannot respond normally to the light-induced intraflagellar changes in Ca^{2+} concentration, which is important for phototaxis. The phototaxis in BBS4 mutant cells is transiently restored after the cell division and the formation of the new flagella (Lechtreck et al., 2009).

Accumulation of the signalling proteins in flagella is caused by the loss of function of some BBSome subunits (Lechtreck et al., 2009). The proteomic analysis revealed the accumulation of Phospholipase D (PLD) (Lechtreck et al., 2009), AMP-activated protein kinase (AMPK) type of Serine/Threonine protein kinase (STPK) (Liu and Lechtreck, 2018), and Putative truncated haemoglobin (THB1) in flagella of BBS1, BBS4 and BBS7 mutants (Lechtreck et al., 2009). As PLD was proposed as a negative regulator of phototaxis (Lechtreck et al., 2009), is plausible that the accumulation of PLD in the flagellum of BBSome-deficient *Chlamydomonas* causes the loss of phototaxis (Liu and Lechtreck, 2018).

PLD enters the flagellum by diffusion (Liu and Lechtreck, 2018) independently on the BBSome and intraflagellar transport (IFT) proteins (Lechtreck et al., 2013). In contrast, the BBSome is required during the export (Lechtreck et al., 2013). The BBSome is a cargo adapter, connecting PLD with the IFT proteins (Lechtreck et al., 2013, 2009). At the flagellar base, the BBSome is bound by an anterograde IFT train. At the tip, the BBSome is released from the train and it is recruited by ARL6-GTP to the membrane to load the PLD (Liu and Lechtreck, 2018; Mourão et al., 2014). PLD-loaded BBSome bounds back to an anterograde IFT train, which forms a complex with retrograde IFT trains (Xue et al., 2020). Export from the flagellum is secured by retrograde IFT trains and dynein (Pazour et al., 1998).

Overall, the BBSome works as a cargo adapter in retrograde flagellar transport in *C. reinhardtii* (Lechtreck et al., 2009). A soluble membrane-associated protein, PLD was described as a *bona fide* BBSome cargo (Lechtreck et al., 2013), whose accumulation in cilia impairs the phototaxis in BBSome-deficient cells (Liu and Lechtreck, 2018).

3. BBSome in *Trypanosoma brucei*

Trypanosoma brucei has all of the BBSome subunits except BBIP (van Dam et al., 2013). There was found 31-40 % identity between the BBSome proteins in *H. sapiens* and *T. brucei*, except BBS7 with only 21 % identity (Table 1). The BBSome localizes to the flagellar pocket membrane, membranes of neighbouring vesicles (Langousis et al., 2016) and the basal body⁴. Unlike in *Chlamydomonas*, the BBSome was not observed to enter the flagellum (Langousis et al., 2016).

LOF mutations in BBS1, BBS4, BBS5, BBS7, or BBS9 were prepared. These mutants did not reveal any defects in the flagella morphogenesis. Moreover, these mutants showed normal motility and viability. However, decreased virulence was observed in BBS1 knockout *T. brucei* in infected mice. Accordingly, the BBS1 subunit was identified as a positive regulator of virulence in *T. brucei* (Langousis et al., 2016) and also in a related organism *Leishmania major* (Price et al., 2013).

As the flagellar pocket is an area of rapid clathrin-mediated endocytosis (Allen et al., 2003), the BBSome might be involved in this process. However, the BBSome is dispensable for clathrin-mediated endocytosis of the transferrin receptor (Langousis et al., 2016). It seems, that the BBSome is mostly responsible for the postendocytic sorting of plasma membrane proteins. Proteomic analysis of BBSome mutants revealed most overabundant proteins on the plasma membrane due to defects in sorting: membrane-bound acidic phosphatase (MBAP), expression-site associated gene 2 (ESAG2), glycosylphosphatidylinositol-anchored variant surface glycoprotein (VSG) related proteins and three members of invariant surface glycoprotein family (ISG) included ISG75 (Langousis et al., 2016).

⁴ <http://tryptag.org/>

It was shown that ISG75 transport to late endosome and lysosome is dependent on ubiquitination (Leung et al., 2011). The BBSome deficiency caused the accumulation of this protein on the plasma membrane. Finally, the pull-down of the *Trypanosoma*'s cell extracts with monoubiquitin beads revealed that the BBSome interacts with ubiquitin. The BBSome can be involved in the trafficking of ubiquitinated proteins in *T. brucei* (Langousis et al., 2016).

Generally, the BBSome in *Trypanosoma brucei* does not localize to the flagellum but mostly to the flagellar pocket membrane. However, the BBSome is involved in the postendocytic sorting of membrane proteins (Langousis et al., 2016).

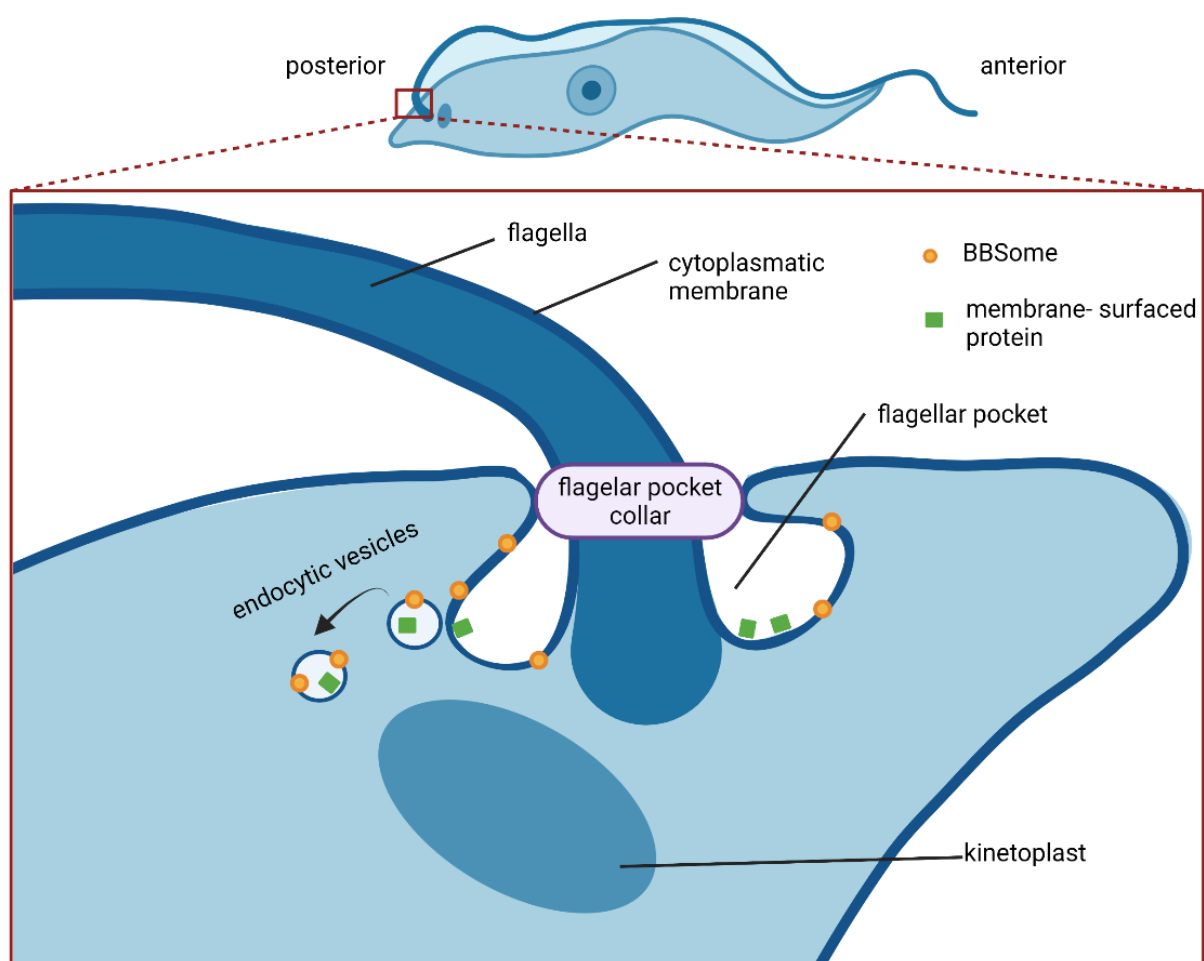


Figure 2 The localization of the BBSome and morphology of the flagellar pocket in *Trypanosoma brucei* (modified from Field and Carrington, 2009).

4. BBSome in *Caenorhabditis elegans*

In *Caenorhabditis elegans*, the BBSome consists of all eight subunits (van Dam et al., 2013). There was found 23-53 % identity between the BBSome proteins in *C. elegans* and *H. sapiens* (Table 1). GFP-tagged BBSome subunits: BBS1, BBS2, BBS7, BBS8 localize mainly at the transition zone and around the axoneme of cilia (Blacque et al., 2004). However, another research group observed the BBS8 subunit only at the basal body (Ansley et al., 2003). Overall, the BBSome enters the cilium in *C. elegans* (Ansley et al., 2003; Blacque et al., 2004).

Primary (non-motile) cilia, established on dendritic endings of sensory neurons in *C. elegans* (White et al., 1986), have mechanosensory (Chalfie and Sulston, 1981) or chemosensory functions (Bargmann and Horvitz, 1991). There were described twenty-five classes of ciliated neurons (White et al., 1986). Sensory neurons are present in small sensory organs, and more complex bilateral organs in the head (amphid) and the tail (phasmid).

LOF mutations in *bbs-1*, *bbs-7*, *bbs-4*, *bbs-5*, and *bbs-8* were prepared (Blacque et al. 2004; Ou et al., 2005; Wei et al., 2012; Xu et al., 2015). LOF mutations in *bbs-8* and *bbs-7* led to structural and functional defects of the cilium, such as cilia shortening, and decreased chemosensory ability (Blacque et al., 2004). DYF-2 protein was described as an important IFT protein that provides structural and functional integrity, and also interacts with anterograde, retrograde train, and with the BBSome (Efimenko et al., 2006). Decreased chemotaxis was observed in the *dyf-2* mutant (Efimenko et al., 2006).

The BBSome was previously described as a *bona fide* IFT protein, which can associate with anterograde (IFT-B)/retrograde (IFT-A) IFT proteins or with both at the same time (Blacque et al., 2004). It was also proposed that the BBSome itself can link IFT-B and IFT-A machineries (Ou et al., 2005). Later, it was suggested that the BBSome regulates the assembly of anterograde and retrograde trains at the ciliary base and their reassembly at the ciliary tip. After the assembly/reassembly, the BBSome is bound as cargo to the anterograde train and the interaction with IFT proteins is stabilized by DYF-2 (Wei et al., 2012).

Defects of the IFT transport in cilia were described in different *bbs* mutants (Blacque et al., 2004; Ou et al., 2005; Wei et al., 2012). In *dyf-2* mutants of *C. elegans*, the BBSome localized at the basal body and the BBSome was not affiliated with IFT proteins. In the *bbs-1* mutant, the same phenotype was observed, as the BBSome were accumulated at the ciliary base. IFT-B were accumulated at the ciliary tip because IFT-B could not be linked with IFT-A during the IFT train reassembly, and IFT-B cannot be removed from the cilium (Wei et al., 2012). In *bbs-7* and *bbs-8* mutants, it was observed that

anterograde and retrograde IFT proteins are moving independently and there is an accumulation of IFT-B in the middle and distal segments of the cilium (Ou et al., 2005).

Accumulations of the sensory receptors in cilia, e.g., mechanosensory osmotic avoidance family member 9 (OSM-9), olfactory serpentine receptor class r-10 (ODR-10), and an orthologue of human polycystin-2 (PKD-2) were detected in *bbs* mutant cells. The accumulation of OSM-9 is caused by defects in the IFT transport connected with the lack of the BBSome (Xu et al., 2015). However, PKD-2 and ODR-10 are not IFT-dependent proteins (Qin et al., 2005). The PKD-2 is a ubiquitin-dependent protein and its transport from the cilia to the lysosome requires the BBSome (Xu et al., 2015), similar to described monoubiquitin dependent transport and postendocytic sorting in *Trypanosoma brucei* (Langousis et al., 2016).

Furthermore, the BBSome is involved in the transport of Rab28 protein. Rab28 is one of the ciliary proteins, which is expressed in ciliary neurons (Jensen et al., 2016). The BBSome regulates the recruitment of GTP-activated Rab28 to the periciliary membrane and its docking with IFT proteins. Rab28 negatively regulates the shedding of extracellular vesicles, which mediate the communication with glia cells (Akella et al., 2020).

The observation of *bbs-1* mutant revealed increased lipid accumulation. It was proposed that lipid homeostasis and accumulation are dependent on the right structure and function integrity of 15 ciliated neurons that are involved in the detection of nutrients (Mak et al., 2006). It has not been described if ciliated neurons work as a regulatory centre or if each neuron can work independently on the others (reviewed in Srinivasan, 2015). Molecular mechanisms of the lipid turnover, connected with the BBSome have not been described in *C. elegans*. It is not clear if the lipid storage is dependent on targeted neuroendocrine signals from ciliated neurons or if it is dependent on the reception pathway in its tissue (Mak et al., 2006).

In conclusion, the BBSome enters the cilium in *C. elegans*. Accumulations of cilia proteins at the ciliary tip were observed in BBS deficient cells (Xu et al., 2015), which is in line with PLD in *C. reinhardtii* (Lechtreck et al., 2009). In contrast to *C. reinhardtii*, the evidence for the cargo adapter function of the BBSome in *C. elegans*, the BBSome was identified as a cargo adapter that mediates interaction with PLD and IFT proteins (Lechtreck et al., 2013, 2009). The BBSome was characterized more as a stabilizer and coupler of IFT-A and IFT-B in *C. elegans*. It was also proposed that the BBSome is involved in monoubiquitin-dependent transport in *C. elegans* (Xu et al., 2015), which parallels the observation in *T. brucei* (Langousis et al., 2016).

5. BBSome in *Drosophila melanogaster*

In *Drosophila melanogaster*, there were described only six subunits of the BBSome. Subunits Bbs2 and Bbs7 are missing (van Dam et al., 2013). There was found 28-45 % percent identity between the corresponding BBSome proteins in *D. melanogaster* and *H. sapiens* (Table 1).

The recombinant expression of the *H. sapiens* BBSome in insect cells revealed that the BBSome is formed from six subunits: BBS1, BBS4, BBS5, BBS8, BBS9, BBIP. This incomplete *H. sapiens* BBSome is more stable than the fully assembled BBSome (eight subunits) in insect cells (Klink et al., 2017). BBS7 and BBS2 form a lobe, which blocks ARL6 interaction with BBS1 in bovine BBSome. This suggests that a conformation change is required for ARL6 binding (Klink et al., 2020), which facilitates the recruitment of the BBSome to the membrane (Jin et al., 2010). It was proposed that this hexameric BBSome is still able to bind ARL6 and its cargo, e.g., GPCRs (G-protein coupled receptors), and polycystin-1. Furthermore, the BBSome binding motifs were observed also in polycystin-2, leptin receptor, and fibrocystin but the binding assay did not reveal interactions with the BBSome (Klink et al., 2017). However, no study has been published that would directly refer to the entry of the hexameric BBSome into the *D. melanogaster* cilium during the elaboration of this bachelor's thesis.

Furthermore, *Drosophila* has two types of cilia, i.e., motile, and non-motile. Non-motile cilia were described at distal parts of sensory neurons, e.g., olfactory neurons. Furthermore, motile cilia are present in chordotonal neurons and spermatids (reviewed in Jana et al., 2016). Since *Drosophila* BBSome mutants have not been prepared, the physiological role of the BBSome in either type of cilia is unknown.

Generally, *Drosophila melanogaster* encodes only six out of eight BBSome genes. It suggests that it has an incomplete BBSome lacking Bbs7 and Bbs2. However, its function has not been described.

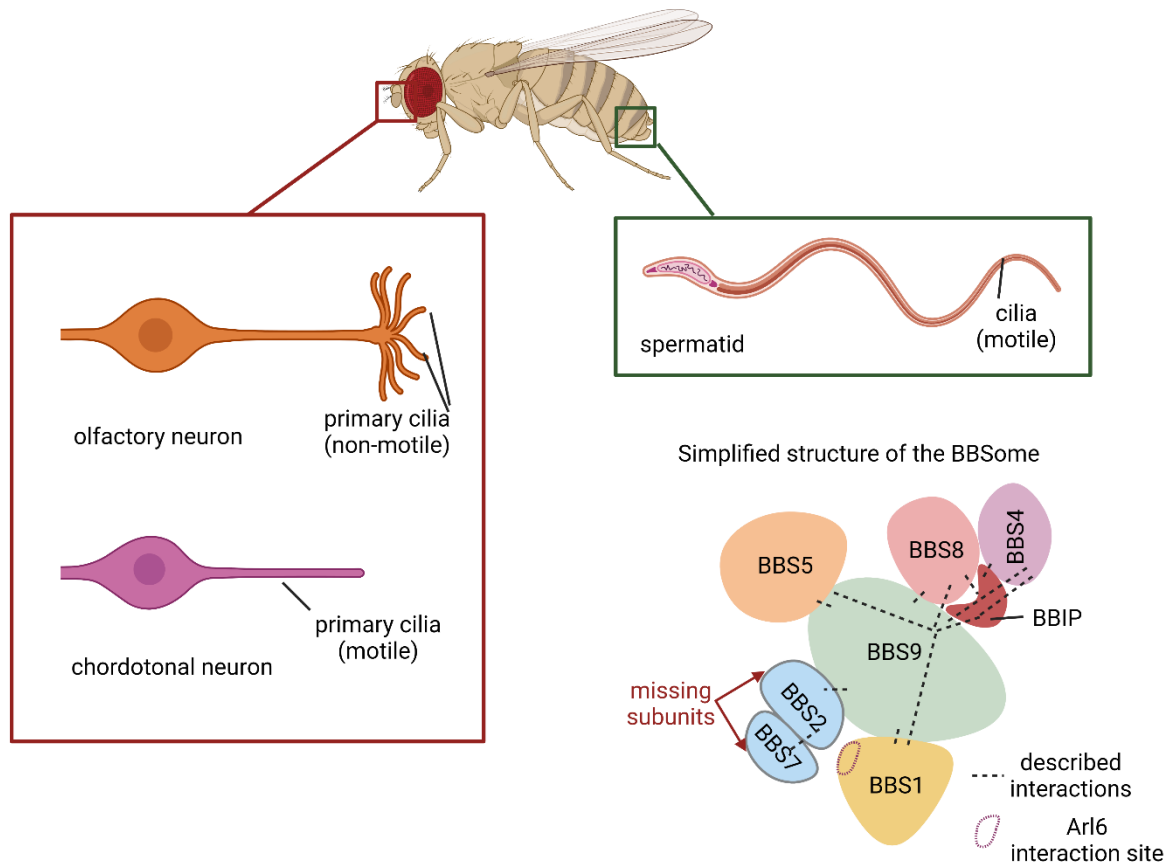


Figure 4 The localization of ciliated cells in *Drosophila melanogaster* and simplified structure of the BBSome (modified from Jana et al., 2016; Klink et al., 2017)

6. Vertebrate models of the BBSome

The BBSome is formed from eight proteins in mammals and *Danio rerio* (van Dam et al., 2013). The percent identity of *Mus musculus* BBSome subunits is from 84-95 % and the percent identity of *Danio rerio* BBSome subunits is only 68-91 % compared to the *Homo sapiens* BBSome subunits (Table 1). The entry of the BBSome into cilia is dependent on ARL6 protein (Zhang et al., 2011), which mediates interactions with the ciliary membrane (Jin et al., 2010; Zhang et al., 2011). However, the ARL6/BBS3 is not a part of the BBSome (Loktev et al., 2008; Nachury et al., 2007).

Two types of cilia – motile and primary are described in vertebrates. Motile cilia were found in sperm, epithelial cells of the respiratory tract and oviduct, or in ependymal cells, which line brain vesicles. A wide variety of cell types with primary cilia were described in many organs, e.g., neurons, epithelial cells, osteoblast, osteocytes, or smooth muscle endothelial cells (Li et al., 2021). Finally, it was shown that defects in the BBSome mainly affect primary cilia and sperm flagella (Shoemark et al., 2015).

BBSome mutations lead to Bardet-Biedl Syndrome (BBS) in humans. The main symptoms of BBS include retinal dystrophy, polydactyly, obesity, genital abnormalities, renal anomalies, and cognitive

impairment (reviewed in Forsythe and Beales, 2013). It is difficult to study molecular mechanisms of BBS symptoms in a complex human body, so other pathways are being sought.

To facilitate the study of BBSome functions, mice models with various *Bbs* gene knockouts were established. It is obvious that differences between phenotypes of various *Bbs* knockout mice models were observed. In general, *Bbs* mice models phenocopy the main symptoms of BBS such as retinal dystrophy (Bales et al., 2020; Hsu, 2021; Hsu et al., 2017; Kretschmer et al., 2019), obesity (Berbari et al., 2008b; Domire et al., 2011; Guo et al., 2016; Kulaga et al., 2004; Rahmouni et al., 2008; Zhang et al., 2013), however, renal defects were not observed so often (Guo et al., 2011), and the polydactyly phenotype has not been reproduced in *Bbs* knockout mice models. ARL6 (ARL6/BBS3 is not a part of the BBSome.) knockout mice developed retinal degeneration and increase body fat (not obesity) as well. In addition, hydrocephalus or hypertension was described in *Arl6* knockout mice, which is in contrast to other *Bbs* mice knockouts (Zhang et al., 2011).

Mice models are also used to establish specific cell lines, which can offer better conditions for observing specific molecular mechanisms. Of course, established human cell lines are also used for this purpose. In addition, *Danio rerio* is sometimes used together with mice models or cell lines in BBSome studies. Finally, functions of the BBSome related to the studied cell type or model will be described below.

6.1. Generic role of the BBSome

The generic role of the BBSome is meant as a function, which can be found in multiple tissues (organs), i.e., it is not unique for a specific cell type.

6.1.1. Cilia dependent and independent roles of the BBSome

Previously, it was hypothesized that the BBSome serves as a dense-planar coat associated with the liposome surface and that it mediates lateral transport between the plasma and the ciliary membrane (Jin et al., 2010). However, the model first described in *C. reinhardtii* is generally accepted. It says that the BBSome is transported by IFT-A/B machinery inside the cilium and that the BBSome is a cargo adapter (Lechtreck et al., 2009). The BBSome is linked to the IFT-B machinery by IFT-27 protein, which is a GTPase (Eguether et al., 2014). IFT-38 protein is another IFT-B (Taschner et al., 2016). It was proposed that IFT-27 protein promotes the ciliary exit of the BBSome, ARL6, and its cargo, e.g., GPCRs (Liew et al., 2014). In mouse olfactory cilia, this model was confirmed and supplemented by the fact that the BBSome also links IFT proteins (Williams et al., 2014). However, whether the BBSome is required mainly for anterograde or retrograde protein transport in cilia remains controversial.

Interestingly, it was described that the BBSome can also have cilia-independent functions. In some studies, it was proposed that the BBSome is required for proper vesicular protein trafficking to the plasma membrane (Guo et al., 2016; Nachury et al., 2007; Starks et al., 2015). This theory is supported by the observation that the BBSome interacts with Rabin8 in the basal body (Nachury et al., 2007). Rabin8 is a Rab8 exchange factor (Hattula et al., 2002). Finally, it was shown that Rab8 and Rabin8 mediate the docking and fusion of post-Golgi vesicles with the plasma membrane (Huber et al., 1993). Overall, these BBSome cilia-independent functions are not well described.

6.1.2. Sonic hedgehog signalling pathway

The Sonic hedgehog (SHH) signalling pathway controls tissue patterning during the development, and post-embryonic tissue regeneration (reviewed in Choudhry et al., 2014). The signalling pathway consists of SHH ligand, Patched (PTCH) receptor, Smoothened (SMO) receptor, Gli transcription factors, and their repressor Suppressor of Fused (SuFu) (reviewed in Wheway et al., 2018). In the inactivated state, the PTCH receptor is located on the ciliary membrane and suppresses SMO receptor ciliary localization (reviewed in Rohatgi et al., 2007), Gli proteins are suppressed by SuFu at the ciliary tip (Zeng et al., 2010). When the pathway is activated, SHH ligands bind to the PTCH receptor. Then, PTCH exits the cilium, and the SMO receptor is allowed to enter the cilium (reviewed in Rohatgi et al., 2007). Finally, the SMO receptor suppresses SuFu and Gli proteins can be translocated to the nucleus to activate transcription (reviewed in Wheway et al., 2018). G-protein coupled receptor 161 (GPR161) is a “model GPCR” and it is a negative regulator of the SHH signalling pathway (Mukhopadhyay et al., 2013). Therefore, GPR161 is removed from the cilium and trafficked to the endosomal sorting compartment when the SHH pathway is activated (Mukhopadhyay et al., 2013).

GPR161 accumulation was detected in the cilium of *Ift27* mutated inner medullary collecting duct 3 cells (IMCD3) (Liew et al., 2014) and *Ift38* knockout retinal pigment epithelium (RPE) cells (Nozaki et al., 2019). Moreover, the accumulation of GPR161 was observed also in *Ar/6* knockout IMCD3 cells, where the pathway was activated (Liew et al., 2014). Taken together, it was suggested that the BBSome and IFT-B are required for the export of GPR161.

However, GPR161 is not the only component of the SHH signalling pathway that was accumulated in the cilia of BBSome-deficient cells. SMO and PTCH were also observed to be accumulated in cilia of *Bbs* knockouts of mouse embryonic fibroblast (MEF) cells as well as in MEF cells with *Bbs* LOF mutation (Zhang et al., 2012). In addition, accumulation of SMO was also observed in *Ar/6* knockout MEF cells, where the SHH signalling pathway was not activated (Liew et al., 2014; Zhang et al., 2012). Finally, it was proposed that the BBSome is involved in the retrograde transport of PTCH, SMO, and GPR161. This theory can be supported by described interactions between the BBSome and SMO (Yang et al.,

2020; Zhang et al., 2012), or PTCH (Zhang et al., 2012). GPR161, SMO, and PTCH receptors are proposed to be BBSome cargoes.

On the other hand, the observation of *Ar/6* knockout IMCD3 cells revealed an increased ubiquitin signal in cilia. Therefore, it was hypothesized that the exit of GPCRs from the cilium is ubiquitin-dependent (Shinde et al., 2020). Indeed, GPR161 was shown to exit the cilium through ubiquitin-dependent transport when the SHH pathway is activated (Shinde et al., 2020) and finally that also the SMO is polyubiquitinated when the pathway is not activated (Desai et al., 2020). Therefore, it seems that the BBSome recognizes these ubiquitinated proteins and together with IFT-B ensures their export from cilia (Desai et al., 2020; Shinde et al., 2020). In general, these observations correspond to the BBSome-mediated monoubiquitin-dependent transport in *C. elegans* (Xu et al., 2015) and *T. brucei*, where the BBSome was shown to interact with ubiquitin (Langousis et al., 2016). In contrast, none of these ubiquitin-dependent transport pathways in *C. elegans* or *T. brucei* was identified as IFT proteins-dependent. In *T. brucei*, the transport is even completely cilia independent. Nevertheless, the interaction between the BBSome and ubiquitin seems to be an interesting parallel across organisms.

All in all, the BBSome is required for IFT-B-dependent retrograde transport of PTCH, SMO, and GPR161. It was shown that the ciliary exit of SMO and GPR161 is ubiquitin-dependent. The BBSome recognizes ubiquitin marks of these cargoes to facilitate their ciliary exit along with IFT-B.

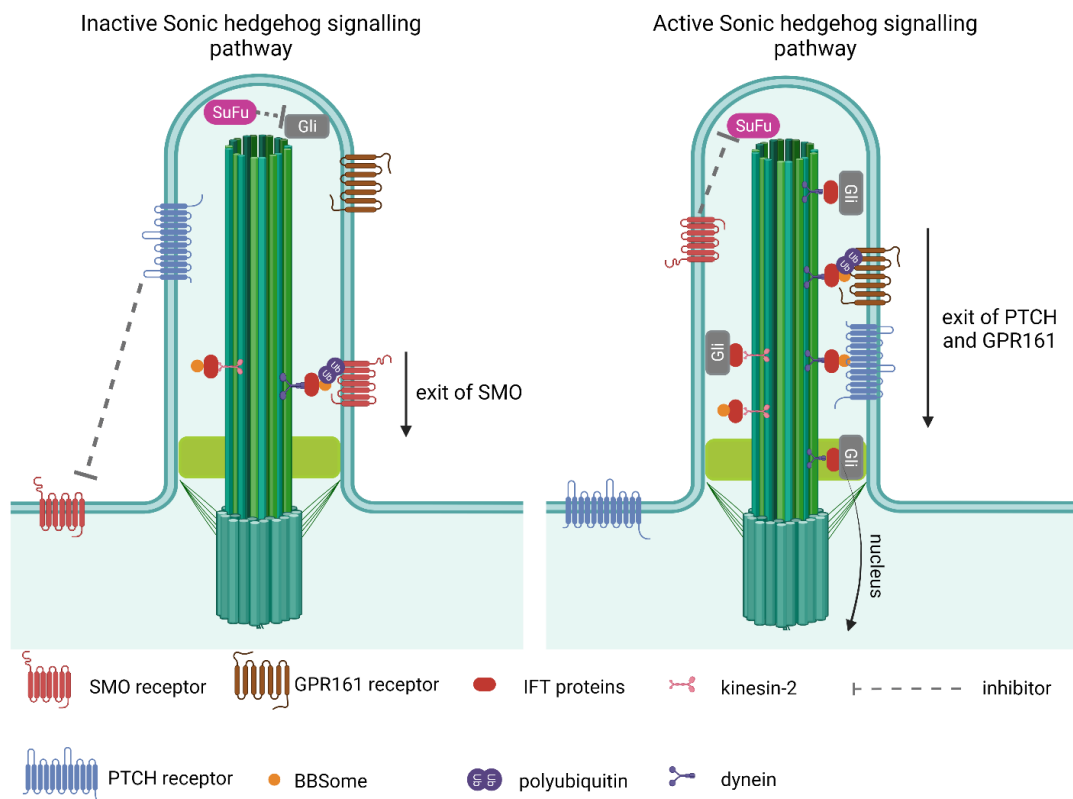


Figure 5 Sonic hedgehog signalling in the primary cilium with the BBSome function (modified from Whewey et al., 2018)

Importantly, it was proposed that polydactyly BBS phenotypes are caused by defects in the SHH signalling pathway (reviewed in Marchese et al., 2020), which corresponds with the fact that *Bbs* LOF mutations showed enhanced SHH signalling resulting in abnormal fin bud patterning in *Danio rerio* (Tayeh et al., 2008).

6.1.3. Other signalling pathways

The depletion of the BBSome was described to affect also other signalling pathways more or less associated with the primary cilium (reviewed in Wheway et al., 2018). In addition to the already mentioned SHH signalling pathway, the BBSome is involved in the WNT signalling pathway (canonical/non-canonical) (Gerdes et al., 2007; Guo and Rahmouni, 2019; Ross et al., 2005) and the Notch signalling pathway (Leitch et al., 2014; Liu et al., 2014).

6.1.3.1. WNT signalling pathway

The WNT signalling pathway is responsible for proper cell polarity, migration, organogenesis, neural patterning, and cell fate determination (reviewed in Komiya and Habas, 2008).

The pathway is activated by the binding of the WNT protein to the Frizzled receptor, the signal is transduced by the Dishevelled protein (reviewed in Komiya and Habas, 2008). The Dishevelled can switch between two WNT pathways (Veeman et al., 2003), non-canonical (β -catenin independent, e.g., planar cell polarity pathway and RhoA pathway), and canonical (β -catenin dependent) (reviewed in Komiya and Habas, 2008). Interestingly, the cilium was shown to suppress canonical WNT signalling and promote non-canonical WNT signalling (Corbit et al., 2008). However, it remains unclear how exactly the cilium is involved in the canonical WNT signalling. In addition, studies in *Danio rerio* and *Mus musculus* revealed that the BBSome depletion amplifies the canonical WNT pathway (Gerdes et al., 2007) and indirectly affects RhoA, which is the component of the non-canonical WNT pathway (Ross et al., 2005).

One of the effects of the enhanced canonical WNT signalling pathway due to the BBSome depletion is an affected B-cell development. Overall, enhanced canonical WNT signalling downregulates the level of the cytokine CXCL12 in bone marrow stromal cells (Tsyklauri et al., 2021) and the chemokine CXCL12 is required for B-cell development in the bone marrow (Zehentmeier and Pereira, 2019).

6.1.3.1.1. Non-canonical WNT signalling pathway

First, *Bbs* mutants have phenotypes as planar cell polarity mutants (e.g., neural tube defects, open eyelids). Interactions with the planar cell polarity gene (*Vangl2*) and the *Bbs* genes were described. However, the exact molecular mechanism of the BBSome effect on the planar cell polarity non-canonical pathway is unknown (Ross et al., 2005).

Second, the BBSome also affects the non-canonical WNT RhoA pathway, which is involved in the cytoskeleton rearrangement. Upregulation of RhoA expression (Guo and Rahmouni, 2019), activity (Guo and Rahmouni, 2019; Hernandez-Hernandez et al., 2013), impaired cell migration, and a disruption of actin stress fibres were observed in *Bbs4*, *Bbs6*, and *Bbs8* deficient renal medullary cells (Hernandez-Hernandez et al., 2013) and MEF or human fibroblast cells with *BBS1* LOF mutation (Guo and Rahmouni 2019). Furthermore, it was proposed that RhoA levels are regulated by polyubiquitination and subsequent proteasomal degradation (Agbor et al., 2016; Ibeawuchi et al., 2015). Therefore, it was suggested that elevated levels of RhoA are due to decreased activity of Cullin-3, which is a subunit of a ubiquitin ligase complex (Guo and Rahmouni, 2019). Finally, levels of Cullin-3 were significantly reduced in MEF cells with *Bbs1* LOF mutation (Guo and Rahmouni, 2019). Taken together, the BBSome seems to affect RhoA levels indirectly through Cullin-3 levels.

However, it should be noted that no additional studies other than those already mentioned have not been found. The mechanism of how the BBSome is involved in this pathway remains to be elucidated.

6.1.3.1.2. Canonical WNT signalling pathway

When the canonical WNT signalling pathway is activated, the β -catenin is stabilized and accumulated in the cytoplasm. β -catenin is then transported to the nucleus, where it affects the transcription (reviewed in Komiya and Habas, 2008). In addition, a higher accumulation of β -catenin was observed in the cytoplasm and the nucleus in BBS4-deficient human embryonic kidney 293 (HEK-293) cells. Moreover, it was suggested that the observed accumulation is due to impaired proteasome degradation because the ubiquitination was not affected. Surprisingly, interactions between the BBSome and the proteasome were described (Gerdes et al., 2007; Liu et al., 2014), which suggests that the BBSome is required for proper proteasomal degradation of β -catenin (Gerdes et al., 2007).

6.1.3.2. Notch signalling pathway

The Notch signalling pathway mediates cellular communication and affects cell differentiation during and after embryogenesis. When the Notch signalling pathway is activated, the Notch receptor is cleaved, and the Notch intracellular domain (NICD) is exposed. The NICD is then translocated to the nucleus and controls the transcription of selected genes (reviewed in Bray, 2016). It was shown that the Notch receptor and Notch-processing enzymes colocalize with the cilium (Ezratty et al., 2011). In general, there are two known models of how the BBSome can be involved in the Notch signalling pathway.

It was suggested that the BBSome is involved in endosomal recycling of the Notch receptor because the decreased plasma membrane localization of the Notch receptor and its accumulation was observed in late endosomes of RPE cells lacking *BBS1*, *BBS4*, or in HEK-293 cells lacking *ARL6* (Leitch et al., 2014).

Furthermore, it was described that *BBS11*, which is one of the BBSome-associated proteins, is a ubiquitin ligase (Chiang et al., 2006). Therefore, it was hypothesized that the BBSome with this associated protein could label proteins themselves with ubiquitin during endosomal recycling (Leitch et al., 2014) and these monoubiquitinated Notch receptors are trafficked to the lysosome for the degradation (reviewed in Le Borgne, 2006). Whereas non-ubiquitinated Notch receptors are trafficked by BBSome-mediated vesicular transport to the plasma membrane (Leitch et al., 2014).

In the second model, it was described that the attenuation of the pathway is dependent on NICD proteasomal degradation (reviewed in Kopan, 1999). Moreover, the *Bbs4* overexpression showed decreased NICD levels in the mouse model, which can be explained by the involvement of the BBSome in the proper proteasomal degradation of NICDs (Liu et al., 2014). To further validate this hypothesis the protein lysate was prepared and biochemical interactions between the BBSome and proteasome components were shown (Gerdes et al., 2007; Liu et al., 2014).

Overall, this suggests that the BBSome is required for the proper membrane localization of the Notch receptor and proteasome localization of polyubiquitinated NICD and its degradation in the proteasome. As with the non-canonical WNT signalling pathway, other studies than those already mentioned are needed to further confirm and better describe this BBSome function.

To conclude, the BBSome seems to affect also other signalling pathways. It is hypothesized that the BBSome is required for proper proteasomal degradation of β -catenin (canonical WNT signalling pathway) and NICD (Notch signalling pathway). Moreover, it was proposed that the BBSome also indirectly affects RhoA (non-canonical WNT pathway) through decreased levels of Cullin-3. Finally, it was suggested that the BBSome is required for endosomal sorting and membrane transport of the Notch receptor. However, these studies cannot help reveal the question of the common function of the BBSome because the effects of the BBSome in these pathways are not well described. There is an opportunity for further studies, which can reveal exactly how the BBSome is involved in these signalling pathways.

6.1.4. Non-ciliary functions of the BBSome

As already mentioned above, the BBSome is involved in vesicular transport to the plasma membrane. One of the proteins thought to be transported by the BBSome-dependent vesicular transport into the plasma membrane is the insulin receptor (IR). Moreover, decreased IR levels were observed on the plasma membrane of *Bbs1* mutant MEF cells. Therefore, it was proposed that the BBSome disruption leads to defective IR transport which leads to insulin resistance associated with diabetes mellitus II (Starks et al., 2015). In contrast with this hypothesis are observations in *Bbs* knockout mice models, where is not referred a significant increase of mice with diabetes mellitus II.

Other proteins transported by the BBSome-dependent vesicular transport such as leptin receptor b (LRb), and serotonin 5-hydroxytryptamine receptor ((HT)_{2c}R) will be described below regarding the specific tissue.

6.2. The role of the BBSome in specific tissues

6.2.1. BBSome in photoreceptors

Rod and cone photoreceptors are light-sensitive polarized sensory neurons of the retina. They consist of an inner and an outer segment, which is itself modified primary cilium (Tokuyasu and Yamada, 1959). Moreover, these two segments are connected by a connecting cilium, which is considered to be analogous to the transition zone in the primary cilium (Besharse and Horst, 1990). There is a constant transport from the inner segment, which is the site of protein synthesis, to the outer segment via the connecting cilium (reviewed in May-Simera, 2017).

The retinal degeneration, reduced retinal function, and structural changes in the outer segment were observed in various *Bbs* knockout mice (Bales et al., 2020; Hsu, 2021; Hsu et al., 2017; Kretschmer et al., 2019) and also in adult mice where the BBSome was disrupted (Hsu et al., 2017). Therefore, it was proposed that the BBSome is essential for the initial acquisition of the outer photoreceptor segment and also for the continuous maintenance and renewal of photoreceptors (Hsu et al., 2017).

Previously, it was hypothesized that rhodopsin (considered a BBSome cargo) mislocalization into the inner segment may be the mechanism of photoreceptor degradation in *Bbs* mutants (Mockel et al., 2011). Later, it has been shown that most of the molecules of rhodopsin localize properly to the outer segment in *Bbs* mutants, suggesting that the primary cause of the retinal degeneration is different (Datta et al., 2015).

To further study the cause of the retinal degeneration, histological and proteomic analysis were prepared. Analysis of *Bbs* mutants revealed accumulations of these proteins: syntaxin-3, syntaxin-binding protein 1 (Datta et al., 2015), M – and S- opsin, arrestin-4, cone receptor (Cyclic Nucleotide Gated Channel Subunit Alpha 3) in the outer segment (Bales et al., 2020). The mislocalization of these non-outer segment proteins suggested that the BBSome is required for the retrograde transport of these proteins from the outer segment back to the cell body (Datta et al., 2015). Moreover, it was observed that the lack of the BBSome caused the entry of non-outer segment proteins into the outer segment of the photoreceptor, e.g., prominin-1 protein, suggesting that the BBSome prevents entry of non-outer segment proteins (Datta et al., 2015). In general, these proteomic changes cause structural changes in the outer segment of the photoreceptors, which impairs the photoreceptor function (Bales et al., 2020; Datta et al., 2015).

Similar structural changes in outer segments of photoreceptors were also described in *Danio rerio bbs1* knockout (Masek et al., 2022). It was suggested that the structure of the outer segment is changed due to altered lipid homeostasis, which plays an important role in phototransduction as has been described (Masek et al., 2022). Finally, accumulations of proteins involved in lipid homeostasis, e.g., apolipoprotein, sterol-binding proteins, and fatty acid-binding proteins were described as the cause of the altered lipid homeostasis in *bbs1* knockout (Masek et al., 2022). However, the molecular mechanism of how exactly the BBSome affects lipid homeostasis remains unclear.

To summarize it, the BBSome is required for the retrograde transport from the outer segment or prevents non-outer segment proteins from entering the outer segment of the photoreceptor. The same phenomenon of the BBSome involvement in the retrograde transport was observed in *C. elegans* (Xu et al., 2015) and *C. reinhardtii* (Lechtreck et al., 2009). Finally, it was proposed that the accumulation of non-outer segment proteins in the outer segment is the primary cause of photoreceptor degradation in mice (Datta et al., 2015). In addition, altered lipid homeostasis may be probably another cause of photoreceptor degradation (Masek et al., 2022), but additional studies are needed.

6.2.2. BBSome in olfactory sensory neurons

It has been described that in addition to ciliated olfactory sensory neurons there are also ciliated horizontal basal cells in the olfactory epithelium. Moreover, cilia on these horizontal basal cells are important for the regeneration of the olfactory epithelium (Joiner et al., 2015).

Bbs LOF mutant mice models revealed a reduction in the length of primary cilia in the olfactory epithelium (Kulaga et al., 2004). In addition, dysfunctions at the level of odor detection were also observed (Kulaga et al., 2004; Tadenev et al., 2011; Uyttingco et al., 2019; Williams et al., 2017).

In the search for the cause of impaired odor detection, it was observed that olfactory proteins important for odor signalling, e.g., Type III adenylyl cyclase, heterotrimeric G-protein subunit gamma 13, stomatin-like protein 3 were decreased on the cilia surface in *Bbs1*, *Bbs4* LOF mutant mice and *Bbs8* knockdown mice (Kulaga et al., 2004; Tadenev et al., 2011). These proteins were mislocalized into the olfactory dendrite. However, there were two hypotheses for the cause of protein mislocalization. Firstly, the previous hypothesis suggested that structural changes in cilia (cilia shortening) are the cause of mislocalization. Secondly, a recent study suggested that the cause was defective IFT transport, which was also observed in the BBS-deficient mice (Williams et al., 2014).

The BBSome was shown to be a constituent of the mammalian olfactory sensory neurons IFT complex (Williams et al., 2014), similar to *C. reinhardtii* (Lechtreck et al., 2009) and *C. elegans* (Wei et al., 2012).

In *Bbs4* mutants, the miscoordination of IFT-A and IFT-B was observed. This suggests that the BBSome may be necessary for IFT trains assembly/reassembly (Uytingco et al., 2019), similar to its function in *C. elegans* (Wei et al., 2012). Taken together, these recent data suggest that the defective IFT transport seems to be more likely the cause of mislocalization.

To conclude, the BBSome acts as a link between IFT-A and IFT-B in olfactory sensory neurons. Therefore, defects in the BBSome cause the disruption of IFT-A and IFT-B, which leads to mislocalization of olfactory proteins and then impaired odor detection.

6.2.3. BBSome in the brain

6.2.3.1. BBSome cargoes associated with the BBS obesity phenotype

Almost all prepared *Bbs* knockout mice developed obesity (Berbari et al., 2008b; Domire et al., 2011; Guo et al., 2011; Kulaga et al., 2004; Rahmouni et al., 2008; Zhang et al., 2013). It was proposed that the depletion of the BBSome affects at least four proteins, which are associated with obesity.

The first protein described to be associated with obesity is leptin. Leptin is a hormone secreted by adipocytes and its function is to mediate energy homeostasis (Brennan and Mantzoros, 2006). It was shown that the energy homeostasis is provided by anorexigenic neurons expressing proopiomelanocortin (POMC) and orexigenic neurons expressing agouti-related peptide (AgRP) in the arcuate nucleus of the hypothalamus (reviewed in Morton et al., 2006). Therefore, the role of leptin is to increase the expression of anorexigenic neuropeptides (Cowley et al., 2001) and to inhibit the expression of orexigenic neuropeptides, e.g., melanin-concentrating hormone (MCH), neuropeptide Y (NPY) (reviewed in Morton et al., 2006).

Bbs knockout mice models revealed higher levels of circulating leptin (Rahmouni et al., 2008), but this leptin was unable to significantly reduce the body weight. Therefore, obesity and hyperleptinemia were suggested to be associated with leptin resistance (Rahmouni et al., 2008). Interestingly, it was described that the BBSome interacts with LRb in spontaneously arising RPE cells (Seo et al., 2009). In addition, further analysis revealed that the deletion of the *Bbs1* in cells expressing just LRb (e.g., in the hypothalamus, not adipocytes) leads to obesity, and decreased levels of the LRb at the membrane were also described (Guo et al., 2016). Finally, it was proposed that the BBSome is involved in LRb vesicular transport from the Golgi vehicle to the plasma membrane (Guo et al., 2016; Seo et al., 2009).

The hypothalamic POMC mRNA was reduced in *Bbs* knockout mice models, therefore it was hypothesized that POMC neurons also participate in the obesity phenotype (Rahmouni et al., 2008). This theory was supported by the observation that the membrane (HT)_{2c}R was stuck in late endosomes of POMC neurons when the BBSome was disrupted. Moreover, this receptor lacks ciliary localization,

but it is important for the energetic homeostasis of the POMC neuron's membrane. Taken together, this suggests that the BBSome is also required for the proper membrane localization of (HT)_{2c}R in POMC neurons (Guo et al., 2019) and that the disruption of the BBSome affects the proper response of POMC neurons to changes in peripheral energy balance (Guo et al., 2019; Seo et al., 2009).

Another receptor, which is associated with the BBS obesity phenotype is the neuropeptide Y receptor type 2, G-coupled receptor (NPY2R). NPY2R is required for energy balance management. NPY2Rs localize to the neuronal cilia of NPY neurons and POMC neurons of the hypothalamus by BBSome-mediated transport (Guo et al., 2019; Loktev and Jackson, 2013). In addition, it was shown that the NPY2R fails to enter cilia in IMCD3 *Bbs1* knockout cells (Guo et al., 2019) and also in *Bbip* knockout mice models (Loktev and Jackson, 2013). However, no accumulations at the ciliary tip were observed. This suggests that the BBSome is involved in the anterograde transport of NPY2R (Guo et al., 2019; Loktev and Jackson, 2013).

The fourth protein, which is associated with the BBS obesity phenotype, is melanin-concentrating hormone receptor 1 (MCHR1). MCHR1 coordinates feeding behaviour and localizes to cilia in parts of the brain, which are involved in feeding (e.g., olfactory bulb, olfactory tubercle, nucleus accumbens, hypothalamus) (Berbari et al., 2008a). Moreover, MCHR1 was observed to lack ciliary localization in *Bbs2* and *Bbs4* knockout mice neurons and IMCD3 cells, where is MCHR1 also expressed (Berbari et al., 2008b), suggesting that the BBSome is required for anterograde transport. However, the involvement of the BBSome during the retrograde transport of MCHR1 still cannot be excluded (Zhang et al., 2011). Overall, the BBSome is probably involved in the anterograde transport of MCHR1 (Berbari et al., 2008b; Zhang et al., 2011). Interestingly, bulges with MCHR1 were observed around the ciliary membrane, suggesting that MCHR1 can be removed from the cilium independently on the BBSome (Zhang et al., 2011).

Interestingly, there is a described mechanism-ectocytosis, which can compensate for the lack of the BBSome. The ectocytosis provides retrieval of activated GPCRs from cilia back to the cell by ectosomes (membrane vesicles) (reviewed in Wood and Rosenbaum, 2015). Finally, the neuronal receptor NPY2R is one of the GPCRs, which was shown to be trafficked by ectocytosis (Nager et al., 2017). Furthermore, described bulges with MCHR1 mentioned above (Zhang et al., 2011) suggest that the MCHR1 can be also ectocytosed from cilia, however, there are no additional studies, which could support this hypothesis. Overall, the lack of NPY2R (and possibly MCHR1) in the BBSome deficient cells may be caused by the ectocytosis and not primary by the BBSome deficiency because these proteins can be removed by ectocytosis in BBSome lacking cells.

In conclusion, it seems that the BBSome is probably required for the anterograde transport of MCHR1 or NPY2R, but additional studies, which could address the potential effect of ectocytosis, are required. Furthermore, it was observed that the BBSome mediates the transport of LRb or (HT)_{2c}R to the plasma membrane. Finally, obesity is caused by dysregulated neuronal function mainly in the hypothalamus (reviewed in Blaess and Wachten, 2021), which is caused by defects in BBSome-dependent transport of the already described proteins. However, it is not clear if all or part of these proteins is involved in the obesity phenotype. On the other hand, it is also hypothesized that obesity may be caused by dysfunctions in adipocytes during early adipogenesis (Marion et al., 2009), this theory will be further described in the chapter devoted to adipocytes.

6.2.3.2. Other neuronal receptors as BBSome cargoes

It was described that the BBSome is involved in the transport of other neuronal receptors such as somatostatin receptor 3 (SSTR3) (Berbari et al., 2008b) and dopamine receptor 1 (D1) (Domire et al., 2011), which are not directly associated with the BBS obesity phenotype.

First, it was observed that SSTR3 levels were decreased in the cilia of hippocampal neurons in *Bbs2* or *Bbs4* knockout mice. It was proposed that the BBSome directly recognizes SSTR3 as cargo for its anterograde transport into cilia (Berbari et al., 2008b; Jin et al., 2010). However, SSTR3 activation increased the amount of IFT-B and BBSomes at the ciliary tip in IMCD3 cells, which suggests that the BBSome is involved in SSTR3 export (Ye et al., 2018). Finally, it was described that the export of activated SSTR3 from cilia is monoubiquitin-dependent and it is mediated by the BBSome, as well as the already mentioned export of GPR161 (Shinde et al., 2020).

Second, D1 localizes to the cilia of mouse central neurons, which are involved in dopamine signalling. *Bbs2* and *Bbs4* null mice mutants revealed a higher abundance of D1 in cilia, especially in the striatum, amygdala, and olfactory tubercle, so this suggests that the BBSome is required for the retrograde transport of D1 (Domire et al., 2011).

Altogether, it seems that the BBSome is required for anterograde transport of NPY2R and MCHR1. However, additional studies considering ectocytosis are needed, as these proteins could be removed by ectocytosis in BBSome-deficient cells. Therefore, it could be wrong to conclude that the BBSome is required for their anterograde transport. In contrast, SSTR3 and D1 receptors require the BBSome for retrograde ciliary transport. Moreover, it was described that the retrograde transport of SSTR3 is ubiquitin-dependent, which corresponds to the hypothesis that the export of all GPCRs from cilia is ubiquitin-dependent. In addition, the BBSome recognizes these ubiquitin marks and provides the export of ubiquitinated proteins from cilia. Finally, it was proposed that the BBSome is also involved in the vesicular transport of LRb and (HT)_{2c}R to the plasma membrane.

6.2.4. BBSome and adipocytes

Bbs4 knockout mice had defective thermogenesis, a reduced amount of ectopic liver fat, and elevated levels of circulating lipids (Gohlke et al., 2021). Therefore, it was suggested that the BBSome is required for proper proliferation and differentiation of adipocytes (Aksanov et al., 2014). It was observed, that the BBSome localizes to primary cilia of preadipocytes (Marion et al., 2009). Moreover, upregulation of BBS proteins during early adipogenesis suggests that the BBSome is mostly required in the early stages of differentiation (Forti et al., 2007). It was further shown that the BBSome deficiency affects more white adipose tissue than brown adipose tissue. This described BBSome function was supported by the observation that silencing of the *BBS4* protein in preadipocyte culture caused the accumulation of triglycerides in smaller droplets and a decrease of lipolysis compared to wild-type cells. Furthermore, it was described that the adipocyte phenotype was changed from a spherical shape to a fibroblast-like shape (Aksanov et al., 2014). Nevertheless, the molecular details of how exactly the BBSome is involved in adipocyte proliferation and differentiation are still unknown.

Generally, it was hypothesized that another cause of the obesity phenotype in BBS might be due to dysfunction during the proliferation and differentiation of adipocytes (Marion et al., 2009). However, this contradicts the already mentioned hypothesis that the obesity is caused by dysregulated neuronal function in the hypothalamus, which is caused by defective transport of proteins associated with obesity, e.g., MCHR1 (Berbari et al., 2008b; Zhang et al., 2011), NPY2R (Guo et al., 2019; Loktev and Jackson, 2013), LRb (Guo et al., 2016; Seo et al., 2009) and (HT)_{2c}R (Guo et al., 2019). In addition, the improbability of the “adipocytes-obesity” hypothesis is further supported by the study where selective *Bbs1* knockout in mice adipocytes do not lead to obesity in mice models (Guo et al., 2019).

6.2.5. BBSome and the renal system

It was already mentioned that one of the symptoms of BBS are renal defects. Changes in the renal system were observed in *Bbs4* mice knockout, e.g., enlarged kidneys, and renal cysts. Moreover, these but milder changes were observed also in *Bbs2* mice knockout (Guo et al., 2011). However, renal changes were not developed in other *Bbs* mice knockout models. One of the most common BBS renal system symptoms is urinary concentration defect, which is caused by renal resistance to vasopressin (Harnett et al., 1988). When the cause of this defect was studied, it was shown that cilia are not formed in human kidney 2 (HK-2) cells and human collecting duct (HCD) cells with *BBS* knockout. Moreover, the lack of cilia causes the vasopressin receptor 2 (AVRP2) to be not properly localized, and these non-ciliated cells cannot target aquaporin 2 (AQP2), which is required for water absorption, to the plasma membrane. Together, it was shown, that non-ciliated HCD cells in cell culture cannot absorb water (Marion et al., 2011). However, AVRP2 and AQP2 proteins have not been identified as BBSome cargoes.

Nevertheless, cilia were formed in the renal epithelium in *Bbs4* knockout mice models, but these cilia were slightly shorter (Guo et al., 2011). Furthermore, a decrease in the expression of transient receptor potential cation channel subfamily V member 1 (TRPV1) and transient receptor potential cation channel subfamily V member 4 (TRPV4) and an increase in the expression level of nitric oxide synthase were observed in the kidney of *Bbs4* and *Bbs2* knockout mice (Guo et al., 2011). The nitric oxide synthase is a vasodilator, whereas the TRPV1 function is to detect and regulate body temperature. TRPV4 is involved in the regulation of systematic osmotic pressure. Finally, it was suggested that changed levels of these molecules may be responsible for another symptom of BBS, which is hypertension (Guo et al., 2011).

Polycystin-1 (PC-1) and polycystin-2 (PC-2) are ciliary membrane proteins that mediate the mechanosensitivity of fluid flow in the renal epithelium. It was proposed that their loss or dysfunctions could lead to mechanosensation defects, e.g., polycystic kidney disease (Nauli et al., 2003). *Arl6* or *Bbs1* knockdown in IMCD3 cells revealed no PC-1 in the cilia and the PC-1 was restricted in the basal body. The same situation was observed also in human fibroblasts with a LOF mutation in *BBS1* (Su et al., 2014). Finally, the hypothesis that the BBSome is required for anterograde transport of PC-1 is further supported by described interactions between the BBSome and PC-1 in HEK-293 cells (Su et al., 2014). Nevertheless, another study on cultured *Bbs7* knockout primary kidney cells proposed that the BBSome is not required for ciliary localization of PC-1 and PC-2 because their function and localization were not affected (Zhang et al., 2013). Taken together, BBSome-dependent transport of PC-1 and PC-2 remains controversial due to conflicting observations in a mammalian model. Interestingly, the accumulation of PKD-2 (orthologue of human PC-2) was observed in *C. elegans bbs* mutant cells. Therefore, it was suggested that the BBSome is required for retrograde transport of PKD-2 (Xu et al., 2015), in contrast to a mammalian model.

However, it seems that the causes of the renal cysts are enhanced SHH signalling and attenuation of the WNT non-canonical pathway (Kawakami et al., 2013; Tran et al., 2014), which have already been explained above.

It was proposed, that the BBSome mediates the transport of many signalling molecules in cilia, however, the BBSome seems to be more involved in the retrograde ciliary protein transport, e.g., D1, PTCH. Moreover, this phenomenon of BBSome-dependent retrograde ciliary transport was described in most specific tissues, e.g., retrograde transport in neurons and retrograde transport from the outer segment in photoreceptors. Furthermore, it was described that retrograde ciliary transport is sometimes associated with ubiquitination and that the BBSome can recognize ubiquitin marks.

Therefore, the BBSome provides the export of ubiquitinated proteins from cilia, e.g., SSTR3, SMO, GPR161.

On the other hand, two neuronal receptors (MCHR1 and NPY2R) were identified as proteins, which require the BBSome for the anterograde transport. Nevertheless, the effect of ectocytosis should be mentioned because the hypothesis that the BBSome is required for their anterograde transport was made based on their lack of ciliary localization. Interestingly, it seems that the lack of ciliary localization of these proteins in BBSome deficient cells can be primarily caused by ectocytosis and not by the BBSome deficiency.

Concerning BBSome-ubiquitin interactions, it was suggested that the BBSome is required for proper proteasomal degradation of NICD and β -catenin, however, this hypothesis needs to be supported by additional data. It was proposed that the BBSome is also involved in the vesicular transport of receptors to the plasma membrane such as LRb, (HT)_{2c}R, Notch receptor, and IR. Nevertheless, these studies need to be supported by further observations.

Taken together, it seems that the BBSome has not one universal cargo type or commonly shared function that can be found in all specific tissues. However, the BBSome-dependent retrograde ciliary transport seems to be a parallel that has been found in most of the specific tissues. Finally, the link between ubiquitination and the BBSome-dependent retrograde ciliary transport appears to be an opportunity for further studies.

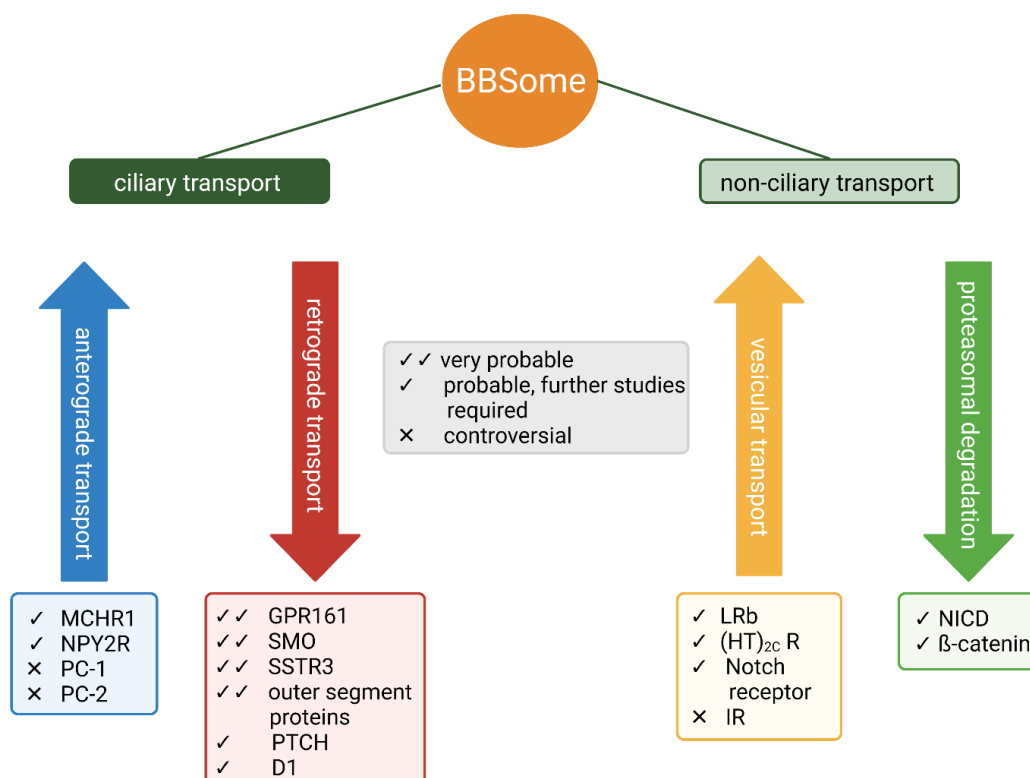


Figure 6 Scheme of BBSome functions with probabilities in a mammalian model. Selected proteins

are marked with symbols indicating the probability that the BBSome is involved in their transport. A higher probability (indicated by ✓✓) means that the BBSome function associated with the selected protein is confirmed by several studies. The symbol ✓ means that additional studies are required to describe the BBSome function in detail. Controversial (indicated by X) means that studies are in conflict.

7. Conclusion

In conclusion, the BBSome has ciliary localization in all described model organisms except *D. melanogaster*, where there is not enough literature available. Overall, proposed BBSome functions can be divided into cilia-dependent and cilia-independent.

In general, the BBSome was described to be carried by IFT proteins and to work as a cargo adapter in the cilium. In *C. elegans*, the BBSome works more as a coupler and stabilizer of IFT proteins. It is widely discussed whether the BBSome is mostly required for retrograde or anterograde transport or both. First, the BBSome is essential for retrograde transport of PLD in *C. reinhardtii* (Lechtreck et al., 2009), sensory receptors, e.g., OSM-9 in *C. elegans* (Xu et al., 2015), and various receptors in a mammalian model. Furthermore, it has been proposed that the BBSome interacts with ubiquitin (Langousis et al., 2016) and mediates the exit of mono and polyubiquitinated proteins such as a PKD-2 in *C. elegans* or GPR161 and SMO in mammals (Desai et al., 2020; Shinde et al., 2020; Xu et al., 2015). However, the ubiquitin-dependent transport, which is mediated by the BBSome, is IFT proteins-independent in *C. elegans* (Xu et al., 2015) in contrast to IFT proteins/ubiquitin-dependent transport in a mammalian model (Desai et al., 2020; Shinde et al., 2020). Finally, these observations are consistent with the hypothesis that the BBSome is essential primarily for retrograde transport. Second, the BBSome is also necessary for ciliary localization and anterograde transport of proteins such as MCHR1, NPY2R in a mammalian model (Berbari et al., 2008b; Guo et al., 2019; Loktev and Jackson, 2013; Zhang et al., 2011), which is in contrast to other model organisms where the BBSome-dependent anterograde transport has not been described. In addition, the number of cargo proteins where the BBSome is required for anterograde transport is smaller than the number of cargo proteins where the BBSome is necessary for retrograde transport in a mammalian model. Furthermore, proteins such as NPY2R and MCHR1 were considered to be trafficked by BBSome-dependent anterograde transport due to their ciliary lack in BBSome deficient cells. An alternative explanation of their ciliary lack could be that they are removed from the cilium by ectocytosis. Taken together, the ciliary lack of these proteins could be caused primarily by ectocytosis and not by the depletion of the BBSome.

The retrograde transport of PLD in *C. reinhardtii* (Lechtreck et al., 2013; Liu and Lechtreck, 2018; Xue et al., 2020) and GPR161 in mammals (Liew et al., 2014; Nozaki et al., 2019; Shinde et al., 2020) is very well described. In both organisms, these proteins were identified as BBSome cargoes, and it was

observed that these proteins accumulate in the cilia of BBSome deficient cells. However, the mechanism by which the BBSome recognizes cargo differs between the two organisms. In *C. reinhardtii*, the BBSome interacts directly with PLD, but in mammals, the BBSome more probably recognizes ubiquitin marks, which have not been described in *C. reinhardtii*. Anyway, these proteins are loaded onto the BBSome and exported from the cilia by BBSome/IFT proteins-dependent retrograde transport.

The second generalized BBSome functions are cilia independent. Interestingly, cilia-independent functions have only been described in *T. brucei* and a mammalian model. These studies showed that the BBSome is involved in the postendocytic sorting of membrane proteins and its function is cilia-independent and associated with ubiquitination (Langousis et al., 2016; Leitch et al., 2014). However, additional cilia-independent functions of the BBSome were observed in mammals such as interactions with the proteasome, and the BBSome-dependent transport of polyubiquitinated proteins, e.g., NICD, β -catenin to the proteasome (Gerdes et al., 2007; Guo et al., 2019; Liu et al., 2014). In addition, the BBSome seems to be also required for vesicular transport of membrane receptors such as LRb, Notch receptor, and (HT)_{2c}R in a mammalian model (Guo et al., 2019, 2016; Leitch et al., 2014; Seo et al., 2009), however, BBSome-dependent vesicular transport to the plasma membrane has not been described in *T. brucei*.

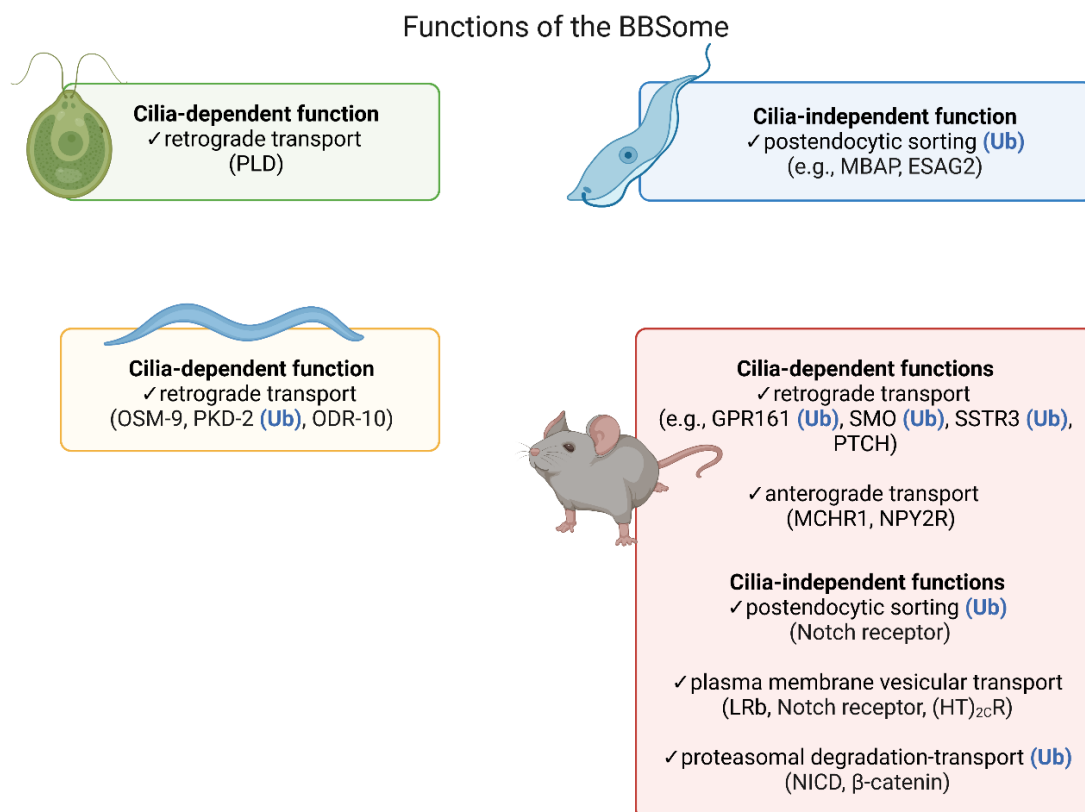


Figure 7 Functions of the BBSome in selected model organisms. Proposed functions associated with ubiquitination are indicated by (Ub).

Model organism	Cilia localization		Cilia-dependent function		Cilia-independent function		
	Yes	No	ciliary transport		postendocytic sorting	plasma membrane transport	proteasomal degradation-transport
			anterograde	retrograde			
<i>Chlamydomonas reinhardtii</i>	✓	✗	✗	✓	✗	✗	✗
<i>Trypanosoma brucei</i>	✓*	✗	✗	✗	✓(Ub)	✗	✗
<i>Caenorhabditis elegans</i>	✓	✗	✗	✓(Ub)	✗	✗	✗
<i>Drosophila melanogaster</i>	✓	✗	✗	✗	✗	✗	✗
<i>Mus musculus</i> <i>Homo sapiens</i>	✓	✗	✓	✓(Ub)	✓(Ub)	✓	✓(Ub)

Table 2 The comparison of functions of the BBSome in model organisms. The functions associated with mono/polyubiquitination are indicated by ✓(Ub). Not enough data are available for *Drosophila melanogaster*; however, it seems that the BBSome has a ciliary localization in *D. melanogaster*. The BBSome localizes to the basal body, transition zone, and flagellar pocket in *T. brucei* ✓*.

Overall, the BBSome is evolutionarily conserved among ciliated organisms. It seems that the depletion of the BBSome affects many signalling pathways, and its functions can be generally divided into cilia-dependent and cilia-independent. The cilia-dependent function, where the BBSome is carried by IFT proteins and works as a cargo adapter, is conserved in *C. reinhardtii*, *C. elegans*, and vertebrates. This function might be conserved also in *T. brucei*, where the BBSome localizes to the cilia base and may provide the ciliary export. Finally, the universal BBSome cargo, which could be shared among model organisms has not been identified. The BBSome-dependent retrograde ciliary transport of proteins is better described than anterograde transport. The BBSome-dependent retrograde transport was described in *C. elegans*, *C. reinhardtii*, *M. musculus*, or *H. sapiens*. However, the BBSome-dependent anterograde transport was proposed to be only in a mammalian model and needs to be further analysed by studies that consider ectocytosis. The lack of proteins (e.g., NPY2R, MCHR1) was observed in BBSome deficient cells. Therefore, it was concluded that the BBSome is required for their anterograde transport. However, the lack of these proteins in cilia in BBSome-deficient cells might be caused indirectly by BBSome-induced non-specific ectocytosis of ciliary vesicles. Cilia-independent functions of the BBSome were described in *T. brucei*, *M. musculus*, or *H. sapiens*, but these functions are slightly different in these organisms. In general, the postendocytic sorting and ubiquitin-dependent transport are parallels, which have been found in these species.

It seems that the last eukaryotic common ancestor (LECA) had two flagella and the BBSome was also likely to be present (reviewed in Mitchell, 2017). However, it remains unclear whether the BBSome in the LECA had cilia-dependent or cilia-independent functions. In my opinion, there are two possible options. First, the BBSome in the LECA could have cilia-dependent and cilia-independent functions, and during the evolution one of the generalized functions could be lost due to adaptations to the new way of life. Second, the BBSome in the LECA had a cilia-dependent function, and during evolution, when a parasitic lifestyle was developed in *T. brucei*, the BBSome localization could be limited to a flagellar base and the BBSome could be involved in the transport of ubiquitinated proteins and postendocytic sorting. In mammals, cilia-independent functions could have been established later in the evolution, when specialized cell types were developed.

The aim of this bachelor's thesis, to assess whether a putative BBSome common function can be identified, was fulfilled. The current knowledge about functions of the BBSome in model organisms was summarized and compared. According to this summarized literature, the putative common function of the BBSome has not been identified. The outcome of this bachelor's thesis is limited by published literature, which is often focused on BBSome derived or specific functions in a multicellular organism, and there are not enough published studies for non-mammalian organisms, e.g., *T. brucei* and *D. melanogaster*. In addition, these derived or specific functions may have arisen by repurposing

the BBSome during its evolution. Therefore, it is difficult to identify the putative common function. Taken together, it seems that the BBSome is conserved due to its complexity because eight subunits must interact to properly assemble the complex. Moreover, interacting partners, e.g., IFT proteins can also be the cause why the BBSome is conserved among model organisms. Taken together, it seems that the only parallel among model organisms (except *T. brucei* and *D. melanogaster*) is the BBSome-dependent retrograde transport. Interestingly, BBSome functions seem to be associated with ubiquitination more than it is generally thought (Table 2). Future research could focus on this issue and a new perspective could be established to further uncover the function of the BBSome associated with ubiquitination.

8. References

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