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The primary cilium as a cellular receiver: organizing ciliary GPCR signaling

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Abstract

The primary cilium is an antenna-like cellular protrusion mediating sensory and neuroendocrine signaling. Its localization within tissue architecture and a growing list of cilia-localized receptors, in particular G-protein-coupled receptors, determine a host of critical physiologies, which are disrupted in human ciliopathies. Here, we discuss recent advances in the identification and characterization of ciliary signaling components and pathways. Recent studies have highlighted the unique signaling environment of the primary cilium and we are just beginning to understand how this design allows for highly amplified and regulated signaling.

Introduction

The primary cilium is a cellular protrusion that functions as a sensory organelle and appears on most mammalian cell types. Its pervasiveness and importance to human development and physiology is highlighted by a wide spectrum of human genetic disorders with ciliary defects collectively called ciliopathies. These syndromes present with broad clinical manifestations, including polydactyly, retinal degeneration, mental retardation, anosmia, obesity, liver fibrosis, and kidney cysts [1]. A combined effort of researchers and clinicians has linked ciliopathies, including nephronophthisis (NPHP), Joubert syndrome (JS), Meckel-Gruber syndrome (MKS), Bardet-Biedl syndrome (BBS), and polycystic kidney disease (PKD), to mutations in genes required for ciliary function [1]. These links have generated a wealth of information informing our understanding of ciliary structural and signaling components. Moreover, the primary cilium is highly conserved throughout eukaryotic evolution, allowing researchers to take advantage of an array of model organisms ranging from *Chlamydomonas* and *Tetrahymena* to worms, flies and mice. These avenues of research have helped illuminate some of the core structures in the primary cilium. In brief, the cilium is comprised of a ciliary membrane surrounding a microtubule-based axoneme (Fig. 1). The axoneme is nucleated by the basal body, including the mother centriole and

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associated pericentriolar material (PCM), which themselves organize signaling pathways in close communication with the cilium (reviewed in detail in [2]). Importantly, the ciliary membrane is highly enriched for receptors, allowing the primary cilium to organize signaling in a highly ordered and concentrated microenvironment. By organizing signaling components in supramolecular complexes, the cilium allows for rapid, regulatable, and highly sensitive signaling. Because the cilium is positioned immediately above the centriole and PCM, the effective microtubule organizing center (MTOC), ciliary signals can couple to processes important for cellular trafficking and movement. Importantly, the orientation and localization of the primary cilium within the tissue architecture allows the cell to present receptors at sites favorable for ligand accumulation and engagement, such as in ducts or the ventricles of the brain. These localized signaling “neighborhoods” are not well described, but may be a critical factor in understanding ciliary signaling in tissues. Intriguingly, primary cilia can vary greatly in length and morphology between different tissues, and this is likely in part due to differences in the repertoire of proteins contained within it as well as the ciliary signaling status [3-5]. In turn, these tissue specific structures may “tune” ciliary signaling, for example by pointing cilia towards a vascular bed that would transport ligands. The detailed structure of cilia appears to be critically important for normal physiology and the timing of signaling. This review focuses on recent advances in both the identification and characterization of ciliary signaling components, in particularly G-protein-coupled receptors (GPCRs; Table 1), as well as our understanding of how the primary cilium conveys signals to the cell body.

Ciliary GPCR signaling

The primary cilium can sense a wide array of signals, including odorants, light, growth factors, and developmental morphogens [6]. As such it plays an integral role both during development and in the adult. The discovery that hedgehog signaling requires primary cilia in mammalian cells has catapulted cilia into the spotlight (Fig. 2A; Box 1) [7]. Intriguingly, many ciliary receptors identified to date are GPCRs (Table 1A). The biology of GPCR signaling has been reviewed in detail elsewhere (see [8]). Briefly, about 800 different GPCRs are expressed in humans and almost half of all therapeutic drugs target GPCRs, creating considerable medical and commercial interest. GPCRs are 7 transmembrane receptors that respond to diverse signals and affect numerous cellular processes. Agonist binding to the receptor results in activation of its GEF activity and dissociation of the bound heterotrimeric G-protein complex into a GTP-bound G_{α} subunit and a $G_{\beta\gamma}$ heterodimer. These components in turn signal through secondary messengers including cAMP. The type of downstream signaling activated depends in part on the specific G_{α} that is coupled to the GPCR ($G_{\alpha s}$, $G_{\alpha i/o}$, $G_{\alpha q}$, and $G_{\alpha 12/13}$). Additionally, GPCRs can signal independently of G-proteins and there is growing literature on GPCR-independent G-protein signaling [9]. GPCR signaling components that to date have been identified as being localized to the primary cilium are summarized in Table 1B. Of note, the advent of proximity labeling proteomics may accelerate the rate of discovery of these ciliary signaling components [10,11].

As reflected in Table 1A, a large body of work has focused on identifying ciliary GPCRs in neurons. Notably, most neurons in the adult mammalian brain possess primary cilia and

intriguingly, different neuronal subpopulations express different ciliary proteins including receptors [12*]. The importance of cilia to neuronal function is highlighted by ciliopathy phenotypes and validated by ciliary knock-out mouse models: loss of ciliary function results in obesity, at least in part due to hyperphagia, as well as learning and memory defects [13-15]. Of note, while there is considerable interest in identifying new ciliary receptors, discovery is relatively slow as it requires generation and expression of fluorescently tagged GPCRs followed by immunofluorescence staining. Nonetheless, the rate of discovery has increased dramatically in recent years, and this is aided by the emergence of central biochemical themes for ciliary GPCRs:

1. **Ciliary targeting sequences:** Targeting of ciliary GPCRs from the *trans*-Golgi Network is thought to be determined by ciliary targeting sequences (CTS). To date, three distinct CTSs have been identified for GPCRs. All three motifs are found in the C-terminal tail or the third intracellular loop (IC3) of ciliary GPCRs, suggesting that these regions are particularly important for ciliary GPCR trafficking. The earliest motif identified was the C-terminal VxPx CTS of rhodopsin. Characterization of known retinal degeneration mutations had narrowed down the region of interest to the C-terminal tail and tagging of membrane-associated GFP with C-terminal rhodopsin fragments defined the distal residues as sufficient for ciliary localization [16]. Another seminal study identified the Ax[S/A]xQ domain in the IC3 domain of Sstr3 and Htr6 by domain swapping with other, non-ciliary family members [17]. However, while the IC3 domain of Sstr3 and Htr6 is sufficient to target non-ciliary family members to the primary cilium, it is not necessary for ciliary localization of Sstr3 and Htr6, suggesting the existence of additional CTSs. Finally, a recent screen for ciliary neuronal GPCRs identified the [R/K][I/L]W motif in the IC3 of NPY2R by domain-swapping with non-ciliary NPY1R [12*]. Of note, receptors containing any of these motifs do not necessarily localize to cilia, nor do all ciliary GPCRs identified to date contain these known CTSs. Moreover, how these motifs couple to known ciliary trafficking pathways is poorly understood.
2. **Ciliary trafficking:** How GPCRs are trafficked to and from the base of the cilium is an active area of investigation. In general, GPCRs can travel to the base of the cilium from the *trans*-Golgi Network, from recycling endosomes, or from the plasma membrane via lateral diffusion. The existence of these multiple pathways is exemplified by trafficking of the GPCR Smoothed: During the first hour after hedgehog signaling, ciliary Smo is thought to primarily originate from the plasma membrane, followed by trafficking of an intracellular pool of Smo [18]. An ever-growing list of proteins involved in these trafficking pathways suggests the existence of multiple mechanisms of ciliary cargo delivery (see [19,20] for recent detailed reviews). One pathway that is critical for delivering vesicles to the ciliary base for ciliary assembly requires Rab8/Rabin8/Rab11. Briefly, the small GTPase Rab8 localizes to the cilium and is thought to activate vesicle fusion to the ciliary pocket, since expression of a GDP-locked mutant results in accumulation of vesicles around the basal body [21] while expression of a GTP-locked version causes elongated cilia [22]. Importantly, the Rab8 GEF, Rabin8, localizes to the

ciliary base [23,24]. Both Rab8 and Rabin8 bind CEP164, a major distal appendage protein that forms a 9-fold symmetric ring required for vesicle docking and organizing ciliary entry [25]. Once ciliary cargo has been deposited at the base of the cilium, it needs to cross the ciliary gate. One model postulates that the intraflagellar transport (IFT) machinery can cross the transition zone and specifically interact with ciliary cargo to mediate delivery into the primary cilium [26]. The IFT machinery is highly conserved and consists of two complexes: IFT-B is required for anterograde transport powered by kinesin and IFT-A is required for retrograde transport powered by dynein [2]. In general, IFT trains are thought to bridge ciliary proteins moving along the length of the cilium to motors that actively transport these proteins along the axoneme (Fig 1).

To date, two different complexes have been implicated to specifically regulate ciliary trafficking of GPCRs (Table 1A; Fig 2B). One complex, the BBSome, was identified by tandem-affinity purification/mass spectrometry of genes mutated in Bardet-Biedl Syndrome [22] and is required for ciliary localization of Sstr3, Mchr1, and Npy2R [12*,27], suggesting that the pathologies of Bardet-Biedl Syndrome are a result of aberrant ciliary signaling. The BBSome may function in vesicular trafficking of GPCRs to and from the cilium. It has been reported to bind liposomes and directly interact with the Ax[S/A]xQ CTS of SSTR3, but as the complex appears punctate in cilia, the context for these interactions is not clear [28]. The Arl6/Bbs3 small GTPase is required for ciliary entry of the BBSome itself [28], whereas Ift25/Ift27 has been suggested to mediate ciliary exit of the BBSome-cargo complex [29,30]. Another family of proteins, the Tubby family, is also critical for ciliary localization of a subset of GPCRs [31,32]. The *tubby* mouse has spontaneous maturity-onset obesity as well as retinal and olfactory degeneration [33,34] and the *Tulp3* knockout mouse is embryonic lethal due to aberrant hedgehog signaling [35,36]. Tandem-affinity purification/mass spectrometry revealed that related Tulp3, Tulp2, and Tubby proteins each bind to ciliary IFT-A and additionally contain a specific phosphoinositide binding domain [31]. Importantly, Tulp3 requires both its IFT-A and PIP₂ binding domains for ciliary localization of the negative regulator of hedgehog signaling Gpr161 (discussed below) [37**]. Yet another pathway has been implicated in ciliary trafficking of lipidated proteins (Fig. 2B): the β -sandwich protein Unc119a/b binds a critical subset of myristoylated proteins in the cytoplasm for targeting to the cilium via a transition zone mediated process [38], whereas the related protein Pde6d functions as the carrier of prenylated proteins [39,40]. Importantly, G α subunits are myristoylated and G γ subunits are prenylated [41], suggesting a coordinated delivery of G proteins via these pathways. Indeed, UNC119 is required for ciliary localization of transducin α , the photoreceptor G α [42]. Once in the cilium, the lipidated cargo-carrier complex binds the small GTPase Arl3^{GTP}, which releases the lipidated protein. Notably, Arl13b was recently identified as the Arl3 GEF [43*]. Both Arl3 and Arl13b are highly conserved in ciliates. Since Arl13b is exclusively ciliary, active Arl3^{GTP} and hence released lipidated ciliary proteins are spatially restricted to the primary cilium.

Intriguingly, most known ciliary GPCRs are subtypes within a larger family of GPCRs, which also contain non-ciliary receptors and which have overlapping expression patterns and ligands. Moreover, a recent study showed isoform specific ciliary localization of DRD2S [44]. These observations are consistent with the idea that ciliary localization of receptors allows for robust and/or differential signaling as discussed in more detail below.

Rationale behind ciliary signaling

We have only a dim understanding of why some cellular signaling pathways are organized into the primary cilium and how the structure of the primary cilium can enable signaling. Given the ancient origin of flagella, the simplest answer is that the efficient mechanisms built in primitive ciliates were preserved even as these structures lost dynein-dependent beating. Here we postulate some advantages of ciliary design:

Processivity of signaling

The average mammalian primary cilium is approximately 3µm in length and its volume is about 1:30,000 that of the cytoplasm [45*]. This small size yet large ratio of sensing surface to volume allows for localized enrichment of both receptors and signaling components, and organization of all components into a chain for rapid propagation of a signal to the centrosome to effect cellular responses [12*]. Moreover, the highly ordered trafficking in cilia provides the opportunity for “pre-organized” signaling complexes, allowing for rapid molecule shuttling, akin to transport in the mitochondria membrane. We and others hypothesize that increased processivity would result in increased ligand sensitivity, which is observed for ciliary IGF1 signaling [46]. Further detailed and quantitative characterization of ciliary versus non-ciliary signaling pathways is needed.

Unique cellular environment

The primary cilium is topologically continuous with the plasma membrane and cytosol and the function of the primary cilium to organize signaling depends on the presence of a diffusion barrier at the base of the cilium. A growing body of work has defined the components of this ciliary gate and the regulation of protein trafficking into and out of the cilium remains an active area of investigation. The consequence of this gate is the formation of a unique signaling environment. Recent work has shown that a high ciliary concentration of PKD1L1-PKD2L1 heteromeric channels results in a ciliary resting calcium concentration approximately 7-fold higher than that of the cytoplasm [45*,47]. Interestingly, this differential calcium environment regulates ciliary trafficking of Gli2, though how calcium achieves this effect is unknown. Similarly, the ciliary membrane is highly enriched for a particular phosphoinositide, PI(4)P, over PI(4,5)P₂ (typical of the plasma membrane), and this ciliary membrane reprogramming is achieved by the ciliopathy-associated phosphoinositide 5-phosphatase Inpp5e [48**,49**,50]. Importantly, Tulp3 had been shown to primarily bind PI(4,5)P₂ [31], and gratifyingly, genetic perturbations altering ciliary phosphoinositol composition (including inactivation of Inpp5e) resulted in deregulation of Tulp3-dependent trafficking of Gpr161 and hedgehog signaling [48**,49**]. It is intriguing to hypothesize that dynamic changes in ciliary phosphoinositide composition in response to stimuli regulate trafficking of signaling components into and out of the cilium. Finally, the

primary cilium allows for compartmentalization of downstream signaling components, co-regulators and/or posttranslational modifiers, which in turn allows for differential ciliary-specific regulation of signaling pathways that is independent of non-ciliary signaling pathways. Indeed, a recent study has shown that the subcellular localization of the bile acid receptor TGR5 determines the functional response of cholangiocytes to bile acid; in non-ciliated cholangiocytes, TGR5 couples to $G_{\alpha s}$ and results in increased cAMP levels and decreased proliferation, while in confluency-arrested, ciliated cholangiocytes, TGR5 also localizes to the primary cilium, couples to $G_{\alpha i}$ and causes a decrease in cAMP levels and increase in proliferation [51*]. Similarly, ciliary GPCRs can activate different downstream pathways depending on the cellular context. Indeed, a recent study uncovered a role of Gpr161 in activating canonical Wnt signaling in addition to its known role in mediating Shh signaling [52].

Integration of signal

The rapidly expanding list of ciliary receptors along with the small size of the primary cilium itself begs the question of how the primary cilium can convey the multitude of signals simultaneously. This is particularly intriguing considering that many signaling pathways impinge on the same downstream effector molecules such as cAMP. We therefore propose that the primary cilium not only serves as a platform to orchestrate signaling pathways, but that its very nature allows for integration of multiple signaling pathways. This coordinated response to a wide array of ligands can then be conveyed to the centrosome at the base of the cilium to effect the appropriate cellular response.

Conclusion

In recent years, our understanding of signaling in the primary cilium has vastly improved, advancing our patchwork understanding into the beginnings of a cohesive picture, revealing distinct themes and tantalizing concepts. These themes center on the unique microenvironment afforded by the primary cilium, and its ability to orient and localize receptors within a tissue's architecture. The gated microenvironment of the primary cilia allows for the localization of supramolecular complexes leading to rapid signal propagation. Additionally, this specialized cilioplasm contains effector molecules and ion concentrations measurably different from the juxtaposed cytoplasm, creating an environment rife for exclusive signaling outcomes. Finally, the small volume of the cilioplasm permits prompt alteration of common GPCR effector concentrations, such as cAMP, possibly coordinating multiple signaling pathways.

Powerful new proteomic techniques under the direction of these defining themes have already yielded key insights into the signaling role of the primary cilium. Decorated by targetable GPCRs, bounded by a singular topography, and made relevant by a host of genetic disorders, the primary cilium stands at the crossroads of human disease and therapeutic intervention.

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Box 1**Lessons learned from Hedgehog Signaling**

Sonic hedgehog (Shh) signaling is a highly conserved pathway that is essential for embryonic development, in particular tissue patterning. Shh signals are also used extensively in adult tissue homeostasis and injury-dependent regeneration [53]. Notably, hedgehog signaling requires the primary cilium in most vertebrates [7]. In fact, pathologies due to mutations affecting hedgehog signaling or the primary cilium have overlapping clinical manifestations including polydactyly. Further, genetic perturbation of key ciliogenesis genes in mice results in neural tube defects, a hallmark of aberrant hedgehog signaling. For a recent review on ciliary hedgehog signaling, see [54]. Briefly, in the OFF state, the receptor Patched 1 is localized in the cilium and in vesicles around the ciliary base [55], while the GPCR Smoothed (Smo) is only transiently ciliary (Fig. 2A) [56,57]. Recently, another GPCR, Gpr161, was shown to also localize to the cilium in the absence of hedgehog and may activate PKA to result in processing of the Gli3 transcription factor to its repressor form [37**]. Importantly, the *Gpr161* knockout mouse is embryonic lethal due to a neural tube defect linked to hyperactive Shh signaling [37**]. In the presence of Shh ligand (ON state), its receptor Ptch1 as well as the orphan receptor Gpr161 exit the cilium while ciliary Smo accumulates and activated Gli2/3 is released at the ciliary tip for translocation to the nucleus [56,58]. Notably, while ciliary localization of vertebrate Smo is required for hedgehog signaling, it is not sufficient, since genetic or pharmacological perturbations that lead to ciliary accumulation of Smo do not always trigger signaling [59,60]. Indeed, how Ptch1 exit regulates Smo activity and how the trafficking and activity of the various ciliary components results in Gli processing versus release remains unclear. Moreover, in flies, where hedgehog was first identified, signaling occurs independent of the primary cilium, arguing for evolutionary divergence [53]. Intriguingly, cilia-mediated hedgehog signaling has however been reported in specific fly sensory tissues [61]. Thus, the connection between the primary cilium and hedgehog signaling remains an active area of investigation that already has already dramatically advanced our molecular understanding in both fields, including the discovery of novel Hedgehog signaling components such as the ciliary GPCR GPR161. We believe that Hedgehog signaling serves as a prime example of the importance of ciliary signaling to human physiology, and that many ciliary signaling pathways, especially tissue specific pathways, remain to be discovered.

Figure 1. Schematic of the Primary Cilium

The cilium is comprised of the ciliary membrane surrounding the microtubule-based axoneme, which is nucleated by the basal body. The ciliary membrane is highly enriched for receptors, including GPCRs. These ciliary signaling components are transported to the base of the cilium along microtubules by the minus-end directed motor dynein, where they cross the transition zone to enter the cilium. Within the primary cilium, IFT-B and IFT-A couple to kinesin and dynein motors, respectively, for anterograde and retrograde transport of signaling components. DA refers to distal appendage.

Figure 2. Signaling in primary cilia

A) Schematic of ciliary Hedgehog signaling. In the absence of Sonic Hedgehog (Shh), Patched1 and Gpr161 are ciliary resulting in activation of PKA and processing of Gli transcription factors to a repressor form. In the presence of Shh, Patched1 and GPR161 exit the cilium allowing for ciliary entry of Smoothed (Smo), release of Gli and its translocation to the nucleus to activate hedgehog target genes

B) Schematic of ciliary GPCR trafficking. The ciliary membrane contains a large repertoire of GPCRs. Trafficking of a subset of these is regulated by the BBSome and/or Tulp3. A subset of myristoylated (including transducin_α) and farnesylated proteins are bound by Unc119 and Pde6d, respectively, in the cytoplasm for targeting to the cilium. Once in the cilium, the lipidated cargo-carrier complex is bound by the small GTPase Arl3_{GTP} to result in release of the cargo. The Arl3 GEF, Arl13b, is exclusively ciliary. The coupling between GPCRs and specific G proteins or other effectors is poorly understood, but GPCRs typically have guanine nucleotide exchange factor activity for Gα subunits.

Table 1A

List of ciliary GPCRs identified to date

GPCR	Ref.	Ligands	General function	General tissue expression	Ciliary localization shown in (overexpression and endogenous)	BBSome/TUBBY dependent trafficking?
DRD1	[62,63]	dopamine	neuronal processes, e.g. memory	multiple subpopulations of CNS	<i>NIH3T3</i> , <i>IMCD3</i> , several regions of brain including primary striatal neurons and amygdala	increased in the absence of BBSome
DRD2 (DRD2S)	[44,63]	dopamine	neuronal processes, e.g. memory	multiple subpopulations of CNS	<i>NIH3T3</i> , primary striatal neurons	
DRD5	[63]	dopamine	neuronal processes, e.g. memory	multiple subpopulations of CNS	<i>NIH3T3</i>	
EP4	[64]	prostaglandin	many functions, e.g. GI homeostasis and cardiac hypertrophy	widely distributed, including cardiovascular and GI system	RPE-1, <i>IMCD3</i>	
GALR2	[12*]	galanin	many functions, e.g. pain sensation and food consumption	widely distributed, including CNS	<i>RPE-1</i>	
GALR3	[12*]	galanin	many functions, e.g. pain sensation, food consumption	widely distributed, including CNS	<i>RPE-1</i> , cultured hypothalamic neurons, hypothalamus section	
GPR161	[29,37**]	orphan	hedgehog signaling	widely distributed, particularly CNS	Variety of cultured cells, e.g. <i>IMCD3</i> , MEFs; primary hippocampal neurons	TULP3 dependent localization; increased ciliary GPR161 in <i>Itih27</i> mutants
GPR175	[65]	orphan	hedgehog signaling	widely distributed	<i>NIH3T3</i> , <i>CO5</i> , S12 cells	
GPR83	[12*]	orphan	energy homeostasis (food consumption)	multiple subpopulations of CNS	<i>RPE-1</i> , several regions of brain, e.g. olfactory tubercle, nucleus accumbens	
GPR88	[66]	orphan	neuronal processes	Cerebral cortex, spleen	<i>IMCD3</i> , cultured striatal neurons	
HTR6	[67,68]	serotonin	neuronal processes, e.g. memory, feeding behavior	multiple subpopulations of CNS	several regions of brain including nucleus accumbens and olfactory tubercles	
KISSR1	[69]	kisspeptin	reproductive function	GnRH neurons	<i>IMCD3</i> , medial hypothalamus	
MCHR1	[17]	melanin-concentrating hormone	energy homeostasis (food consumption)	multiple subpopulations of CNS	<i>IMCD3</i> , nucleus accumbens	BBSome and TUBBY dependent localization
NMUR1	[44]	neuromedin U	energy homeostasis	widely expressed, including GI tract and CNS	<i>NIH3T3</i>	
NPFER1	[44]	neuropeptide FF	pain sensation	subpopulations of CNS	<i>NIH3T3</i>	
NPY2R	[12*,44]	neuropeptides NPY, peptide YY	energy homeostasis (food consumption)	wide distribution; highly expressed in CNS and	<i>NIH3T3</i> , <i>RPE-1</i> , cultured hypothalamic neurons, hypothalamus	BBSome dependent localization

GPCR	Ref.	Ligands	General function	General tissue expression	Ciliary localization shown in (overexpression and endogenous)	BBSome/TUBBY dependent trafficking?
				colon	section (arcuate nucleus)	
NPY5R	[12*]	neuropeptides NPY, peptide YY	energy homeostasis (food consumption)	wide distribution; highly expressed in CNS and colon	RPE-1, cultured hypothalamic neurons, hypothalamus section	
olfactory GPCRs	[70,71]	odorant	smell	olfactory sensory neurons		
opsins (e.g. rhodopsin)	[32,72]	light	vision	retina	retina	TUBBY dependent localization
P2Y12	[73]	ADP	many functions, e.g. platelet function	widely distributed, including platelets and regions of brain	rat liver section (cholangiocytes)	
PRLHR	[44]	prolactin-releasing hormone	neuronal processes, e.g. food consumption, pain sensation	subpopulations of CNS; adrenal gland	NIH3T3, third ventricle mouse brain	
QRFP	[12*]	neuropeptide QRFP	many functions, e.g. food consumption	subpopulations of CNS; bone	RPE-1, cultured hypothalamic neurons, hypothalamus section	
SMO	[29,58]	unknown	hedgehog signaling	widely expressed	MDCK; MEFs, IMCD3, nodal cells	Increased ciliary Smo in <i>Hh27</i> mutants
SSTR3	[74,75]	somatostatin	many functions, including energy homeostasis	primarily CNS, also pancreatic islets	several regions of brain including hypothalamus, amygdala and cerebellum; pancreatic islets; anterior pituitary	BBSome and TUBBY dependent localization
TGR5	[76]	bile acid	energy homeostasis	widely expressed	Isolated cholangiocytes, liver sections	

Table 1B. List of ciliary GPCR signaling components identified to date

	signaling component	Reference
G proteins	G _{αs}	[77]
	G _{αil}	[65]
	G _{αi3}	[78]
	G _{oif}	[79,80]
	transducin	[81]
	G _β	[79]
adenylate cyclases	Adenylate cyclase type III, class 3	[82-84]
	Adenylate cyclase type III, class 4	[73]
	Adenylate cyclase type III, class 5	[85]

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	signaling component	Reference
	Adenylate cyclase type III, class 6	[10,73,85]
	Adenylate cyclase type III, class 8	[73]
secondary effectors	PKA	[10,73]
	PKC	[79]
G-protein independent	β -arrestin2	[10,86-88]
	GRK3	[86,89]