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HYPOTHESIS

SUBJECT COLLECTION: CILIA AND FLAGELLA

The conserved ancestral signaling pathway from cilium to nucleus

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ABSTRACT

Many signaling molecules are localized to both the primary cilium and nucleus. Localization of specific transmembrane receptors and their signaling scaffold molecules in the cilium is necessary for correct physiological function. After a specific signaling event, signaling molecules leave the cilium, usually in the form of an endocytic vesicle scaffold, and move to the nucleus, where they dissociate from the scaffold and enter the nucleus to affect gene expression. This ancient pathway probably arose very early in eukaryotic evolution as the nucleus and cilium co-evolved. Because there are similarities in molecular composition of the nuclear and ciliary pores the entry and exit of proteins in both organelles rely on similar mechanisms. In this Hypothesis, we propose that the pathway is a dynamic universal cilia-based signaling pathway with some variations from protists to man. Everywhere the cilium functions as an important organelle for molecular storage of certain key receptors and selection and concentration of their associated signaling molecules that move from cilium to nucleus. This could also have important implications for human diseases such as Huntington disease.

KEY WORDS: Primary cilium, Signaling scaffold molecule, Transmembrane receptor, Nuclear pore, Huntingtin

Introduction

The primary cilium is a generally non-motile, membrane-bound centriolar extension of the cell with a 9+0 microtubule-based axoneme (a ring with nine outer doublet microtubules without a central pair of microtubules) that is found on many diverse mammalian cell types, including fibroblasts and neurons. It acts as a sensory antenna of these cells (Singla and Reiter, 2006; Satir et al., 2010; Anvarian et al., 2019) and is the basis of sensory organs, such as the vertebrate eye, and certain insect and invertebrate sensilla. It is closely related to motile 9+2 cilia, which are widely distributed from protists to man and also have sensory functions (Sigg et al., 2017). Mutations in ciliary proteins have been linked to human diseases including polycystic kidney disease (PKD) and primary ciliary dyskinesia (PCD), now collectively called ciliopathies (Fliegauf et al., 2007; Waters and Beales, 2011).

Below the ciliary membrane, the matrix around the axoneme is open to the cytoplasm, but the base of the cilium (transition zone) contains a barrier (the ciliary pore) that restricts unfettered entry of cytoplasmic constituents. Because of the ciliary pore (also called ciliary necklace), the primary cilium provides a sequestered compartment for both membrane proteins, including important receptors, and for cytoplasmic molecules, including signaling proteins and signaling scaffold proteins. We seek to explain why so many cilia-localized signaling molecules are also found in the nucleus. We propose here that the cilium is a dynamic storage organelle for these molecules, some of which are specifically targeted to the organelle upon their synthesis and concentrated in the cilium for effective interactions. Signaling and signaling scaffold molecules leave this storage compartment after specific stimulation of a ciliary membrane receptor at an appropriate physiological or developmental time, and could then move from cilium to nucleus to reprogram gene expression (Fig. 1). Since entry and exit of signaling molecules must be facilitated in both the cilium and the nucleus, this hypothesis is based on the probability of co-evolution of the two organelles at the beginning of eukaryotic evolution (Satir et al., 2015), which is supported by homologies of molecular composition and mechanisms between nuclear pores and ciliary pores that have recently been described (Kee et al., 2012; Del Viso et al., 2016; Endicott and Brueckner, 2018). We have put forward previously incomplete versions of this hypothesis (Satir et al., 2015; Satir, 2017). However, we have now incorporated recent concepts provided by others, in particular Maxence Nachury (Nachury, 2014), who calculated the concentration factor for receptors and signaling molecules, which explains why the pathway begins in the cilium, as well as findings from the Copenhagen group (Christensen et al., 2017; Morthorst et al., 2018; Anvarian et al., 2019), who have delineated the details of the signaling pathways, some of which end in the nucleus.

We know of many molecules that are localized to both the cilium and nucleus, including the SMAD transcription factors, p90Rsk (also known as RPS6KA1) (Clement et al., 2013a; Clement et al., 2013b), Jade 1, a Wnt-related ubiquitin ligase (Borgal et al., 2012), certain ciliate GEFs (Bell et al., 2009), RSP3 (also known as RSPH3) (Yan et al., 2015), the parafusin (PFUS; see https://www.ncbi.nlm.nih.gov/nuccore/AY970820) (Satir et al., 2015) (Fig. 2) and Huntingtin (Htt) (Maiuri et al., 2013). A comprehensive survey of molecules found in both organelles suggests that there are many additional examples yet to be confirmed (McClure-Begley and Klymkowsky, 2017).

A plausible scenario is that cytoplasmic molecules, such as SMADs or PFUS, move along microtubules (Caviston et al., 2007; Caviston and Holzbaur, 2009) in the form of a scaffold for secretory or other vesicles (Monis et al., 2017). While traditional scaffold molecules, for example, clathrin, are major structural components of the vesicle coat, the signaling scaffold molecules we broadly consider are diverse (including G-proteins, transcription factors etc.), usually more minor components that bind to and move with the coat. Our hypothesis (Fig. 1) is that the vesicles move from their site of synthesis to the ciliary base, before being exocytosed into the cilium (Monis et al., 2017). This simplified interpretation is consistent with a previous report that showed that axonemal proteins are found on cytoplasmic vesicles during ciliary growth (Wood and Rosenbaum, 2014) and the direct demonstration that the delivery of ciliary membrane proteins to primary cilia involves several different

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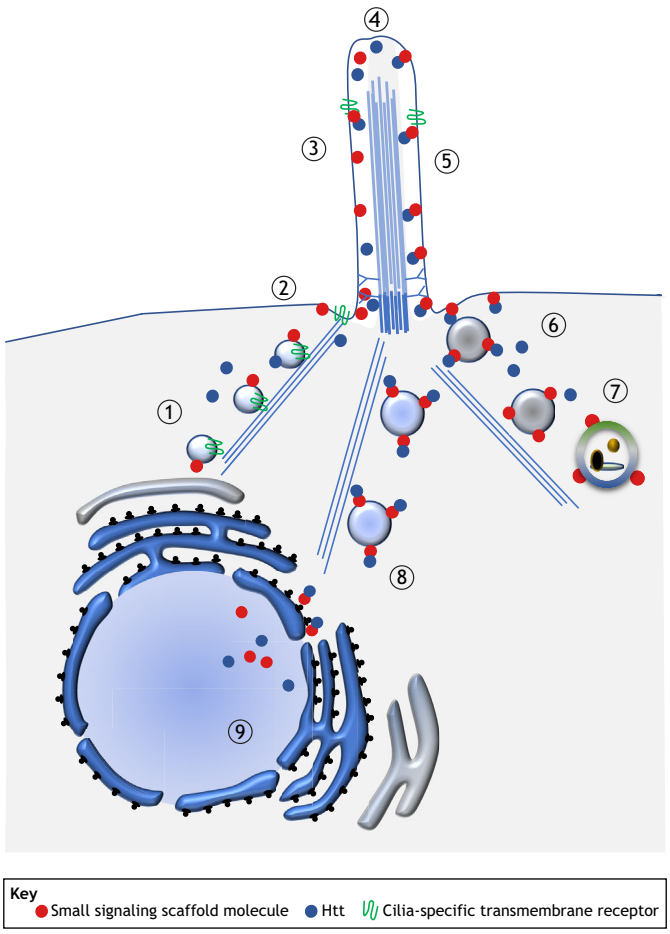

Fig. 1. Proposed translocation of signaling scaffold molecules to and from the cilium. Illustrated here is our proposed translocation of signaling molecules (e.g. SMAD4 or Htt) into the cilium, following their synthesis in the cytoplasm. At the cilium, they are stored before an appropriate ciliary transmembrane receptor is stimulated, which results in their ciliary exit and movement through the cytoplasm and into the nucleus. Detailed steps are as follows: (1) synthesis on ribosomes followed by release into cytoplasm and the formation of a vesicle scaffold; (2) transport to the cilium; here exocytosis occurs near the ciliary pocket, and signaling molecules are transported as peripheral membrane proteins past the ciliary pore; (3) IFT or diffusion to the ciliary tip for storage; (4) signal initiation owing to receptor activation; (5) IFT, in conjunction with BBsome proteins, out of the cilium past the ciliary pore; (6) endocytosis from the ciliary pocket into vesicular scaffolds. Here, post-translational modifications might occur; (7) transport to autophagosomes, an alternative destination that affects ciliogenesis; (8) transport to the nucleus; (9) entry into the nucleus through the nuclear pore. Signaling proteins might bind to chromatin as has been shown for Htt, or affect gene expression indirectly.

pathways of vesicular trafficking (Monis et al., 2017). For instance, the intraflagellar transport (IFT) complex A has been shown to be critical for such transport (Picariello et al., 2019). Furthermore, transmembrane receptors (e.g. polycystin-2) become localized to the ciliary membrane (Pazour et al., 2002; Monis et al., 2017). Small signaling molecules, such as GTP and cAMP, and signaling scaffold molecules, such as SMADs, enter the cilium proper to be stored and processed along the axoneme as matrix molecules or peripheral ciliary membrane proteins (Pazour et al., 2005). Upon a specific external signaling event or trigger, which often is a transmembrane receptor ligand but could also be a physical stimulus such as light, signaling proteins then leave the cilium, typically through a complex process that involves the BBSome and retrograde IFT (Lechtreck et al., 2009; Ye et al., 2018) to become part of an endocytic or other vesicular scaffold (Christensen et al., 2017). From the ciliary base, these factors then move to the nucleus, where they dissociate from the scaffold and enter the nucleus.

In this Hypothesis, we argue that, based on the present evidence, this plausible sequence of events constitutes an ancient universal cilia-based signaling pathway. In this context, we also consider evolutionary aspects of the signaling scaffold protein PFUS and the possible intracellular movement of Htt, the signaling scaffold protein whose mutation leads to Huntington disease (HD), from the cilium to the nucleus.

Specific receptors must be in the primary cilium for them to function correctly

The importance of the primary cilium for human disease first became clear when it was understood that in order to function properly, the polycystins, important transient receptor potential (TRP) receptors for the prevention of PKD, had to enter the ciliary membrane (Pazour et al., 2000, 2002). Entry into the cilium requires the biogenesis of lysosome-related organelles complex-1 (BLOC-1) (Monis et al., 2017). After synthesis, the polycystins reside in the ER – but they are non-functional and do not prevent cystogenesis if they remain there and do not enter the cilium (Nauli and Zhou, 2004).

This finding was confirmed for other receptors, for example the receptor tyrosine kinase (RTK) platelet-derived growth factor receptor α (PDGFRα) (Schneider et al., 2005). Upon serum starvation, fibroblasts enter G0, the non-cycling quiescent state where primary cilia grow and remain stable. Quiescent fibroblasts newly synthesize PDGFRα, and the receptor is specifically targeted to the ciliary membrane. By 24 h of starvation, the receptor has entered the cilium and becomes localized all along the ciliary membrane (Schneider et al., 2005).

In cells incapable of growing a primary cilium, although PDGFRα is sometimes synthesized, it cannot respond to a normal stimulus, such as the presence of its ligand PDGF-AA (Schneider et al., 2005). Indeed, PDGFRα requires the IFT protein IFT20 for its proper localization to the cilium (Schmid et al., 2018). IFT20 modulates ciliary PDGFRα signaling by regulating the stability, ciliary localization and activity of Cbl E3 ubiquitin ligases. In the absence of IFT20, no cilium is built and PDGFRα is found dispersed throughout the cell, with some localization to the plasma membrane. When PDGF-AA is added, the plasma membrane receptor becomes phosphorylated, but because Cbl is degraded, resulting activation is not shut off properly, thereby producing an aberrant signal. Only localization of both the receptor and Cbl in the primary cilium allows the signaling to proceed normally to completion (Schmid et al., 2018).

The serine threonine kinase receptors TGFβRI and TGFβRII, members of the transforming growth factor β superfamily, provide another example of receptors that need to localize to the cilium for correct functioning. In the absence of ligand, these receptors localize to the tips and bases of fibroblast primary cilia (Clement et al., 2013b). In mutant fibroblasts with defective primary cilia, the production of receptor is unchanged, but signaling is abnormal (Christensen et al., 2017).

Cilia of fibroblasts can be long lived, but potentially they are transient structures, which are resorbed if the cell is stimulated to divide (Tucker et al., 1979; Ford et al., 2018). More permanent cilia are found on neurons, including olfactory sensing neurons or the outer segments of photoreceptors. Although components of these cilia, such as the rod discs turn over, the cilia themselves, as with the cells they project from, can persist for months (Mackay-Sim and Kittel, 1991), or in the case of photoreceptors, almost a lifetime (Perkins and Fadool, 2010). In these cilia, the sequestration and

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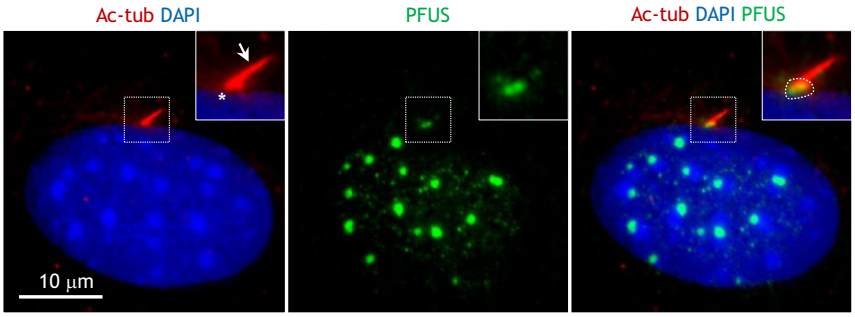

Fig. 2. Example of the localization of a molecule to both the cilium and nucleus. Parafusin (PFUS; green) is a small signaling scaffold molecule that localizes to the ciliary base and to the nucleolus in MEFs. The nucleus is DAPI stained (blue). The cilium is identified by acetylated α-tubulin (Ac-tub, red).

storage of receptors and signaling molecules (e.g. G proteins) is particularly obvious and critically important (Insinna and Besharse, 2008; Pearring et al., 2013; Molday and Moritz, 2015).

The vertebrate photoreceptor cilium is among the best-studied example of molecular segregation and sequestration in a cilium. Opsins, the photoreceptor proteins, are made in the inner segment and continuously transported, mainly through IFT (Luby-Phelps et al., 2008), into the forming outer segment discs (Insinna and Besharse, 2008). Opsins are G-protein-coupled receptors (GPCRs), which are targeted, sequestered, stored and primed for activity in the greatly exaggerated photoreceptor ciliary membrane that forms the outer segment discs (Keady et al., 2011; Pearring et al., 2013; Molday and Moritz, 2015).

There are numerous odor receptors for sensory reception in mammalian olfactory cilia, which are also GPCRs (Jenkins et al., 2009); each is produced by a specific gene (over 400 in humans) and localizes to a ciliary membrane. As in the case for opsins, odor receptors are targeted to and stored in the ciliary membrane. Signaling requires this localization because odorants are external ligands that will not normally reach intracellular compartments.

Primary cilia are also particularly important in brain neurons, as illustrated by the highly complex interactions between receptors of the different classes of neurons in the hypothalamus that are involved in control of eating. Loss of primary cilia on pro-opiomelanocortin (POMC) neurons produces hyperphagia and obesity as no signal to stop eating is generated (Davenport et al., 2007; Satir, 2007). At least seven GPCRs, including the neuropeptide Y (NPY) receptors NPY2R and NPY5R, and the melanocortin receptor MC4R localize to primary cilia in the hypothalamus and control energy homeostasis (Loktev and Jackson, 2013). MC4R colocalizes with a second ciliary transmembrane receptor, adenylyl cyclase 3 (AC3; also known as ADCY3), in the primary cilia of a subset of hypothalamic neurons (Siljee et al., 2018). Mutations that cause mis-localization of MC4R lead to faulty activation of AC3, which in turn leads to obesity (Siljee et al., 2018).

The take-home lesson from these examples is that at least four types of transmembrane receptors that act in physiologically significant signaling pathways, namely TRP channels, RTKs, TGFβ/BMP receptors and GPCRs, must be targeted to and inserted into the membrane of the primary cilium in order to signal correctly. As illustrated in the example of polycystin, these receptors usually move through the vesicular compartments of the cell, from the ER and the Golgi, on their way to the cilium. Indeed, if these receptors remain in a vesicle or are exocytosed at the plasma membrane, they are non-functional or misfunctional (see, for example, Nauli and Zhou, 2004, Schmid et al., 2018). For correct physiological function, targeted exocytosis near the ciliary base leads to translocation of the receptors, likely together with any signaling scaffold molecules, into the ciliary membrane for their storage, or perhaps further covalent modifications until the proper signal arrives. Here, the sensory modes and stimuli such as the ligands for each receptor are specific to the differentiated cell type.

In this context, signaling scaffold molecules, for example G proteins, could either enter the cilium on the same vesicle scaffold as the receptor or independently. Small molecules such as GTP or ATP, which interact with the receptor, the G proteins or signaling scaffold molecules, could also enter by other means, including diffusion (Breslow et al., 2013). All these molecules thus come to surround the ciliary transmembrane receptor (Pazour et al., 2005).

Ciliary receptor activation drives molecules out of the cilium

Hh signaling in mammalian cells is the canonical illustration of a specific signal inducing the movement of a protein (Gli family proteins) that is localized in the cilium to the nucleus to alter gene expression (Haycraft et al., 2005; Rohatgi et al., 2007; Goetz, et al., 2009; Nozawa et al., 2013). As ciliogenesis proceeds, for example in human embryonic stem cells (Kiprilov et al., 2008), the factors involved in Hh signaling are synthesized and distributed to the cilium or cytoplasm. Defects in IFT proteins, including IFT27, attenuate Hh signaling (Eguether et al., 2018). In the unstimulated cell, the transmembrane receptor Patched 1 (Ptch1) is stored along the entire ciliary membrane, whereas Gli proteins and other modifiers of the pathways are stored at the ciliary tip. In the absence of Hh ligand, certain Gli transcription factors are processed into repressor forms (GliR), leave the cilium and move to the nucleus to act on a set of genes that affect cell differentiation (Goetz et al., 2009). When a ligand, such as sonic hedgehog (Shh) binds to Ptch1, it is activated, triggering its exit from the cilium (Nozawa et al., 2013).

Concurrently with Ptch1 leaving the cilium, the transmembrane receptor, Smoothened (Smo) localizes to the plasma membrane and moves through the ciliary pore to enter the ciliary membrane (Wang et al., 2009; Milenkovic, et al., 2009). Smo is a GPCR that is activated by cholesterol or oxysterols (Huang et al., 2016; Raleigh et al., 2018; Qi et al., 2018); however, is it not clear whether or why this occurs specifically in the ciliary membrane in vertebrates. Here, Smo changes the processing of Gli, giving rise to the Gli variant GliA, which then leaves the cilium and enters the nucleus, where it activates a different set of genes that change the differentiation program. Although this process and the end products in terms of neural differentiation have been studied extensively (Goetz et al., 2009), the transport of Gli through the cytoplasm is less well understood.

A second example of a direct path from the cilium to the nucleus is TGF-β signaling in growth-arrested human foreskin fibroblasts (Clement et al., 2013b). Here, several molecules in the cilium and ciliary base, for example, receptor SMADs (SMAD2 and SMAD3) and helper SMAD (SMAD4), are activated and processed together. Stimulation of the TGF-β receptor by TGFβ1 leads to

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phosphorylation of SMAD2/3, followed by translocation of SMAD4 and phosphorylated SMAD2/3 into the nucleus (Clement et al., 2013b). However, beyond these examples, the evidence for such direct movement is inconclusive.

Broader effects of cilia-based signaling

It is worth emphasizing that cilia-based signaling has much broader effects than direct transport of signaling molecules from the cilium to the nucleus. Because a high concentration of receptor and of signaling molecules are sequestered in the cilium (Nachury, 2014) and initiate a timed series of signaling events, many different pathways that originate there can be stimulated concurrently to extend the outcome of the stimulus to achieve different purposes. In this regard, the relationship between cilia and autophagy should be noted (Pampliega et al., 2013; Kaliszewski et al., 2015). Ciliary signaling, for example, from the Hedgehog pathway, induces autophagy, while blockage of autophagy enhances cilia-associated signaling.

As an example of multiple pathway stimulation, TGFβ also activates non-SMAD signaling pathways, including MAPK pathways, Rho-like GTPase signaling pathways and phosphatidylinositol 3-kinase (PI3K)/AKT pathways, some of which have origins in the cilium. Although these cilia-localized kinases do not themselves enter the nucleus, their downstream targets do and can alter gene expression, thereby having wide-ranging physiological and developmental effects (reviewed in Zhang, 2009).

PDGFRα signaling also has a broader effect. After the receptor enters the cilium, it dimerizes and two tyrosine residues in each of the PDGFRα chain become phosphorylated in the presence of its ligand PDGF-AA (Schneider et al., 2005). One of the phosphorylated tyrosine residues acts as a docking site for adaptor proteins with SH2 domains, which become phosphorylated and activate Erk1 and Erk2 (Erk1/2, also known as MAPK3 and MAPK1, respectively). The other phosphorylated tyrosine residue is a docking site for p85 PI3K regulatory subunit (also known as PIK3R1), which acts to mediate the phosphorylation of Akt proteins (Schneider et al., 2005). In this manner, along the cilium, two important signaling pathways, Mek1/2–Erk1/2 and PI3K–Akt, are both activated by PDGF-AA stimulation. Furthermore, activation of Mek1/2 (also known as MAP2K1 and MAP2K2, respectively) and Erk1/2 kinases at the ciliary base induces changes in cytoskeletal organization including microtubule bundling and vesicular transport of a Na^+/H^+ exchanger (NHE1) to a forming lamellipodium to help initiate actin polymerization (Clement et al., 2013a).

In tissues, PDGF-AA is released in a wound, and upon reaching the fibroblasts, activates the ciliary responses to help cause directional cell migration to close the wound (Schneider et al., 2009; Schneider et al., 2010). Aberrant PDGFRα signaling delays wound closure. In other cell types, mistrafficking of PDGFRα leads to defects such as hydrocephalus (Carter et al., 2012) or other developmental abnormalities.

Downstream of Mek1/2–Erk1/2, p90Rsk, which is localized at the ciliary base, is activated by phosphorylation. p90Rsk is typically found in the nucleus (Clement et al., 2013a), where it has been shown to regulate cell growth and cell cycle progression (Anjum and Blenis, 2008). However, some of the nuclear activated p90Rsk might originate from the ciliary base, thus providing another potential example of a direct path from the cilium to the nucleus.

The question remains what would be the difference between signaling and signaling scaffold molecules in the cilium and their non-ciliary counterparts? One possibility is that the signaling molecules that reside in the cilium interact with specific enzymes at the ciliary base as they leave the cilium and become modified, such as, for instance, through phosphorylation, acetylation and sumoylation (Maiuri et al., 2013), or O-GlcNAcylation (Hart, 2014), which would distinguish them from their cytoplasmic counterparts and make the transport pathway they take unique.

Our hypothesis is more speculative for sensory cilia. Here, the immediate physiological response is a change in the membrane potential (Molday and Moritz, 2015; Jenkins et al., 2009). In photoreceptors and olfactory cells, molecules such as G-proteins (e.g. transducin) leave the cilium. In rod photoreceptors, transducin translocation into the cell body (inner nuclear layer) contributes to survival and enhances synaptic transmission (Majumder et al., 2013). Whether transducin enters the cell nucleus is unclear. However, for our model the physiological response may not be the significant response. The signal scaffold protein Htt is localized along the photoreceptor cilium (Karam et al., 2015). As we discuss later, Htt is involved in neuronal survival but also in ciliogenesis (Keryer et al., 2011). In these sensory cells, molecular movement from the cilium to the nucleus could operate during embryogenesis, cell differentiation and survival. In the hypothalamus, where the main physiological changes relate to neurosecretion and synapse formation controlling eating, we propose that the signaling pathway from the cilium to the nucleus is likely to affect these developmental and survival processes, as well as long term changes in eating behavior.

We conclude that activation of signaling molecules sequestered in the cilium often induces major immediate physiological changes in the cell, as well as a pathway of movement of some of the signaling molecules from the cilium to the nucleus to effect long-term changes in gene expression that affect differentiation, survival, longer term physiology and division.

Ciliary exit of signaling molecules as scaffolds

When they exit the cilium, the signaling molecules that will reach the nucleus must find their way. Very little is known about this process. Although diffusion cannot be ruled out, a probable direct pathway would be by trafficking along the cytoskeleton as scaffold molecules on a membrane vesicle.

In TGF-β signaling, upon ligand interaction, the dimerized receptor leaves the cilium, followed by endocytosis into clathrin-coated vesicles at the ciliary pocket (Clement et al., 2013b; Christensen et al., 2017). Here, SMAD2/3 transcription factors have been found to associate with the coated vesicles at the ciliary base (Clement et al., 2013b). However, it remains to be seen whether the SMAD molecules that move from the cilium to the nucleus indeed piggyback onto forming endocytic vesicles all the way to the nucleus.

In the case of Hh signaling, Ptch1 has been shown to exit the cilium through endocytosis into caveolin-containing vesicles (Yue et al., 2014); this is dependent on the specific ubiquitin ligases Smurf1 and Smurf2, SMAD-related signaling scaffold molecules, which help to deliver Ptch1 to lysosomes for degradation and could themselves translocate into the nucleus (Koganti et al., 2018).

Moreover, the roles of the cytoskeleton and of vesicular trafficking in the transport of signaling molecules from the cilium to nucleus are less well studied and may be less important in protists and invertebrate cilia (Sengupta, 2017; Sigg et al., 2017) than in mammalian primary cilia.

Co-evolution of the cilium and nucleus for signaling integration

Major support for our hypothesis of a direct signaling pathway between the cilium and the nucleus comes from evolutionary findings. The cilium originated early in eukaryotic evolution,

probably as an enveloped virus that then became a basal body (Satir et al., 2007), and, importantly, this occurred concurrently with the evolution of the nucleus and nuclear membrane (Gupta and Golding, 1996; Jékely and Arendt, 2006; Satir et al., 2015). The pathway from cilia to the nucleus might have evolved to allow the concentration of receptors and signaling molecules in a compartment that was effectively sequestered from the rest of the cell. This enabled signaling to be triggered for an accurate timing of gene expression. Primary evidence supporting this view comes from the mechanisms of molecular import and export into and out of the two organelles, which involves many of the same molecules, as well as the similarities between the nuclear and ciliary pores (Kee et al., 2012; Del Viso et al., 2016; Endicott and Brueckner, 2018; Satir, 2017). Indeed, both the nuclear and ciliary pores consist of the same or closely related nucleoporins (Kee et al., 2012; Del Viso et al., 2016). Of these, NUP98, a component of the phenylalanine-glycine (FG) permeability barrier at the nuclear pore, also limits the diffusion of soluble molecules greater than 70 kDa into the mammalian primary cilium (Endicott and Brueckner, 2018). In addition, scaffold proteins of exocytic vesicles, nuclear pores and cilia are often structurally related (Jékely and Arendt, 2006; Dacks and Field, 2007; Dacks et al., 2008). Furthermore, nuclear localization sequences (NLS) and ciliary localization sequences (CLS) are also similar (Kee et al., 2012), and both organelles use a Ran-mediated importin mechanism for cargo transport (Dishinger et al., 2010; Maiuri et al., 2013).

Since this universal pathway presumably evolved near the beginning of the eukaryotic cell lineage, and other important signaling pathways emerged as evolution proceeded (Sigg et al., 2017), variations on some aspects of this direct signaling pathway between the two organelles are to be expected, as notable in motile cilia of protists such as *Chlamydomonas*. In *Chlamydomonas*, ciliary membrane trafficking does not significantly depend on endocytosis, but rather relies on shedding in the form of ectosomes (Cao et al., 2015). Interestingly, shedding of ciliary membrane is also part of photoreceptor disc turnover (Pearring et al., 2013), suggesting it might be a more general signaling mechanism of ciliated cells. Moreover, in *Chlamydomonas*, Ca^{2+} influx through ciliary receptors can directly trigger changes in gene expression (Cheshire and Keller, 1991); perhaps, other second messenger molecules such as cAMP may do this as well.

Nevertheless, despite such variations, the direct molecular cilium-nucleus pathway is probably present in protists. In *Tetrahymena*, the signaling scaffold and DNA repair protein Rad51 is localized to both the cilium and the nucleus and may move from a ciliary receptor to the macronucleus (Christensen et al., 2003; Satir and Satir, 2016); however, it is not known whether endosomal trafficking is part of the pathway.

Studies on the localization of the signaling scaffold molecule PFUS also support the notion that the direct pathway from the cilium to the nucleus is present in protists. PFUS is an ancient paralog of phosphglucomutase that is found in protists, such as *Paramecium* and *Toxoplasma*, as well as its homologs in mammalian cells (Wyroba et al., 1995). In *Paramecium*, PFUS is predominantly found as a signaling scaffold protein on exocytic vesicles that is critical for the initiation of membrane fusion (Subramanian and Satir, 1992); however, it also localizes to the cilium tip and the macronucleus (Satir et al., 2015). Inhibition of PFUS by RNA interference (RNAi) entirely inhibits the production of new secretory proteins (Liu et al., 2011), which suggests that nuclear PFUS affects gene expression. In mouse fibroblasts, PFUS also localizes to both the ciliary base and the nucleus, and specifically to the nucleolus (Satir et al., 2015) (Fig. 2). It is possible that the features of PFUS signaling present in protists have been retained in mammalian evolution.

Taken together, there is ample evidence for the early co-evolution of the cilium and the nucleus that would explain the origin of direct signaling pathways between the two organelles to account for ciliary signaling scaffold proteins found in the nucleus. This supports our hypothesis that the cilium is a dynamic storage organelle for these molecules, which after their activation, leave the cilium and enter the nucleus to affect gene expression.

The Htt conundrum

To illustrate the importance of the pathway we are proposing, we address one example of a prominent molecule that has been found both in the cilium and the nucleus and thus might shuttle between these organelles: Htt, a ubiquitous cytoplasmic protein, whose mutant form in neurons is responsible for HD, a devastating neurogenerative disorder (Maiuri et al., 2013; Gasset-Rosa et al., 2017). Htt is a membrane-associated scaffold protein that enters the nucleus to affect gene expression (Benn et al., 2008). However, Htt also localizes to neuronal primary cilia and the ciliary base, as well as the connecting cilium of the photoreceptor (Karam et al., 2015). Furthermore, Htt has been shown to be involved in regulating ciliogenesis (Keryer et al., 2011). This raises the intriguing conundrum of why is Htt in cilia (Nowogrodzki, 2018; Saudou and Humbert, 2016).

Our hypothesis predicts that after synthesis in the cytoplasm, Htt molecules will localize into the cilium along with specific membrane receptors, and, at some defined developmental or physiological time, then will relocate from the cilium to the nucleus with subsequent changes in gene expression that affect neuronal survival (Atwal et al., 2007; Benn et al., 2008; Gasset-Rosa et al., 2017).

The structure of Htt permits interaction with many different proteins, which is particularly dependent on its phosphorylation state (Maiuri et al., 2013); in fact, Htt has been considered as a master scaffold hub (Saudou and Humbert, 2016). Htt utilizes dynein–dynactin and kinesin to move back and forth along microtubules with vesicles in neurons (Saudou and Humbert, 2016). It is likely that dynein–dynactin is used to reach the basal body of the cilium (Kubo et al., 1999), whereas kinesin may be used to move toward the nucleus.

The ciliary localization of mammalian Htt depends on N17, a 17-amino-acid phosphorylatable amphipathic α-helix at the N-terminal region of the protein just prior to the polyglutamine sequences, which, when expanded, causes HD. Only nonphosphorylated N17 is found in striatal cell line cilia (Maiuri et al., 2013), which suggests to us that *de novo* synthesized and transported Htt is not phosphorylated. The authors proposed that N17 is a master regulator of Htt transport in and out of the cilium and nucleus that is dependent on ER stress and subsequent N17 phosphorylation (Maiuri et al., 2013).

The ciliary receptor or receptors, which when stimulated by the appropriate ligand, could result in the exit of Htt out of the cilium are unknown, but there are intriguing possibilities. We will briefly consider three possible ciliary signaling pathways that could trigger ciliary exit of Htt: TGFβ, brain-derived neurotrophic factor (BDNF) and Hh.

As discussed above, TGFβ signaling relies on SMAD transcription factors to move through and out of the primary cilium and into the nucleus. Interestingly, TGFβ signaling is dysfunctional in HD (Bowles et al., 2017). Indeed, mutant Htt has been shown to alter the extent of SMAD2/3 phosphorylation and its translocation into the nucleus (Bowles et al., 2017). Therefore, Htt could be part of the

Box 1. Unanswered questions on cilia-to-nucleus transport

- Is there a common cytoskeletal or cytosolic pathway that signaling molecules use to travel between cilium and nucleus in mammalian cells?
- Which features of this pathway are evolutionarily conserved?
- Are specific signaling molecules that localize to the cilium absent from the nucleus before stimulation of the appropriate ciliary transmembrane receptor?
- Do signaling molecules such as Htt enter or leave the primary cilium on a vesicular scaffold?
- Is there a specific ligand that drives Htt from the cilium to the nucleus?
- Is a specific covalent modification of Htt necessary for this process?
- Does mutant Htt that produces HD follow the same pathway?

scaffold that normally accompanies the SMADs as they move from cilium to nucleus.

Htt also upregulates the transcription of BDNF (Zuccato et al., 2001), a ligand for the TrkB receptor. Moreover, neuronal survival in HD requires BDNF (Zuccato and Cattaneo, 2007; Molero and Mehler, 2016). In cultured cells in the presence of BDNF, the TrkB receptor localizes to cilia and its activation and signaling after ligand binding depend at least partially on ciliary localization (Leitch and Zaghloul, 2014). The signaling pathways from the TrkB receptor are similar to those of other RTKs (Christensen et al., 2012). Hence, BDNF could be the ligand that drives Htt out of the cilium and into the nucleus.

Finally, there might also be a link between Htt and Hh signaling. Neuronal ciliogenesis and differentiation are both regulated in part by Htt (Keryer et al., 2011). However, differentiation also depends largely on Hh signaling that operates through the primary cilium (Goetz et al., 2009). Since the Gli effector that is necessary for Hh signaling translocates from cilium to nucleus, is it possible that movement of Htt through the cytoplasm is induced in the same way as that of Gli upon Smo activation, or that both proteins indeed move concurrently. In support of this, the G-protein receptor trafficking protein Gprasp2, which is involved in the movement of Smo (Jung et al., 2016), has been shown to interact with Htt (Horn et al., 2006).

The hypothesis proposed here thus points to possible experiments to determine which, if any, of these proposed pathways is correct and to follow Htt as it traverses the cell from the cilium to the nucleus (Box 1). Furthermore, findings from such future studies might also help us to shed light on the pathogenesis of HD.

Conclusions

Many signaling and signaling scaffold molecules associated with transmembrane receptors of the TRP, RTK, TGFβ/BMP and GPCR families localize to both the primary cilium and nucleus. In fact, a number of transmembrane receptors must be targeted to and stored in the primary cilium to function correctly. We propose here, that as a response to complementary external stimuli, signaling molecules that specify immediate physiological changes leave the cilium together with certain signaling scaffold molecules that move, often in conjunction with endocytic vesicles, to the nucleus to affect gene expression. As detailed above, Htt is a prominent signal scaffold protein that might follow this pathway from the cilium to the nucleus. The proposed universal nature of the pathway, which is based on the common early evolution of the ciliary pore and the nuclear pore, should permit its extrapolation to signaling systems that have not yet been fully recognized as being based on cilia. We therefore anticipate that future research efforts to characterize this pathway might elucidate the growing connections between cilia and cancer (Liu et al., 2018) and cilia and cognitive disease (Marley and von Zastrow, 2012; Pruski and Lang, 2019).

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Competing interests

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References

Anjum, R. and Blenis, J. (2008). The RSK family of kinases: emerging roles in cellular signalling. *Nat. Rev. Mol. Cell Biol.* **9**, 747-758. doi:10.1038/nrm2509

Anvarian, Z., Mykytyn, K., Mukhopadhyay, S., Pedersen, L. B. and Christensen, S. T. (2019). Cellular signalling by primary cilia in development, organ function and disease. *Nat. Rev. Nephrol* **15**, 199-219. doi:10.1038/s41581-019-0116-9

Atwal, R. S., Xia, J., Pinchev, D., Taylor, J., Epand, R. M. and Truant, R. (2007). Huntingtin has a membrane association signal that can modulate huntingtin aggregation, nuclear entry and toxicity. *Human Mol. Genetics* **16**, 2600-2615. doi:10.1093/hmg/ddm217

Bell, A. J., Guerra, C., Phung, V., Nair, S., Seetharam, R. and Satir, P. (2009). GEF1 is a ciliary Sec7 GEF of Tetrahymena thermophila. *Cell Motil. Cytoskeleton* **66**, 483-499. doi:10.1002/cm.20348

Benn, C. L., Sun, T., Sadri-Vakili, G., McFarland, K. N., DiRocco, D. P., Yohrling, G. J., Clark, T. W., Bouzou, B. and Cha, J.-H. J. (2008). Huntingtin modulates transcription, occupies gene promoters in vivo and binds directly to DNA in a polyglutamine-dependent manner. *J. Neurosci.* **28**, 10720-10733. doi:10.1523/JNEUROSCI.2126-08.2008

Borgal, L., Habbig, S., Hatzold, J., Liebau, M. C., Dafinger, C., Sacarea, I., Hammerschmidt, M., Benzing, T. and Schermer, B. (2012). The ciliary protein nephrocystin-4 translocates the canonical Wnt regulator Jade-1 to the nucleus to negatively regulate beta-catenin signaling. *J. Biol. Chem.* **287**, 25370-25380. doi:10.1074/jbc.M112.385658

Bowles, K. R., Stone, T., Holmans, P., Allen, N. D., Dunnett, S. B. and Jones, L. (2017). SMAD transcription factors are altered in cell models of HD and regulate HTT expression. *Cell. Signal.* **31**, 1-14. doi:10.1016/j.cellsig.2016.12.005

Breslow, D. K., Koslover, E. F., Seydel, F., Spakowitz, A. J. and Nachury, M. V. (2013). An in vitro assay for entry into cilia reveals unique properties of the soluble diffusion barrier. *J. Cell Biol.* **203**, 129-147. doi:10.1083/jcb.201212024

Cao, M., Ning, J., Hernandez-Lara, C. I., Belzile, O., Wang, Q., Dutcher, S. K., Liu, Y. and Snell, W. J. (2015). Uni-directional ciliary membrane protein trafficking by a cytoplasmic retrograde IFT motor and ciliary ectosome shedding. *Elife* **4**, e05242. doi:10.7554/eLife.05242

Carter, C. S., Vogel, T. W., Zhang, Q., Seo, S., Swiderski, R. E., Moninger, T. O., Cassell, M. D., Thedens, D. R., Keppler-Noreuil, K. M., Nopoulos, P. et al. (2012). Abnormal development of NG2+PDGFRα+ neural progenitor cells leads to neonatal hydrocephalus in a ciliopathy mouse model. *Nat. Med.* **18**, 1797-1804. doi:10.1038/nm.2996

Caviston, J. P. and Holzbaur, E. L. (2009). Huntingtin as an essential integrator of intracellular vesicular trafficking. *Trends Cell Biol.* **19**, 147-155. doi:10.1016/j.tcb.2009.01.005

Caviston, J. P., Ross, J. L., Antony, S. M., Tokito, M. and Holzbaur, E. L. (2007). Huntingtin facilitates dynein/dynactin-mediated vesicle transport. *Proc. Natl. Acad. Sci. USA* **104**, 10045-10050. doi:10.1073/pnas.0610628104

Cheshire, J. L. and Keller, L. R. (1991). Uncoupling of Chlamydomonas flagellar gene expression and outgrowth from flagellar excision by manipulation of Ca2+. *J. Cell Biol.* **115**, 1651-1659. doi:10.1083/jcb.115.6.1651

Christensen, S. T., Guerra, C. F., Awan, A., Wheatley, D. N. and Satir, P. (2003). Insulin receptor-like proteins in Tetrahymena thermophila ciliary membranes. *Curr. Biol.* **13**, R50-R52. doi:10.1016/S0960-9822(02)01425-2

Christensen, S. T., Clement, C. A., Satir, P. and Pedersen, L. B. (2012). Primary cilia and coordination of receptor tyrosine kinase (RTK) signalling. *J. Pathol.* **226**, 172-184. doi:10.1002/path.3004

Christensen, S. T., Morthorst, S. K., Mogensen, J. B. and Pedersen, L. B. (2017). Primary cilia and coordination of receptor tyrosine kinase (RTK) and transforming growth factor beta (TGF-beta) signaling. *Cold Spring Harb. Perspect Biol.* **9**, a028167. doi:10.1101/cshperspect.a028167

Clement, D. L., Mally, S., Stock, C., Lethan, M., Satir, P., Schwab, A., Pedersen, S. F. and Christensen, S. T. (2013a). PDGFRalpha signaling in the primary cilium regulates NHE1-dependent fibroblast migration via coordinated differential activity of MEK1/2-ERK1/2-p90RSK and AKT signaling pathways. *J. Cell Sci.* **126**, 953-965. doi:10.1242/jcs.116426

Clement, C. A., Ajbro, K. D., Koefoed, K., Vestergaard, M. L., Veland, I. R., Henriques de Jesus, M. P., Pedersen, L. B., Benmerah, A., Andersen, C. Y.,

Larsen, L. A. et al. (2013b). TGF-beta signaling is associated with endocytosis at the pocket region of the primary cilium. *Cell Rep.* **3**, 1806-1814. doi:10.1016/j.celrep.2013.05.020
Dacks, J. B. and Field, M. C. (2007). Evolution of the eukaryotic membrane-trafficking system: origin, tempo and mode. *J. Cell Sci.* **120**, 2977-2985. doi:10.1242/jcs.013250
Dacks, J. B., Poon, P. P. and Field, M. C. (2008). Phylogeny of endocytic components yields insight into the process of nonendosymbiotic organelle evolution. *Proc. Natl. Acad. Sci. USA* **105**, 588-593. doi:10.1073/pnas.0707318105
Davenport, J. R., Watts, A. J., Roper, V. C., Croyle, M. J., van Groen, T., Wyss, J. M., Nagy, T. R., Kesterson, R. A. and Yoder, B. K. (2007). Disruption of intraflagellar transport in adult mice leads to obesity and slow-onset cystic kidney disease. *Curr. Biol.* **17**, 1586-1594. doi:10.1016/j.cub.2007.08.034
del Viso, F., Huang, F., Myers, J., Chalfant, M., Zhang, Y., Reza, N., Bewersdorf, J., Lusk, C. P. and Khokha, M. K. (2016). Congenital Heart disease genetics uncovers context-dependent organization and function of nucleoporins at cilia. *Dev. Cell* **38**, 478-492. doi:10.1016/j.devcel.2016.08.002
Dishinger, J. F., Kee, H. L., Jenkins, P. M., Fan, S., Hurd, T. W., Hammond, J. W., Truong, Y. N., Margolis, B., Martens, J. R. and Verhey, K. J. (2010). Ciliary entry of the kinesin-2 motor KIF17 is regulated by importin-beta2 and RanGTP. *Nat. Cell Biol.* **12**, 703-710. doi:10.1038/ncb2073
Eguether, T., Cordelieres, F. P. and Pazour, G. J. (2018). Intraflagellar transport is deeply integrated in hedgehog signaling. *Mol. Biol. Cell* **29**, 1178-1189. doi:10.1091/mbc.E17-10-0600
Endicott, S. J. and Brueckner, M. (2018). NUP98 Sets the size-exclusion diffusion limit through the ciliary base. *Curr. Biol.* **28**, 1643-1650.e3. doi:10.1016/j.cub.2018.04.014
Fliegauf, M., Benzing, T. and Omran, H. (2007). When cilia go bad: cilia defects and ciliopathies. *Nat. Rev. Mol. Cell Biol.* **8**, 880-893. doi:10.1038/nrm2278
Ford, M. J., Yeyati, P. L., Mali, G. R., Keighren, M. A., Waddell, S. H., Mjoseng, H. K., Douglas, A. T., Hall, E. A., Sakaue-Sawano, A., Miyawaki, A. et al. (2018). A cell/cilia cycle biosensor for single-cell kinetics reveals persistence of cilia after G1/S transition is a general property in cells and mice. *Dev. Cell* **47**, 509-523.e5. doi:10.1016/j.devcel.2018.10.027
Gasset-Rosa, F., Chillon-Marinas, C., Goginashvili, A., Atwal, R. S., Artates, J. W., Tabet, R., Wheeler, V. C., Bang, A. G., Cleveland, D. W. and Lagier-Tourenne, C. (2017). Polyglutamine-expanded huntingtin exacerbates age-related disruption of nuclear integrity and nucleocytoplasmic transport. *Neuron* **94**, 48-57. doi:10.1016/j.neuron.2017.03.027
Goetz, S. C., Ocbina, P. J. and Anderson, K. V. (2009). The primary cilium as a Hedgehog signal transduction machine. *Methods Cell Biol.* **94**, 199-222. doi:10.1016/S0091-679X(08)94010-3
Gupta, R. S. and Golding, G. B. (1996). The origin of the eukaryotic cell. *Trends Biochem. Sci.* **21**, 166-171. doi:10.1016/S0968-0004(96)20013-1
Hart, G. W. (2014). Three decades of research on O-GlcNAcylation-a major nutrient sensor that regulates signaling, transcription and cellular metabolism. *Front. Endocrinol.* **5**, 183. doi:10.3389/fendo.2014.00183
Haycraft, C. J., Banizs, B., Aydin-Son, Y., Zhang, Q., Michaud, E. J. and Yoder, B. K. (2005). Gli2 and Gli3 localize to cilia and require the intraflagellar transport protein polaris for processing and function. *PLoS Genet.* **1**, e53. doi:10.1371/journal.pgen.0010053
Horn, S. C., Lalowski, M., Goehler, H., Droge, A., Wanker, E. E. and Stelzl, U. (2006). Huntingtin interacts with the receptor sorting family protein GASP2. *J. Neural. Transm.* **113**, 1081-1090. doi:10.1007/s00702-006-0514-6
Huang, P., Nedelcu, D., Watanabe, M., Jao, C., Kim, Y., Liu, J. and Salic, A. (2016). Cellular cholesterol directly activates smoothened in hedgehog signaling. *Cell* **166**, 1176-1187. doi:10.1016/j.cell.2016.08.003
Insinna, C. and Besharse, J. C. (2008). Intraflagellar transport and the sensory outer segment of vertebrate photoreceptors. *Dev. Dyn.* **237**, 1982-1992. doi:10.1002/dvdy.21554
Jékely, G. and Arendt, D. (2006). Evolution of intraflagellar transport from coated vesicles and autogenous origin of the eukaryotic cilium. *BioEssays* **28**, 191-198. doi:10.1002/bies.20369
Jenkins, P. M., McEwen, D. P. and Martens, J. R. (2009). Olfactory cilia: linking sensory cilia function and human disease. *Chem. Senses* **34**, 451-464. doi:10.1093/chemse/bjp020
Jung, B., Padula, D., Burtscher, I., Landerer, C., Lutter, D., Theis, F., Messias, A. C., Geerlof, A., Sattler, M., Kremmer, E. et al. (2016). Pitchfork and Gprasp2 target smoothened to the primary cilium for hedgehog pathway activation. *PLoS ONE* **11**, e0149477. doi:10.1371/journal.pone.0149477
Kaliszewski, M., Knott, A. B. and Bossy-Wetzel, E. (2015). Primary cilia and autophagic dysfunction in Huntington's disease. *Cell Death Differ.* **22**, 1413-1424. doi:10.1038/cdd.2015.80
Karam, A., Tebbe, L., Weber, C., Messaddeq, N., Morle, L., Kessler, P., Wolfrum, U. and Trottier, Y. (2015). A novel function of Huntingtin in the cilium and retinal ciliopathy in Huntington's disease mice. *Neurobiol. Dis.* **80**, 15-28. doi:10.1016/j.nbd.2015.05.008
Keady, B. T., Le, Y. Z. and Pazour, G. J. (2011). IFT20 is required for opsin trafficking and photoreceptor outer segment development. *Mol. Biol. Cell* **22**, 921-930. doi:10.1091/mbc.e10-09-0792
Kee, H. L., Dishinger, J. F., Blasius, T. L., Liu, C.-J., Margolis, B. and Verhey, K. J. (2012). A size-exclusion permeability barrier and nucleoporins characterize a ciliary pore complex that regulates transport into cilia. *Nat. Cell Biol.* **14**, 431-437. doi:10.1038/ncb2450
Keryer, G., Pineda, J. R., Liot, G., Kim, J., Dietrich, P., Benstaali, C., Smith, K., Cordelières, F. P., Spassky, N., Ferrante, R. J. et al. (2011). Ciliogenesis is regulated by a huntingtin-HAP1-PCM1 pathway and is altered in Huntington disease. *J. Clin. Invest.* **121**, 4372-4382. doi:10.1172/JCI57552
Kiprilov, E. N., Awan, A., Desprat, R., Velho, M., Clement, C. A., Byskov, A. G., Andersen, C. Y., Satir, P., Bouhassira, E. E., Christensen, S. T. et al. (2008). Human embryonic stem cells in culture possess primary cilia with hedgehog signaling machinery. *J. Cell Biol.* **180**, 897-904. doi:10.1083/jcb.200706028
Koganti, P., Levy-Cohen, G. and Blank, M. (2018). Smurfs in protein homeostasis, signaling, and cancer. *Front. Oncol.* **8**, 295. doi:10.3389/fonc.2018.00295
Kubo, A., Sasaki, H., Yuba-Kubo, A., Tsukita, S. and Shiina, N. (1999). Centriolar satellites: molecular characterization, ATP-dependent movement toward centrioles and possible involvement in ciliogenesis. *J. Cell Biol.* **147**, 969-980. doi:10.1083/jcb.147.5.969
Lechtreck, K.-F., Johnson, E. C., Sakai, T., Cochran, D., Ballif, B. A., Rush, J., Pazour, G. J., Ikebe, M. and Witman, G. B. (2009). The Chlamydomonas reinhardtii BBSome is an IFT cargo required for export of specific signaling proteins from flagella. *J. Cell Biol.* **187**, 1117-1132. doi:10.1083/jcb.200909183
Leitch, C. C. and Zaghloul, N. A. (2014). BBS4 is necessary for ciliary localization of TrkB receptor and activation by BDNF. *PLoS ONE* **9**, e98687. doi:10.1371/journal.pone.0098687
Liu, L., Wyroba, E. and Satir, B. H. (2011). RNAi knockdown of parafusin inhibits the secretory pathway. *Eur. J. Cell Biol.* **90**, 844-853. doi:10.1016/j.ejcb.2011.06.002
Liu, H., Kiseleva, A. A. and. Golemism, E. A. (2018). Ciliary signalling in cancer. *Nat. Rev. Cancer* **18**, 511-524. doi:10.1038/s41568-018-0023-6
Loktev, A. V. and Jackson, P. K. (2013). Neuropeptide Y family receptors traffic via the Bardet-Biedl syndrome pathway to signal in neuronal primary cilia. *Cell Rep.* **5**, 1316-1329. doi:10.1016/j.celrep.2013.11.011
Luby-Phelps, K., Fogerty, J., Baker, S. A., Pazour, G. J. and Besharse, J. C. (2008). Spatial distribution of intraflagellar transport proteins in vertebrate photoreceptors. *Vision Res.* **48**, 413-423. doi:10.1016/j.visres.2007.08.022
Mackay-Sim, A. and Kittel, P. W. (1991). On the life span of olfactory receptor neurons. *Eur. J. Neurosci.* **3**, 209-215. doi:10.1111/j.1460-9568.1991.tb00081.x
Maiuri, T., Woloshansky, T., Xia, J. and Truant, R. (2013). The huntingtin N17 domain is a multifunctional CRM1 and Ran-dependent nuclear and cilial export signal. *Hum. Mol. Genet.* **22**, 1383-1394. doi:10.1093/hmg/dds554
Majumder, A., Pahlberg, J., Boyd, K. K., Kerov, V., Kolandaivelu, S., Ramamurthy, V., Sampath, A. P. and Artemyev, N. O. (2013). Transducin translocation contributes to rod survival and enhances synaptic transmission from rods to rod bipolar cells. *Proc. Natl. Acad. Sci. USA* **110**, 12468-12473. doi:10.1073/pnas.1222666110
Marley, A. and von Zastrow, M. (2012). A simple cell-based assay reveals that diverse neuropsychiatric risk genes converge on primary cilia. *PLoS ONE* **7**, e46647. doi:10.1371/journal.pone.0046647
McClure-Begley, T. D. and Klymkowsky, M. W. (2017). Nuclear roles for cilia-associated proteins. *Cilia* **6**, 8. doi:10.1186/s13630-017-0052-x
Milenkovic, L., Scott, M. P. and Rohatgi, R. (2009). Lateral transport of Smoothened from the plasma membrane to the membrane of the cilium. *J. Cell Biol.* **187**, 365-374. doi:10.1083/jcb.200907126
Molday, R. S. and Moritz, O. L. (2015). Photoreceptors at a glance. *J. Cell Sci.* **128**, 4039-4045. doi:10.1242/jcs.175687
Molero, A. and Mehler, M. (2016). Huntington's disease. In *Neuroscience in the 21st Century* (ed. D. Pfaff and N. Volkow), pp. 3863-3892. Springer.
Monis, W. J., Faundez, V. and Pazour, G. J. (2017). BLOC-1 is required for selective membrane protein trafficking from endosomes to primary cilia. *J. Cell Biol.* **216**, 2131-2150. doi:10.1083/jcb.201611138
Morthorst, S. K., Christensen, S. T. and Pedersen, L. B. (2018). Regulation of ciliary membrane protein trafficking and signalling by kinesin motor proteins. *FEBS J.* **285**, 4535-4564. doi:10.1111/febs.14583
Nachury, M. V. (2014). How do cilia organize signalling cascades? *Phil. Trans. R. Soc. Lond. B* **369**, 20130465. doi:10.1098/rstb.2013.0465
Nauli, S. M. and Zhou, J. (2004). Polycystins and mechanosensation in renal and nodal cilia. *BioEssays* **26**, 844-856. doi:10.1002/bies.20069
Nowogrodzki, A. (2018). Huntington's disease: 4 big questions. *Nature* **557**, S48. doi:10.1038/d41586-018-05180-3
Nozawa, Y. I., Lin, C. and Chuang, P.-T. (2013). Hedgehog signaling from the primary cilium to the nucleus: an emerging picture of ciliary localization, trafficking and transduction. *Curr. Opin. Genet. Dev.* **23**, 429-437. doi:10.1016/j.gde.2013.04.008
Pampliega, O., Orhon, I., Patel, B., Sridhar, S., Diaz-Carretero, A., Beau, I., Codogno, P., Satir, B. H., Satir, P. and Cuervo, A. M. (2013). Functional

interaction between autophagy and ciliogenesis. *Nature* **502**, 194-200. doi:10.1038/nature12639

Pazour, G. J., Dickert, B. L., Vucica, Y., Seeley, E. S., Rosenbaum, J. L., Witman, G. B. and Cole, D. G. (2000). Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J. Cell Biol.* **151**, 709-718. doi:10.1083/jcb.151.3.709

Pazour, G. J., San Agustin, J. T., Follit, J. A., Rosenbaum, J. L. and Witman, G. B. (2002). Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. *Curr. Biol.* **12**, 3778-3380. doi:10.1016/S0960-9822(02)00877-1

Pazour, G. J., Agrin, N., Leszyk, J. and Witman, G. B. (2005). Proteomic characterization of a eukaryotic cilium. *J. Cell Biol.* **170**, 103-113. doi:10.1083/jcb.200504008

Pearring, J. N., Salinas, R. Y., Baker, S. A. and Arshavsky, V. Y. (2013). Protein sorting, targeting and trafficking in photoreceptor cells. *Prog. Retin. Eye Res.* **36**, 24-51. doi:10.1016/j.preteyeres.2013.03.002

Perkins, B. D. and Fadool, J. M. (2010). Photoreceptor structure and development analyses using GFP transgenes. *Methods Cell Biol.* **100**, 205-218. doi:10.1016/B978-0-12-384892-5.00007-4

Picariello, T., Brown, J. M., Hou, Y., Swank, G., Cochran, D. A., King, O. D., Lechtreck, K., Pazour, G. J. and Witman, G. B. (2019). A global analysis of IFT-A function reveals specialization for transport of membrane-associated proteins into cilia. *J. Cell Sci.* **132**, jcs220749. doi:10.1242/jcs.220749

Pruski, M. and Lang, B. (2019). Primary cilia–an underexplored topic in major mental illness. *Front. Psychiatry* **10**, 104. doi:10.3389/fpsyt.2019.00104

Qi, X., Schmiege, P., Coutavas, E. and Li, X. (2018). Two Patched molecules engage distinct sites on Hedgehog yielding a signaling-competent complex. *Science* **362**, aas8843. doi:10.1126/science.aas8843

Raleigh, D. R., Sever, N., Choksi, P. K., Sigg, M. A., Hines, K. M., Thompson, B. M., Elnatan, D., Jaishankar, P., Bisignano, P., Garcia-Gonzalo, F. R. et al. (2018). Cilia-associated oxysterols activate smoothened. *Mol. Cell* **72**, 316-327.e5. doi:10.1016/j.molcel.2018.08.034

Rohatgi, R., Milenkovic, L. and Scott, M. P. (2007). Patched1 regulates hedgehog signaling at the primary cilium. *Science* **317**, 372-376. doi:10.1126/science.1139740

Satir, P. (2007). Cilia biology: stop overeating now! *Curr. Biol.* **17**, R963-R965. doi:10.1016/j.cub.2007.09.006

Satir, P. (2017). CILIA: before and after. *Cilia* **6**, 1. doi:10.1186/s13630-017-0046-8

Satir,P. and Satir, B. H. (2016). Intracytoplasmic signaling from cilia in ciliates. In *Biocommunication of Ciliates* (ed. G. Witzany and M. Nowacki), pp. 51-63. Switzerland Chapter 4: Springer.

Satir, P., Guerra, C. and Bell, A. J. (2007). Evolution and persistence of the cilium. *Cell Motil. Cytoskeleton* **64**, 906-913. doi:10.1002/cm.20238

Satir, P., Pedersen, L. B. and Christensen, S. T. (2010). The primary cilium at a glance. *J. Cell Sci.* **123**, 499-503. doi:10.1242/jcs.050377

Satir, B. H., Wyroba, E., Liu, L., Lethan, M., Satir, P. and Christensen, S. T. (2015). Evolutionary implications of localization of the signaling scaffold protein parafusin to both cilia and the nucleus. *Cell Biol. Int.* **39**, 136-145. doi:10.1002/cbin.10337

Saudou, F. and Humbert, S. (2016). The biology of huntingtin. *Neuron* **89**, 910-926. doi:10.1016/j.neuron.2016.02.003

Schmid, F. M., Schou, K. B., Vilhelm, M. J., Holm, M. S., Breslin, L., Farinelli, P., Larsen, L. A., Andersen, J. S., Pedersen, L. B. and Christensen, S. T. (2018). IFT20 modulates ciliary PDGFRalpha signaling by regulating the stability of Cbl E3 ubiquitin ligases. *J. Cell Biol.* **217**, 151-161. doi:10.1083/jcb.201611050

Schneider, L., Clement, C. A., Teilmann, S. C., Pazour, G. J., Hoffmann, E. K., Satir, P. and Christensen, S. T. (2005). PDGFRalphaalpha signaling is regulated through the primary cilium in fibroblasts. *Curr. Biol.* **15**, 1861-1866. doi:10.1016/j.cub.2005.09.012

Schneider, L., Stock, C. M., Dieterich, P., Jensen, B. H., Pedersen, L. B., Satir, P., Schwab, A., Christensen, S. T. and Pedersen, S. F. (2009). The Na+/H+ exchanger NHE1 is required for directional migration stimulated via PDGFR-alpha in the primary cilium. *J. Cell Biol.* **185**, 163-176. doi:10.1083/jcb.200806019

Schneider, L., Cammer, M., Lehman, J., Nielsen, S. K., Guerra, C. F., Veland, I. R., Stock, C., Hoffmann, E. K., Yoder, B. K., Schwab, A., Satir, P. and Christensen, S. T. (2010). Directional cell migration and chemotaxis in wound healing response to PDGF-AA are coordinated by the primary cilium in fibroblasts. *Cell Physiol. Biochem.* **25**, 279-292. doi:10.1159/000276562

Sengupta, P. (2017). Cilia and sensory signaling: The journey from “animalcules” to human disease. *PLoS Biol.* **15**, e2002240. doi:10.1371/journal.pbio.2002240

Sigg, M. A., Menchen, T., Lee, C., Johnson, J., Jungnickel, M. K., Choksi, S. P., Garcia, G., III, Busengdal, H., Dougherty, G. W., Pennekamp, P. et al. (2017). Evolutionary proteomics uncovers ancient associations of cilia with signaling pathways. *Dev. Cell* **43**, 744-762.e11. doi:10.1016/j.devcel.2017.11.014

Siljee, J. E., Wang, Y., Bernard, A. A., Ersoy, B. A., Zhang, S., Marley, A., Von Zastrow, M., Reiter, J. F. and Vaisse, C. (2018). Subcellular localization of MC4R with ADCY3 at neuronal primary cilia underlies a common pathway for genetic predisposition to obesity. *Nat. Genet.* **50**, 180-185. doi:10.1038/s41588-017-0020-9

Singla, V. and Reiter, J. F. (2006). The primary cilium as the cell’s antenna: signaling at a sensory organelle. *Science* **313**, 629-633. doi:10.1126/science.1124534

Subramanian, S. V. and Satir, B. H. (1992). Carbohydrate cycling in signal transduction: parafusin, a phosphoglycoprotein and possible Ca(2+)-dependent transducer molecule in exocytosis in Paramecium. *Proc. Natl. Acad. Sci. USA* **89**, 11297-11301. doi:10.1073/pnas.89.23.11297

Tucker, R. W., Pardee, A. B. and Fujiwara, K. (1979). Centriole ciliation is related to quiescence and DNA synthesis in 3T3 cells. *Cell* **17**, 527-535. doi:10.1016/0092-8674(79)90261-7

Wang, Y., Zhou, Z., Walsh, C. T. and McMahon, A. P. (2009). Selective translocation of intracellular Smoothened to the primary cilium in response to Hedgehog pathway modulation. *Proc. Natl. Acad. Sci. USA* **106**, 2623-2628. doi:10.1073/pnas.0812110106

Waters, A. M. and Beales, P. L. (2011). Ciliopathies: an expanding disease spectrum. *Pediatr. Nephrol.* **26**, 1039-1056. doi:10.1007/s00467-010-1731-7

Wood, C. R. and Rosenbaum, J. L. (2014). Proteins of the ciliary axoneme are found on cytoplasmic membrane vesicles during growth of cilia. *Curr. Biol.* **24**, 1114-1120. doi:10.1016/j.cub.2014.03.047

Wyroba, E., Widding Hoyer, A., Storgaard, P. and Satir, B. H. (1995). Mammalian homologue of the calcium-sensitive phosphoglycoprotein, parafusin. *Eur. J. Cell Biol.* **68**, 419-426.

Yan, R., Hu, X., Zhang, W., Song, L., Wang, J., Yin, Y., Chen, S. and Zhao, S. (2015). The mouse radial spoke protein 3 is a nucleocytoplasmic shuttling protein that promotes neurogenesis. *Histochem. Cell Biol.* **144**, 309-319. doi:10.1007/s00418-015-1338-y

Ye, F., Nager, A. R. and Nachury, M. V. (2018). BBSome trains remove activated GPCRs from cilia by enabling passage through the transition zone. *J. Cell Biol.* **217**, 1847-1868. doi:10.1083/jcb.201709041

Yue, S., Tang, L. Y., Tang, Y., Tang, Y., Shen, Q. H., Ding, J., Chen, Y., Zhang, Z., Yu, T. T., Zhang, Y. E. et al. (2014). Requirement of Smurf-mediated endocytosis of Patched1 in sonic hedgehog signal reception. *Elife* **3**, e02555. doi:10.7554/eLife.02555

Zhang, Y. E. (2009). Non-Smad pathways in TGF-beta signaling. *Cell Res.* **19**, 128-139. doi:10.1038/cr.2008.328

Zuccato, C. and Cattaneo, E. (2007). Role of brain-derived neurotrophic factor in Huntington’s disease. *Prog. Neurobiol.* **81**, 294-330. doi:10.1016/j.pneurobio.2007.01.003

Zuccato, C., Ciammola, A., Rigamonti, D., Leavitt, B. R., Goffredo, D., Conti, L., MacDonald, M. E., Friedlander, R. M., Silani, V., Hayden, M. R. et al. (2001). Loss of huntingtin-mediated BDNF gene transcription in Huntington’s disease. *Science* **293**, 493-498. doi:10.1126/science.1059581