

---

# Olfactory Cilia: Linking Sensory Cilia Function and Human Disease

---

Paul M. Jenkins, Dyke P. McEwen and Jeffrey R. Martens

Department of Pharmacology, University of Michigan, 1301 MSRB III, 1150 W. Medical Center  
Dr. Ann Arbor, MI 48109-5632, USA

Correspondence to be sent to: Jeffrey R. Martens, Department of Pharmacology, University of Michigan, 1301 MSRB III, 1150 W. Medical Center Dr. Ann Arbor, MI 48109-5632, USA. e-mail: martensj@umich.edu

---

## Abstract

The olfactory system gives us an awareness of our immediate environment by allowing us to detect airborne stimuli. The components necessary for detection of these odorants are compartmentalized in the cilia of olfactory sensory neurons. Cilia are microtubule-based organelles, which can be found projecting from the surface of almost any mammalian cell, and are critical for proper olfactory function. Mislocalization of ciliary proteins and/or the loss of cilia cause impaired olfactory function, which is now recognized as a clinical manifestation of a broad class of human diseases, termed ciliopathies. Future work investigating the mechanisms of olfactory cilia function will provide us important new information regarding the pathogenesis of human sensory perception diseases.

**Key words:** anosmia, cilia, ciliopathies, olfactory, trafficking

## Introduction

Inhalation of odorants across the surface of the olfactory epithelium (OE) initiates the olfactory signaling cascade, which involves the binding of odorants to receptors localized on the cilia of olfactory sensory neurons (OSNs). In the well-described canonical pathway, activated odorant receptors (ORs) act through a stimulatory G protein–coupled mechanism to activate adenylyl cyclase type III (ACIII) and increase the ciliary concentration of cAMP. Olfactory cyclic nucleotide-gated (CNG) channels open in response to cAMP binding and allow the depolarization of the OSN that is further amplified by the Ca<sup>2+</sup>-activated Cl<sup>-</sup> channel. All of these components necessary for odorant detection are enriched in olfactory cilia, and perturbation in the localization of these components or in the cilia themselves causes impaired olfactory function (Kulaga et al. 2004; McEwen et al. 2007). Despite this critical ciliary compartmentalization, there is a relative paucity of information regarding membrane transport to this microtubule-based organelle. The revelation that many neuronal cells types possess a cilium, a unique cellular compartment whose function remains obscure, has stimulated interest in the segregation of proteins and the functional specialization of these membrane subdomains (reviewed in Fuchs and Schwark 2004). One cell type where the ciliary function is well described is the OSN. In this review, we will focus on olfactory cilia including structure and function, developmental formation, and relation to human disease.

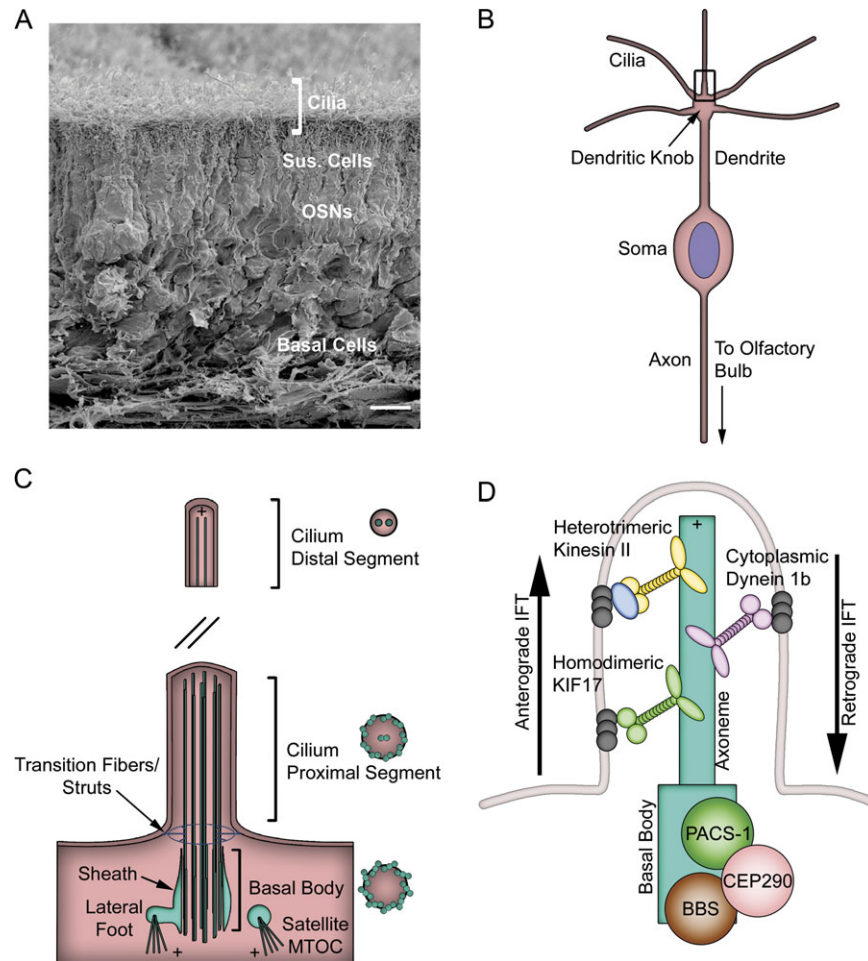
## Location of olfactory cilia

The main OE is a pseudostratified epithelium composed of several cell types (Figure 1A). The OSN is the main sensory cell, which contains the elements of the olfactory sensory cascade (Figure 1B, Figure 2 reference). These neurons are bounded apically by the somata of sustentacular cells and basally by a heterogeneous population of proliferative basal cells. OSN dendrites extend apically between the sustentacular cells toward the nasal cavity, ending in a knob containing multiple basal bodies from which the cilia project into the mucous of the OE (Cuschieri and Bannister 1975a, 1975b) (Figure 1A,B).

## Olfactory cilia structure

### Axoneme

Cilia are nearly ubiquitous organelles that can be found projecting from the surface of most mammalian cell types (reviewed in Wheatley et al. 1996). These cilia are generally divided into classes based on their axonemal structure and motility. The structural component of the cilium, the axoneme, is most often composed of 9 doublets of microtubules arranged symmetrically around a central core. Cilia that contain a pair of microtubules within the central core are said to be in the (9 + 2) configuration, whereas those that lack the



**Figure 1** Anatomy of the OE and OSN. **(A)** Side view of a scanning electron micrograph at 1030 $\times$  magnification from mouse OE. Mouse OE was dissected, fixed in glutaraldehyde, and processed for scanning electron microscopy as previously described (McEwen et al. 2007). Scanning electron micrographs were captured using an Amray 1910FE field emission scanning electron microscope at 5 kV. Images were recorded digitally with Semicaps software. Layers of the OE are labeled in white. Cilia layer marked by bracket. Sus. Cells = sustentacular cells. Scale bar represents 10  $\mu$ m. (Image generously provided by Wanda Layman and Dr Donna Martin, Department of Human Genetics, University of Michigan). **(B)** Diagram of a single OSN. Boxed region of interest at base of cilium shown at higher magnification in panel **C**. **(C)** Diagram of olfactory cilium and associated organelles. Cross sections of axonemal configuration from distal segment (top), proximal segment (middle), and basal body (bottom) shown on the right. Solidi (//) mark transition from proximal to distal segments. MTOC = Microtubule Organizing Center. +s indicate plus ends of microtubules. **(D)** Diagram of the ciliary and basal body components involved in olfactory ciliary transport. Anterograde motors move cargo to the distal tip of the cilium. Heterotrimeric kinesin-II is composed of KIF3a, KIF3b, and KAP3, whereas KIF17 comprises a homodimer. The retrograde motor, cytoplasmic dynein 1b moves cargo back out of the cilium. IFT complexes are shown as gray circles. BBS proteins, CEP290, and PACS-1 are discussed in detail in the Selectivity of Ciliary Protein Entry section.

central pair contain the (9 + 0) configuration. Although OSNs possess cilia that have the (9 + 2) microtubule configuration normally found in motile cilia, they lack the dynein arms necessary for movement and are thus rendered immotile (Menco 1984). Interestingly, some nonmammalian vertebrates, such as goldfish and frogs (Reese 1965; Lidow and Menco 1984), display motile olfactory cilia, which have an axoneme resembling that of respiratory cilia in their proximal segments and are suggested to play a role in odorant clearance (Bronshstein and Minor 1973; Mair et al. 1982).

Much of the current knowledge about the structure of olfactory cilia can be credited to early electron microscopy

studies (Reese 1965; Cuschieri and Bannister 1975a, 1975b; Menco 1980a, 1980c; Menco and Morrison 2003). These reports demonstrated that the mammalian olfactory cilium is approximately 50–60  $\mu$ m in length and is divided into 2 distinct sections termed the proximal and distal segments. The thicker proximal segment projects 2–3  $\mu$ m from the basal body in a (9 + 2) configuration with a thickness of around 300 nm (Menco 1997). The distal segment projects the remaining  $\sim$ 50  $\mu$ m and dwindles down to an axonemal configuration of 1–4 singlet microtubules, most commonly consisting of a pair of singlet microtubules (Figure 1C) (Menco 1997). The distal segments of the olfactory cilia

are oriented parallel to the epithelial surface. Because there are numerous cilia (10–30) per cell and because of the large distance they project from the dendritic knob, there is substantial overlap of cilia from different OSNs (Menco 1997). This intertwined mat of cilia increases the sensory surface of the OE by over 40 times thus increasing our ability to detect odorants (Menco 1992).

The ciliary axoneme is composed of long strands of  $\alpha$  and  $\beta$  tubulin dimers, which form the structural backbone for the cilium (reviewed in Rosenbaum and Witman 2002; Scholey 2003). These microtubules provide the roadway for molecular motors, such as kinesins and dyneins, to transport cargo into and out of the cilium. Olfactory ciliary axonemes are oriented with the microtubule plus end located in the distal tip of the cilium (Figure 1C), which means that plus end-directed motors carry cargo to the tip of the cilium, whereas minus end-directed motors are responsible for the return of cargo (reviewed in Rosenbaum and Witman 2002; Scholey 2003).

Recently, a number of posttranslational modifications of tubulin have been discovered to play functional roles in the regulation of cargo transport (reviewed in Hammond et al. 2008). Many modifications to tubulin have been found on the ciliary axoneme, including acetylation ( $\alpha$ ), polyglutamylation ( $\alpha + \beta$ ), polyglycylation ( $\alpha + \beta$ ), and detyrosination ( $\alpha$ ). Although all of these modifications have been detected in olfactory cilia, their precise functional relevance is poorly understood (Schwarzenbacher et al. 2005; Pathak et al. 2007). However, a recent study found that zebra fish lacking an enzyme responsible for polyglutamylation exhibited a loss of olfactory cilia (Pathak et al. 2007), indicating a role for posttranslational tubulin modifications in assembly or maintenance of olfactory cilia. It is unclear if these modifications are uniform along the length of the ciliary axoneme.

In addition to changes in the axoneme structure, there is evidence for heterogeneity in protein content along the length of the cilium. The proximal and distal cilia segments may represent distinct subcellular compartments. During development, signaling proteins appear to localize differentially between these 2 regions. In newly formed cilia, the olfactory signaling proteins are more evenly distributed between the proximal and distal segments. In mature cilia, the signaling molecules, such as  $G\alpha_{olf}$ , ACIII, and CNG channel, appear to preferentially localize to the long distal segment where the odorant presumably first makes contact with the OSN (Menco 1997; Matsuzaki et al. 1999; Flannery et al. 2006). This clustering of signaling molecules at the site of odorant exposure may increase the efficiency of odorant-stimulated signaling.

### Lipid composition

The microtubule-based axoneme is encased by the lipid bilayer in the form of a membrane sheath, which, given the importance of lipids in cellular signaling, most certainly plays a critical role in olfactory signaling. The canonical

olfactory signaling pathway includes several peripheral and transmembrane proteins; therefore, there likely exists a dynamic reciprocity between odorant signaling proteins and membrane lipids in olfactory cilia such that perturbation of membrane lipids can affect olfactory signaling.

Recently, there is growing evidence for the role of lipid membrane microdomains, enriched in cholesterol and sphingolipids, in the organization of olfactory signaling proteins (Schreiber et al. 2000; Kobayakawa et al. 2002; Brady et al. 2004). In OE, Schreiber et al. (2000) demonstrated that the G protein and adenylyl cyclase isoforms involved in odorant signaling associate with lipid rafts. They also reported that  $G_{olf}$  and ACIII interact with the cholesterol binding protein, caveolin, and that disruption of the caveolin interaction inhibits odorant-induced cAMP production in OSNs. Additionally, the recently identified stomatin-related olfactory (SRO) protein (Kobayakawa et al. 2002; Goldstein et al. 2003) has been shown to associate with lipid rafts in olfactory cilia and bind both caveolin and ACIII. Importantly, anti-SRO antibodies stimulated cAMP production in fractionated cilia membranes suggesting that rafts and/or a caveolin/lipid/protein complex regulate odorant signaling (Kobayakawa et al. 2002). However, the study of lipid rafts and membrane organization in cilia and membranes in general has been hampered by the lack of quantitative biophysical approaches. Nevertheless, early ultrastructural data from the Menco laboratory comparing olfactory cilia membranes to that of respiratory cilia led them to conclude that the outer leaflet membranes of olfactory cilia are thicker than inner leaflets (Lidow and Menco 1984). This is consistent with a potential enrichment of sphingolipids that are localized almost exclusively to the outer leaflet (Tillman and Cascio 2003). The enrichment of certain lipids is further supported by work in invertebrates that has shown that the ciliary membrane of *Paramecium* is highly enriched with sphingolipids (Andrews and Nelson 1979). These investigators later showed that ciliary membrane excitability in the same invertebrate model was sensitive to sterol composition (Hennessey et al. 1983). Others have reported that there is an enrichment of cholesterol in the ciliary shaft, but not the necklace region, of epithelial cilia that extends during ciliogenesis (Chailley et al. 1983). Surprisingly, however, there is virtually no information regarding the precise lipid composition of this important membrane structure in the olfactory system.

### Ciliary necklace

One clearly delineated microdomain of the ciliary membrane can be found where the lipid membrane sheath meets the dendritic knob. This membrane specialization is termed the “ciliary necklace” and likely represents the transition zone between the cytoplasm and the ciliary compartment. This highly ordered domain is marked by a spiraling array of membrane particles (Andres 1969; Gilula and Satir 1972; Menco 1980d), which connect to the basal body just

below the ciliary axoneme (Satir and Christensen 2007). Although most cilia types possess a ciliary necklace, olfactory cilia typically have more strands per cilium than their respiratory counterparts, although the physiological relevance of this is unknown (Menco 1980d). The formation of the ciliary necklace precedes ciliogenesis as a patch of membrane, and in malformed cilia, there are still necklace-like structures (Menco 1980d; Carson et al. 1981). Interestingly, some ciliary transport proteins have been found to be localized at the ciliary necklace indicating that it may serve as a cargo-docking site connecting the ciliary shaft to the protein complexes at the base of the cilium (Deane et al. 2001).

### Basal body

The basal body is a modified centriole that migrates to the plasma membrane prior to ciliogenesis (Figure 3). The basal bodies are duplicated en masse in the cell body of the OSN before they migrate to the dendritic knob (Figure 3A) (Dirksen 1974; Cuschieri and Bannister 1975a, 1975b; Hagiwara et al. 2004; Schwarzenbacher et al. 2005). Basal bodies, like the ciliary axoneme, are composed of 9 sets of microtubules arranged in a radial symmetry (Figure 1C). However, basal bodies are composed of polymers of triplet microtubules of  $\gamma$  tubulin rather than the doublet microtubules of  $\alpha$  and  $\beta$  tubulin seen in the axoneme. The basal body serves as the microtubule organizing center (MTOC) in the dendritic knob with the axonemal tubules projecting from the basal body, such that the plus ends orient toward the distal tip of the cilium (Burton 1992).

In addition to serving as MTOCs for the ciliary axoneme, the basal bodies are associated with electron-dense satellite particles that appear to also be MTOCs (Burton 1992). These organizing centers serve as nucleation sites for microtubules that project from the dendritic knob back through the dendrite toward the cell body (Burton and Laveri 1985). Some of the MTOCs are connected to the basal body through a sheath of material that surrounds the basal body and thickens at its proximal end. The basal bodies and sheath are connected to the plasma membrane through 9 struts that correspond to the electron-dense endings, which anchor to the plasma membrane (Figure 1C) (Menco 1980d).

### Ciliary rootlet

The ciliary rootlet is a cytoskeletal feature found projecting from the basal body in many ciliated cells and believed to participate in anchoring of cilium (Engelmann 1880). Although the structural components of the ciliary rootlet are beginning to be elucidated (Yang et al. 2002), still very little is known about its function. It seems unclear if olfactory cilia possess a rootlet (Farbman and Gesteland 1974; Yamamoto 1976; Naguro and Iwashita 1992); however, OSNs have been shown to express components of the ciliary rootlet in a localization consistent with the dendritic knob/basal body region

(Yamamoto 1976; McClintock et al. 2008). More work is necessary to definitively demonstrate the presence or absence of an olfactory ciliary rootlet.

### Ciliogenesis

The olfactory placode first appears in the mouse at embryonic day 9 (E9) postfertilization (Cuschieri and Bannister 1975a, 1975b; Menco 1980a, 1980b; Menco and Morrison 2003; Schwarzenbacher et al. 2005). At E10, the olfactory placode invaginates and forms the olfactory pit, which is composed primarily of 2 cell types: a population that is electron dense (proliferative basal cells) and those that appear light (differentiated OSNs) (Cuschieri and Bannister 1975a, 1975b; Menco 1980a, 1980b; Menco and Morrison 2003). At E11, the dendrites begin to form and extend toward the apical surface. Also, the olfactory pit deepens and forms recesses that will eventually become the olfactory turbinates (Cuschieri and Bannister 1975a, 1975b; Menco 1980a, 1980b; Menco and Morrison 2003). During this time, the primary site of OSN growth and maturation is the deep recesses of the olfactory pit (Cuschieri and Bannister 1975a, 1975b; Menco 1980a, 1980b; Menco and Morrison 2003).

By E11, several morphological changes occur in OSNs, representing the initial stages of ciliogenesis. First, in the perinuclear region of these neurons, numerous microtubules and microfilaments form and extend vertically toward the apical surface (Cuschieri and Bannister 1975a; Menco and Farbman 1985; Menco and Morrison 2003). Second, the distal end of the dendrite now extends into the lumen of the nasal cavity, where it begins to swell and form the dendritic knob (Figure 3B,C) (Cuschieri and Bannister 1975a; Menco and Farbman 1985; Menco and Morrison 2003). Finally, and perhaps most importantly, centriole duplication occurs and groups of centrioles accumulate in the perinuclear region of the neuron (Figure 3A) (Cuschieri and Bannister 1975a; Menco and Farbman 1985; Menco and Morrison 2003).

By E12, the rate of OSN proliferation increases, and these OSNs begin to develop well-formed dendrites and dendritic knobs filled with mitochondria, small coated vesicles, and numerous microtubules (Cuschieri and Bannister 1975a; Menco and Farbman 1985; Menco and Morrison 2003). The microtubules in the dendritic knob are arranged in 2 distinct populations; 1 is arranged concentrically around the periphery of the knob, whereas the other is arranged longitudinally and extends deep into the dendrite (Cuschieri and Bannister 1975a; Menco and Farbman 1985; Menco and Morrison 2003). In addition, the centrioles that were duplicated at E11 begin to migrate to the dendritic knob and eventually disperse singly around the knob periphery where they associate with the plasma membrane (Figure 3C). Ciliogenesis commences when a single, primary cilium of approximately 1  $\mu\text{m}$  extends into the nasal cavity (Cuschieri



and Bannister 1975a; Menco and Farbman 1985; Menco and Morrison 2003; Schwarzenbacher et al. 2005). As new cilia form, their microtubule-based axoneme elongates and the basal body, formed by the migrating centrioles, matures and is anchored at the plasma membrane (Figure 3C,D) (Dirksen 1974; Cuschieri and Bannister 1975a, 1975b; Hagiwara et al. 2004; Schwarzenbacher et al. 2005). By E13 or E14, multiple cilia up to 2  $\mu\text{m}$  in length can be seen extending from a single dendritic knob (Figure 3D). Over the next several days, olfactory cilia continue to elongate and can reach up to 60  $\mu\text{m}$  prior to birth. Intraflagellar transport (IFT), which will be discussed in more detail below, plays a key role in the transport of cargo responsible for the growth and maintenance of cilia (Rosenbaum and Witman 2002; Scholey 2003). In some species, the cilia will continue to grow and can reach up to 200  $\mu\text{m}$  in length (Reese 1965; Seifert 1971; Menco and Morrison 2003).

In addition to ciliogenesis, the proper delivery of ciliary signaling proteins is essential for normal olfactory function. Most of the work examining developmental expression of olfactory signaling molecules has probed for mRNA expression using either Reverse transcriptase Polymerase chain reaction, northern blot, or in situ hybridization analysis (Margalit and Lancet 1993; Strotmann et al. 1995; Saito et al. 1998; Schwarzenbacher et al. 2004, 2005). Interestingly, there appears to be a differential temporal expression of the components necessary for odor detection. ORs, of which a subset begin to be expressed at E11, appear to be the first member of the signaling cascade to be expressed as determined by both mRNA and protein expression (Saito et al. 1998; Schwarzenbacher et al. 2004, 2005). This expression occurs prior to ciliogenesis, and thus the OR protein localizes in high density at the dendritic knob (Schwarzenbacher et al. 2005). Eventually the diversity of expression continues to increase during development thus allowing the expression of hundreds of ORs (Margalit and Lancet 1993; Saito et al. 1998; Schwarzenbacher et al. 2004).

The downstream components of the olfactory signaling cascade appear to be expressed later in embryonic development. ACIII expression is first detected around E15, whereas  $G_{\text{olf}}$  and the CNG channel expression initiates at E16 and E19, respectively (Margalit and Lancet 1993). It is assumed that odor detection cannot occur until all proteins are present in olfactory cilia; thus, the relevance of this temporal expression pattern remains unknown.

The protein expression of 1 specific OR, mOR256-17, has been used to track ciliogenesis (Schwarzenbacher et al. 2004). As mentioned above, ORs begin their expression prior to the initiation of ciliogenesis. mOR256-17 accumulates at the dendritic knob in high density at this stage. Only after the cilia form and elongate ( $\sim$ E11) can the OR be properly localized initially to the knob and at the very proximal portions of the cilia. Once the cilia reach 2  $\mu\text{m}$  or longer ( $\sim$ E12–13), mOR256-17 migrates almost exclusively to OSN cilia (Schwarzenbacher et al. 2004). Interestingly, this work indi-

cates that, at least in the case of mOR256-17, the protein can localize to the dendritic knob independent of any signal from the cilium.

### Intraflagellar transport

Cargo transport in cilia occurs through an evolutionarily conserved process termed intraflagellar transport, which was first discovered in the laboratory of Joel Rosenbaum in *Chlamydomonas* (Kozminski et al. 1993). Because cilia lack the necessary components for protein synthesis and no obvious vesicular structures have been observed within the cilium, cargo must be synthesized in the cell and carried into the cilia through IFT, which involves movement along microtubules by molecular motors in complex with transport molecules, called IFT particles (reviewed in Rosenbaum and Witman 2002; Scholey 2003, 2008). Because the basic mechanisms of IFT are widely conserved not only between cilia types, but also often between species, we presume that these mechanisms studied in invertebrates are also acting in mammalian olfactory cilia.

IFT involves bidirectional transport into and out of the cilium by molecular motors that utilize the energy from ATP hydrolysis to generate processive movement. The transport of cargo out of the cilium back into the cell is accomplished via the cytoplasmic dynein motor (Pazour et al. 1998), whereas anterograde transport toward the distal, plus end of the cilium microtubules has been shown to involve kinesin motors (Cole et al. 1998) (Figure 1D). Work in *Caenorhabditis elegans* has shown that the formation and maintenance of the chemosensory ciliary axoneme and the delivery of cargo is accomplished through coordination of 2 anterograde kinesin motors: the heterotrimeric kinesin-II motor and the homodimeric OSM-3 (Snow et al. 2004). The mammalian kinesin-II motor, consisting of KIF3a, KIF3b, and the accessory protein, KAP3, has also been found to be necessary for ciliogenesis (Figure 1D) (Lin et al. 2003). However, differences are beginning to be recognized between specialized cilia types in invertebrates and mammals (Ou et al. 2005; Jenkins et al. 2006). Expression of a dominant-negative KIF17, the mammalian homolog of OSM-3, impaired ciliary trafficking of the olfactory CNG channel; however, it had no effect on cilia length as predicted by work in *C. elegans* (Ou et al. 2005; Jenkins et al. 2006). Future studies are necessary to determine if these motors are also responsible for cargo transport in mammalian olfactory cilia. Interestingly, OSM-3 operates on singlet microtubules of the distal segments of *C. elegans* cilia (Ou et al. 2005). Because olfactory cilia have such prominent distal segments and several signaling proteins, including the CNG channel, are found to be enriched in the distal segment, it seems likely that KIF17 is also functioning on distal segments in the mammalian olfactory cilium. Nevertheless, the differences in kinesin-2 motor coordination between the cilia of *C. elegans* and mammals highlight the need to further explore the mechanisms of IFT in mammalian olfactory cilia.

Using electron microscopy, IFT particles can be seen as electron-dense regions consisting of motors and IFT complexes found along the olfactory ciliary axoneme (Reese 1965). It is known that IFT motors associate with 2 distinct complexes of transport proteins called IFT proteins, which are named according to their molecular weight. These 2 complexes comprise 17 highly conserved proteins, termed complex A and complex B (Cole 2003). Complex A consists of IFT144, 140, 139, 122, and possibly 43, whereas complex B consists of IFT 172, 88, 81, 80, 74/72, 57/55, 52, 46, 27, and 20. Defects in either complex can impair IFT and cause a host of human diseases including Jeune syndrome, polycystic kidney disease, retinal degeneration, and situs inversus (reviewed in Blacque et al. 2008). A recent report demonstrated that mutation of the locus encoding the zebra fish homolog of IFT88 caused a loss of cilia from OSNs (Tsujikawa and Malicki 2004). Although the function of IFT proteins in many cases remains elusive, some IFT proteins have been shown to share significant homology with Golgi-localized clathrin trafficking machinery (Avidor-Reiss et al. 2004). Interestingly, the clathrin AP-1  $\mu$  adaptor, UNC-101, has been shown to be responsible for the localization of ORs to the cilia of *C. elegans* (Dwyer et al. 2001). In most cases, however, the precise role of the IFT complexes in mammalian olfactory cilia transport remains undefined.

Recent work in *C. elegans* suggests that there is a dynamic reciprocity between ciliary signaling and IFT-mediated ciliary structure maintenance. Mukhopadhyay et al. (2008) have shown that the loss of activation of the sensory signaling cascade modulates the structure of the AWB neuron modified sensory cilia. This sensory signaling-dependent remodeling was shown to be dependent on kinesin-II as well as Bardet–Biedl Syndrome (BBS) proteins (Mukhopadhyay et al. 2008). This is similar to a previous study showing that structure of AWC neuron cilia is also linked to sensory function (Roayaie et al. 1998). Although gross structural changes have been reported in mice deprived of odorant stimulation by naris occlusion (Farbman et al. 1988), it would be interesting to examine ultrastructural changes in cilia architecture due to loss of olfactory cues. Regardless, this suggests a potential feedback interaction between the IFT proteins involved in ciliary assembly and maintenance and those involved in odorant-induced signaling.

Although we have learned a great deal about IFT from invertebrate models, the olfactory systems of invertebrates may not be homologous with those found in vertebrates (Northcutt and Gans 1983). Additionally, differences are beginning to be recognized even between cilia types within an organism. Therefore it is critical that we continue to elucidate the function of vertebrate, and specifically mammalian, olfactory cilia.

### Selectivity of ciliary protein entry

Common to all organisms is the fact that only a subset of cellular proteins is able to gain access to the cilium, because

it contains a protein population distinct from the extraciliary compartment (Inglis et al. 2006). It is widely believed that there must be a barrier to diffusion that restricts entry into the cilium. This selective gate is thought to occur at the basal body through interactions with a large complex of proteins (Figure 1D) (Rosenbaum and Witman 2002; Scholey 2003). One family of proteins that has been shown to be involved in the regulation of ciliary transport is the BBS family of proteins. BBS is a pleiotropic ciliopathy that include phenotypes such as retinal degeneration, polydactyly, obesity, anosmia, and others (discussed in more detail below). There are 12 known BBS proteins (BBS1–12), which encode proteins involved in different stages of cilia transport. Although there are a variety of ciliary phenotypes associated with defects in BBS proteins, loss of function of BBS1 and BBS4 caused impaired olfactory function (Kulaga et al. 2004; Iannaccone et al. 2005). Interestingly, mice null for BBS1 or BBS4 may exhibit defects in olfactory cilia maintenance or assembly, although the mechanism for this defect remains unknown (Kulaga et al. 2004; Iannaccone et al. 2005).

Mutation of the cilia/centrosomal protein CEP290 has been implicated in the specific mislocalization of olfactory G proteins (McEwen et al. 2007). Importantly, mutation in CEP290 did not globally alter cilia structure and all other olfactory signaling molecules tested were localized normally, indicating that in olfactory cilia, regulation of cargo entry is distinct for different proteins. Interestingly, CEP290 was recently shown to interact with the centriolar satellite protein PCM-1 in a retinal epithelial cell line (Kim et al. 2008). PCM-1 has been shown to interact with BBS4 (Kim et al. 2004) and is dependent on the presence of BBS4 for proper ciliary localization (Kulaga et al. 2004). Together, these reports represent the beginning of the discovery of the mechanisms of basal body/cilia function in the OSN, though much work remains to further elucidate this process.

Recently, the intracellular trafficking protein, phosphofurin acidic cluster sorting protein 1 (PACS-1) has been shown to localize to the base of human respiratory cilia and control the localization of nephrocystin 1 to the transition zone of respiratory cilia (Schermer et al. 2005). Although PACS-1 has been shown to interact with acidic cluster-containing ion channels such as polycystin-2/TRPP2, TRPV4, and CLC-7 (Kulaga et al. 2004; Youker et al. 2008), no direct role has been demonstrated in the control of ciliary localization of ion channels. Recent unpublished work from the Martens laboratory has found that this protein localizes to the dendritic knob of OSNs and is necessary for the localization of the olfactory CNG channel, but not ACIII, to olfactory cilia. Interestingly, this mechanism is dependent on phosphorylation of PACS-1 and CNGB1b by CK2, thus providing a mechanism for the subunit-dependent trafficking of the olfactory CNG channel (Jenkins et al. 2006). Phosphorylation-dependent trafficking of olfactory signaling proteins may represent a mechanism for the tuning of the olfactory response. Another report demonstrated that

ORs interact with  $\beta$ -arrestin in a phosphorylation-dependent manner, and that this interaction may be responsible for trafficking of ORs out of the cilium on prolonged exposure to odorant (Mashukova et al. 2006). Despite these reports, very little is known regarding the dynamic trafficking of proteins into and out of olfactory cilia either under normal conditions or in response to stimuli.

Growing interest in ciliary protein trafficking has led to the identification of amino acid sequences necessary for entry of cargo into cilia. For example, the “RVxP” motif originally identified in polycystin-2 (Geng et al. 2006) was found to be necessary for the ciliary delivery of the olfactory CNG channel (Jenkins et al. 2006). Interestingly, a recent report demonstrated that the homologous “xVxP” motif in rhodopsin interacts with the small GTPase Arf4 and regulates trafficking of a ciliary targeting complex from the trans-Golgi network (Mazelova et al. 2009). Additionally, several ORs were recently found to contain another ciliary targeting motif consisting of (AX[S/A]XQ), which was sufficient to drive ciliary localization of nonciliary receptors (Berbari et al. 2008). The precise mechanisms by which these motifs control ciliary localization remain unknown. Interestingly, only a subset of ciliary proteins expresses these motifs indicating that there are multiple potential ciliary targeting motifs that most likely act through distinct ciliary entry mechanisms.

Due to the lack of rough endoplasmic reticulum, the dendritic knob is not a site for protein synthesis. This suggests that ciliary cargo must be synthesized in the soma and transported down the length of the dendrite in order to gain access to the cilium. Therefore, a loss of somatodendritic trafficking of ciliary cargo could also cause ciliary dysfunction. For example, in *C. elegans*, mutation in the membrane protein ODR-4 causes a mislocalization of a subset of chemosensory receptors (Dwyer et al. 1998). Because of the localization of ODR-4 to intracellular membranes within the soma, the authors conclude that this mislocalization could be due to a loss of ODR-4-mediated OR folding, sorting, or transport from the soma. Although this mechanism has yet to be seen in mammalian OSNs, given the difficulty expressing ORs in heterologous systems, it seems likely that mammalian OSNs possess a similar set of proteins necessary for proper OR transport.

#### Dynamics of protein movement within olfactory cilia

Although we are beginning to understand some of the mechanisms of ciliary cargo entry, we have virtually no information regarding the dynamics of cargo movement once inside the cilium. One might expect that members of signaling cascades, especially transmembrane proteins, would move relatively slowly within the cilium and display long half-lives in order to increase the efficiency of the signaling cascade. Indeed, in fluorescence recovery after photobleaching experiments, the olfactory CNG channel moved within the cilium at a rate consistent with slow diffusion ( $t_{1/2}$  of recovery

$\sim 10$  min) in a model system (Jenkins et al. 2006). However, in *C. elegans*, the transient receptor potential channel OSM-9 moves along the ciliary membrane at rates comparable to IFT ( $\sim 1\text{--}2$   $\mu\text{m}$  per second) (Qin et al. 2005). One potential reason for the rapid movement of cargo in cilia would be the recycling of damaged signaling proteins; however, at this point, the dynamics of protein movement within olfactory cilia remains relatively unexplored.

#### Fate of mistargeted ciliary cargo

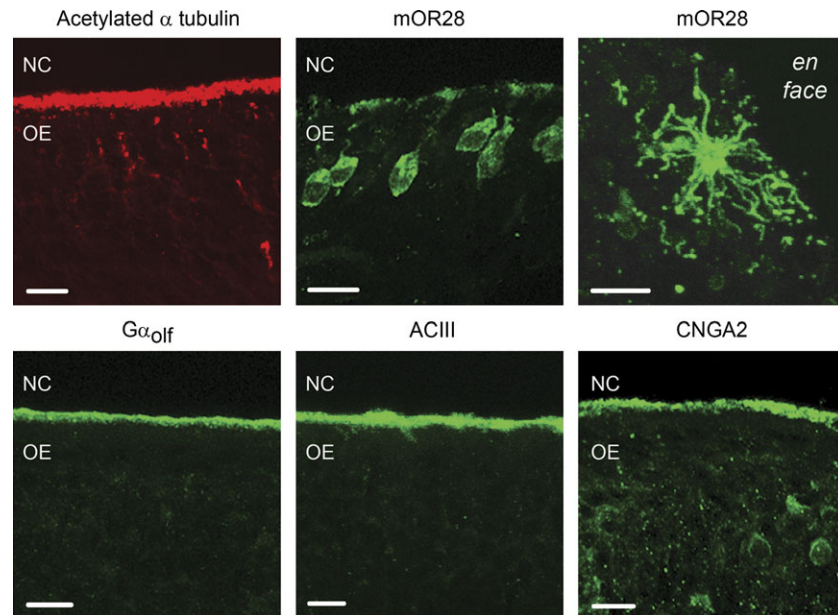
As discussed earlier, the basal body serves as the nucleation site for the ciliary axoneme and appears to serve as a scaffold for a complex of proteins that regulate entry of cargo into the cilium. In addition to these functions, it appears that the basal body also acts as a site of organization of proteolytic machinery. For example, it has been shown that proteolytic enzymes are enriched around the centrosome (Wigley et al. 1999). Recently, another group demonstrated that disruption of basal body function by suppression of BBS4 impairs proteasome function (Gerdes et al. 2007). This basal body/proteasome complex may serve to degrade improperly folded or mistrafficked ciliary cargo. For example, mice null for CNGB1b demonstrate very low levels of the remaining CNG subunits (Michalakakis et al. 2006). However, when these mice are treated with a proteasome inhibitor, the remaining channel subunits can be readily detected at the dendritic knob suggesting that the knob is serving as the site of proteolytic degradation.

#### Ciliary genomics and proteomics

Cilia contain a set of proteins distinct from the remainder of the cell (Inglis et al. 2006). In addition, the components necessary for odorant detection are all highly localized to cilia (Figure 2). Although we understand the function of a handful of these proteins, emerging areas of research are yielding new insights into other cilia-related genes and novel proteins that may be involved in olfactory signaling or ciliary structure and maintenance (McClintock et al. 2008). Recent advances in technology have vastly improved our ability to use bioinformatics as a tool to identify novel genes involved in various cellular processes, such as cilia formation and function. Hundreds of genes present in numerous ciliated species have recently been identified to be important in cilia-related functions (Avidor-Reiss et al. 2004; Li et al. 2004; Pazour et al. 2005; Smith et al. 2005; Stolc et al. 2005; Blacque et al. 2006; McClintock et al. 2008).

Although it is believed that cilia are widely conserved between cilia types, it is now becoming clear that differences exist both between species and between cilia types within 1 organism. Only a few studies have concentrated on identifying ciliary genes in mammals, with only a few focusing on olfactory cilia (Ostrowski et al. 2002; Su et al. 2004; Sammeta et al. 2007; Klimmeck et al. 2008; Mayer, Kuller, et al. 2008;





**Figure 2** Localization of Olfactory Signaling Proteins to Cilia. Immunocytochemistry for olfactory signaling proteins was performed on 14-mm-thick sections of frozen mouse OE as described previously (McEwen et al. 2007). Acetylated  $\alpha$  tubulin is a marker for the cilia layer. The odorant receptor mOR28 (top middle, antibody courtesy of Dr Richard Axel) is localized throughout the OSN including the cilia, which can be more easily visualized in an en face section (top right). The olfactory G protein  $G\alpha_{olf}$  (bottom left), adenylyl cyclase III (bottom middle), and CNG channel CNGA2 subunit (bottom right) are all enriched in the cilia layer. NC = nasal cavity, OE = olfactory epithelium. Scale bars represent 10  $\mu$ m for all images except en face mOR28 (bar represents 5  $\mu$ m).

Mayer, Ungerer, et al. 2008; McClintock et al. 2008). Using genomics and proteomics, these studies have identified hundreds of cilia-related genes of known and unknown function in OSNs. These studies represent a solid starting point for the elucidation of ciliary proteome; however, improved cilia purification methods should facilitate further study. Nevertheless, the challenge remains to demonstrate the function and physiological relevance of these ciliary proteins, especially in relation to human ciliopathies.

### Olfactory cilia and human disease

Although the olfactory system is necessary for detecting odors and crucial for our sense of taste, it also plays important roles in our quality of life, health, and safety. Dysosmia (impaired sense of smell) or anosmia (loss of ability to smell) can prevent us from detecting signs of danger such as smoke or spoiled food, and also can lead to medical problems such as weight gain and poor nutrition (Toller 1999). Impaired olfactory function is estimated to affect 3–6 million Americans and over 50% of those over the age of 65 (Murphy et al. 2002; Nguyen-Khoa et al. 2007); however, this may be a gross underestimate given that olfactory dysfunction frequently goes unreported (Nguyen-Khoa et al. 2007). Although the leading causes of smell disorders in patients are injury due to head trauma, upper respiratory tract infections, and chronic rhinosinusitis, in at least 20% of cases, the underlying etiology remains unknown (Jafek 2000). Olfactory dysfunction due to genetic mutations or neurode-

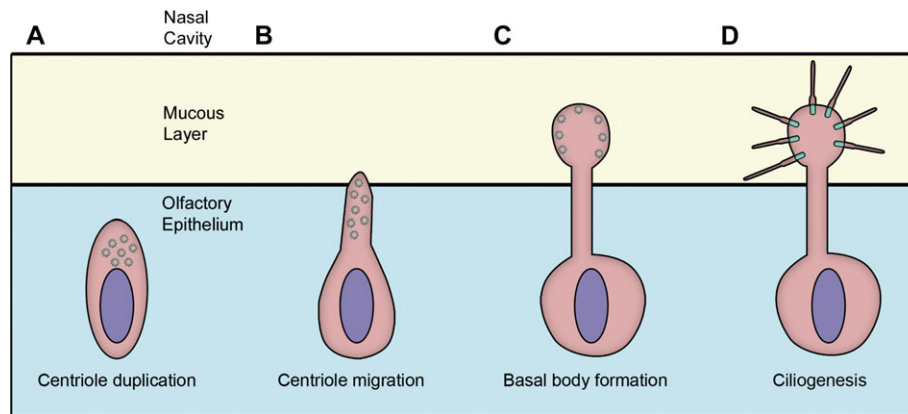
generative disorders affecting cilia is becoming increasingly recognized and better studied.

### Olfactory ciliopathies

One of the first documented cases of a human patient with anosmia presumably due to ciliary defects was in 1975 (Douek et al. 1975; Afzelius 2004). A biopsy from this patient, who suffered from congenital anosmia, revealed that, although the global architecture of the epithelium appeared normal, his OSNs were devoid of cilia, the cause of which is unknown (Douek et al. 1975). It has only been within the past 5 years that patients with deficits in olfaction due to ciliary defects have been clearly identified (Kulaga et al. 2004; Iannaccone et al. 2005; McEwen et al. 2007). In these cases, the olfactory deficits were shown to occur in 2 different pleiotropic diseases, BBS and Leber congenital amaurosis (LCA).

BBS is highly pleiotropic with patients exhibiting mental disabilities, obesity, retinal degeneration, polycystic kidneys, hypertension, and hypercholesterolemia, which together may lead to premature death (Bardet 1995; Biedl 1995; Beales et al. 1999; Klysiak 2008). The varied effects are dependent on mutations in 1 of 12 members of the BBS gene family, with the most severe mutations occurring in either BBS1 or BBS10 (Beales et al. 2003; Mykytyn et al. 2003; Hichri et al. 2005; Stoetzel et al. 2006; Klysiak 2008). Several BBS proteins, BBS1–8, have been characterized as basal body proteins that are thought to regulate protein entry into the cilium (Klysiak 2008). Human mutations in 2 BBS proteins, BBS1 and BBS4, and genetic deletion of BBS1, BBS2, or





**Figure 3** Steps of Ciliogenesis in the OSN. **(A)** In a developing OSN (at approximately E10–E11) centrioles are duplicated en masse from the mother/daughter centrioles in the cell body before migrating along the developing dendrite toward the eventual dendritic knob **(B)**. **(C)** In the dendritic knob (at approximately E12–E14), centrioles are converted to basal bodies and anchored to the plasma membrane, and ciliogenesis begins as the ciliary axonemes extend from the basal body and elongate the cilia into the mucous layer where odorant transduction occurs **(D)** Interestingly, 1 single primary cilium of approximately 1  $\mu\text{m}$  in length forms before the appearance of the remaining cilia.

BBS4 in mice resulted in severely impaired olfactory function (Kulaga et al. 2004; Mykityn et al. 2004; Nishimura et al. 2004; Iannaccone et al. 2005). However, mutations in the BBS proteins do not seem to share a common underlying mechanism of olfactory dysfunction. For example, patients with mutations in BBS1 are anosmic, most likely due to a loss of olfactory cilia, as the cilia are absent in the BBS1 null mouse model (Kulaga et al. 2004; Iannaccone et al. 2005). In the BBS2 null mouse, the status of olfactory cilia has not been examined, but both renal and retinal cilia are able to assemble (Nishimura et al. 2004). Finally, in 2 different studies, patients with mutations in BBS4 exhibited decreased olfaction or anosmia (Kulaga et al. 2004; Iannaccone et al. 2005). The divergent phenotypes observed with mutation or loss of specific BBS proteins highlight the complex regulation of ciliogenesis as well as the assembly and maintenance of the axoneme. This is also true when comparing different ciliated cells from a single BBS null animal. For example, mice deficient in BBS4 have a diminished cilia layer in the OE, which causes an almost complete loss of electroolfactogram response (Kulaga et al. 2004). Similarly sperm from these null mice completely lack flagella (Mykityn et al. 2004). However, these mice are still capable of ciliogenesis (Mykityn et al. 2004). Renal epithelial cells from these animals possess cilia but demonstrate altered timing for axoneme extension (Mokrzan et al. 2007), which may allow the age-dependent development of polycystic kidneys (Eichers et al. 2006). These results demonstrate the sensitivity of different cell types to alterations in BBS function and suggest that the manifestation of disease may reflect the extent to which complete elaboration of normal cilia is essential for a wildtype phenotype

A second example of a ciliary defect leading to olfactory impairment is a recent study investigating olfactory function in patients with LCA (McEwen et al. 2007). LCA, first dis-

covered by Theodor Leber almost 140 years ago (Leber 1869), is a congenital retinal dystrophy accounting for more than 5% of inherited retinopathies (Koenekoop 2004). LCA can occur due to mutations in several proteins of varying function, from retinoid metabolism and phototransduction to cell-cycle progression (Koenekoop 2004). Recent reports have also shown that LCA can be caused by mutations in the centrosomal/basal body protein, CEP290 (den Hollander et al. 2006; Cideciyan et al. 2007). Olfactory function was tested in the original LCA patient population with mutations in CEP290 using the Brief Smell Identification Test (McEwen et al. 2007). For all patients tested, mutations in CEP290 resulted in severely impaired olfactory function despite a self-described normal sense of smell (McEwen et al. 2007). Using a mouse model, it was determined that the olfactory impairment was due to a mislocalization of the olfactory G-protein rendering the signaling pathway nonfunctional, despite cilia remaining intact (McEwen et al. 2007). Together, these studies suggest that olfactory dysfunction due to ciliary defects can occur by 2 separate mechanisms: 1) a complete loss of olfactory cilia and 2) a defect in protein trafficking leading to a loss in olfactory signaling.

Although it is now clear that olfactory dysfunction is a clinical manifestation of a subset of ciliopathies, there appears to be a selective penetrance of phenotypes between different cilia in the body. For example, hypomorphic mutation in CEP290 causes anosmia and early-onset retinal degeneration without a renal phenotype (Chang et al. 2006; McEwen et al. 2007). In addition, KIF17 is not essential for renal cilia maintenance; however it is necessary for the maintenance of the rod outer segment, which is an extension of the modified connecting cilium (Jenkins et al. 2006; Insinna et al. 2008). Despite the varying penetrance of ciliopathies, the assessment of olfactory function represents an attractive tool for pregenetic screening due to the low cost and minimally invasive nature

of the procedure. In addition, screening for olfactory dysfunction may lead to the discovery of previously undescribed ciliary diseases. Finally, the finding that loss of olfactory cilia or perturbation in ciliary protein localization can represent the underlying cause of olfactory dysfunction highlights the necessity for further elucidation of the mechanisms and molecular machinery necessary for ciliary transport in OSNs.

### The OSN as a site for pathogen entry

The mammalian olfactory system is unique in that it is the only region of the central nervous system that is directly exposed to the external environment (Doty et al. 1991; Doty 2008). It is estimated that the exposed surface of the OE, comprising the dendritic knob plus cilia, is around 23 cm<sup>2</sup> (Doty et al. 1991; Doty 2008). Together, this makes the OE a unique and vulnerable target for the entry of pathogens directly into the brain. Even though the OE is partially protected by the presence of the nasal mucous as well as high levels of metabolizing enzymes, such as cytochrome P450s, evidence exists that pathogens can enter the brain through the OE (Baker and Genter 2003; Ding and Dahl 2003; Doty 2008). In the early 20th century, it was shown that viruses could enter the monkey brain and that this was prevented by lesioning either the OE, the axon tracts, or the olfactory bulb (Flexner 1917; Brodie and Elvidge 1934; Schultz and Gebhardt 1936; Doty 2008). One of the major debilitating viruses shown to enter the brain via the OE was the poliomyelitis virus (Flexner 1917; Brodie and Elvidge 1934; Schultz and Gebhardt 1936). Today, the list of viruses able to infect the OE has expanded and includes some major viruses, such as adenovirus, herpes simplex, hepatitis, influenza A, and rabies, as well as many others (Doty 2008). A subset of these pathogens may enter exclusively through OSNs and specifically the cilia. For example, the olfactory cilia from a patient with sporadic Creutzfeldt–Jakob disease were positive for protease-resistant prion protein (Tabaton et al. 2004). Following death, a neuropathological examination revealed nerve loss and gliosis in cerebral cortex, striatum, and cerebellum, suggesting that the olfactory cilia served as a site for pathogen entry (Tabaton et al. 2004). Therefore, the OE, specifically the OSN cilia, is likely a major target for pathogenic transmission of xenobiotics directly into the brain.

### Conclusions

Olfactory dysfunction in the general population is frequent, affecting at least 2.5 million people in the United States alone. In at least 20% of the cases, the etiology of the chemosensory disturbance cannot be identified. Recent evidence demonstrates that olfactory dysfunction is a clinical manifestation of an emerging class of human genetic disorders, termed ciliopathies, which involve defects in ciliary assembly and/or protein transport. Given the plasticity of the olfac-

tory system and its regenerative properties, OSNs undergo a continual process of ciliogenesis and protein transport that is critical for olfactory function. Intrinsic mechanisms are present in OSNs that direct cell surface localization and selective ciliary compartmentalization of olfactory transduction proteins. Remarkably, the mechanisms and molecular machinery necessary for ciliary transport in OSNs are poorly understood. Future work in this area will afford new insights into the regulation of sensory perception while emphasizing that olfactory dysfunction represents an important clinical manifestation of ciliary disease.

### Funding

National Institutes of Health (grants DC009606 to J.R.M., GM007767 to P.M.J., DC00011 to P.M.J. and D.P.M.) and NRSA fellowship (DC009524 to P.M.J.).

### References

- Afzelius BA. 2004. Cilia-related diseases. *J Pathol.* 204:470–477.
- Andres KH. 1969. Der olfaktorische Saum der Katze. *Z Zellforsch Mikrosk Anat.* 96:140–154.
- Andrews D, Nelson DL. 1979. Biochemical studies of the excitable membrane of *Paramecium tetraurelia*. II. Phospholipids of ciliary and other membranes. *Biochim Biophys Acta.* 550:174–187.
- Avidor-Reiss T, Maer AM, Koundakjian E, Polyanovsky A, Keil T, Subramaniam S, Zuker CS. 2004. Decoding cilia function: defining specialized genes required for compartmentalized cilia biogenesis. *Cell.* 117:527–539.
- Baker H, Genter MB. 2003. The olfactory system and the nasal mucosa as portals of entry of viruses, drugs, and other exogenous agents into the brain. In: Doty RL, editor. *Handbook on olfaction and gustation*. New York: Informa Health Care. p. 909–950.
- Bardet G. 1995. On congenital obesity syndrome with polydactyly and retinitis pigmentosa (a contribution to the study of clinical forms of hypophyseal obesity). 1920. *Obes Res.* 3:387–399.
- Beales PL, Badano JL, Ross AJ, Ansley SJ, Hoskins BE, Kirsten B, Mein CA, Froguel P, Scambler PJ, Lewis RA, et al. 2003. Genetic interaction of BBS1 mutations with alleles at other BBS loci can result in non-Mendelian Bardet–Biedl syndrome. *Am J Hum Genet.* 72:1187–1199.
- Beales PL, Elcioglu N, Woolf AS, Parker D, Flinter FA. 1999. New criteria for improved diagnosis of Bardet–Biedl syndrome: results of a population survey. *J Med Genet.* 36:437–446.
- Barbari NF, Johnson AD, Lewis JS, Askwith CC, Mykytyn K. 2008. Identification of ciliary localization sequences within the third intracellular loop of G protein-coupled receptors. *Mol Biol Cell.* 19:1540–1547.
- Biedl A. 1995. A pair of siblings with adiposo-genital dystrophy. 1922. *Obes Res.* 3:404.
- Blaquie OE, Cevik S, Kaplan OI. 2008. Intraflagellar transport: from molecular characterisation to mechanism. *Front Biosci.* 13:2633–2652.
- Blaquie OE, Li C, Inglis PN, Esmail MA, Ou G, Mah AK, Baillie DL, Scholey JM, Leroux MR. 2006. The WD repeat-containing protein IFTA-1 is required for retrograde intraflagellar transport. *Mol Biol Cell.* 17:5053–5062.
- Brady JD, Rich TC, Le X, Stafford K, Fowler CJ, Lynch L, Karpen JW, Brown RL, Martens JR. 2004. Functional role of lipid raft microdomains

- in cyclic nucleotide-gated channel activation. *Mol Pharmacol*. 65: 503–511.
- Brodie M, Elvidge AR. 1934. The portal of entry and transmission of the virus of poliomyelitis. *Science*. 79:235–236.
- Bronshstein AA, Minor AV. 1973. [Significance of flagellae and their mobility for olfactory receptor function]. *Dokl Akad Nauk SSSR*. 213: 987–989.
- Burton PR. 1992. Ultrastructural studies of microtubules and microtubule organizing centers of the vertebrate olfactory neuron. *Microsc Res Tech*. 23:142–156.
- Burton PR, Laveri LA. 1985. The distribution, relationships to other organelles, and calcium-sequestering ability of smooth endoplasmic reticulum in frog olfactory axons. *J Neurosci*. 5:3047–3060.
- Carr VM, Menco BP, Yankova MP, Morimoto RI, Farbman AI. 2001. Odorants as cell-type specific activators of a heat shock response in the rat olfactory mucosa. *J Comp Neurol*. 432:425–439.
- Carson JL, Collier AM, Knowles MR, Boucher RC, Rose JG. 1981. Morphometric aspects of ciliary distribution and ciliogenesis in human nasal epithelium. *Proc Natl Acad Sci USA*. 78:6996–6999.
- Chailley B, Boisvieux-Ulrich E, Sandoz D. 1983. Evolution of filipin-sterol complexes and intramembrane particle distribution during ciliogenesis. *J Submicrosc Cytol*. 15:275–280.
- Chang B, Khanna H, Hawes N, Jimeno D, He S, Lillo C, Parapuram SK, Cheng H, Scott A, Hurd RE, et al. 2006. In-frame deletion in a novel centrosomal/ciliary protein CEP290/NPHP6 perturbs its interaction with RPGR and results in early-onset retinal degeneration in the rd16 mouse. *Hum Mol Genet*. 15:1847–1857.
- Cideciyan AV, Aleman TS, Jacobson SG, Khanna H, Sumaroka A, Aguirre GK, Schwartz SB, Windsor EA, He S, Chang B, et al. 2007. Centrosomal-ciliary gene CEP290/NPHP6 mutations result in blindness with unexpected sparing of photoreceptors and visual brain: implications for therapy of Leber congenital amaurosis. *Hum Mutat*. 28: 1074–1083.
- Cole DG. 2003. The intraflagellar transport machinery of *Chlamydomonas reinhardtii*. *Traffic*. 4:435–442.
- Cole DG, Diener DR, Himelblau AL, Beech PL, Fuster JC, Rosenbaum JL. 1998. *Chlamydomonas* kinesin-II-dependent intraflagellar transport (IFT): IFT particles contain proteins required for ciliary assembly in *Caenorhabditis elegans* sensory neurons. *J Cell Biol*. 141: 993–1008.
- Cuschieri A, Bannister LH. 1975a. The development of the olfactory mucosa in the mouse: electron microscopy. *J Anat*. 119:471–498.
- Cuschieri A, Bannister LH. 1975b. The development of the olfactory mucosa in the mouse: light microscopy. *J Anat*. 119:277–286.
- Deane JA, Cole DG, Seeley ES, Diener DR, Rosenbaum JL. 2001. Localization of intraflagellar transport protein IFT52 identifies basal body transitional fibers as the docking site for IFT particles. *Curr Biol*. 11:1586–1590.
- den Hollander AI, Koenekoop RK, Yzer S, Lopez I, Arends ML, Voeseke KE, Zonneveld MN, Strom TM, Meitinger T, Brunner HG, et al. 2006. Mutations in the CEP290 (NPHP6) gene are a frequent cause of Leber congenital amaurosis. *Am J Hum Genet*. 79:556–561.
- Ding X, Dahl AR. 2003. Olfactory mucosa: composition, enzymatic localization, and metabolism. In: Doty RL, editor. *Handbook on olfaction and gustation*. New York: Informa Health Care. p. 98–135.
- Dirksen ER. 1974. Ciliogenesis in the mouse oviduct. A scanning electron microscope study. *J Cell Biol*. 62:899–904.
- Doty RL. 2008. The olfactory vector hypothesis of neurodegenerative disease: is it viable? *Ann Neurol*. 63:7–15.
- Doty RL, Perl DP, Steele JC, Chen KM, Pierce JD, Jr., Reyes P, Kurland LT. 1991. Odor identification deficit of the parkinsonism–dementia complex of Guam: equivalence to that of Alzheimer's and idiopathic Parkinson's disease. *Neurology*. 41:77–80; discussion 80–71.
- Douek E, Bannister LH, Dodson HC. 1975. Recent advances in the pathology of olfaction. *Proc R Soc Med*. 68:467–470.
- Dwyer ND, Adler CE, Crump JG, L'Etoile ND, Bargmann CI. 2001. Polarized dendritic transport and the AP-1 mu1 clathrin adaptor UNC-101 localize odorant receptors to olfactory cilia. *Neuron*. 31:277–287.
- Dwyer ND, Troemel ER, Sengupta P, Bargmann CI. 1998. Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. *Cell*. 93:455–466.
- Eichers ER, Abd-El-Barr MM, Paylor R, Lewis RA, Bi W, Lin X, Meehan TP, Stockton DW, Wu SM, Lindsay E, et al. 2006. Phenotypic characterization of Bbs4 null mice reveals age-dependent penetrance and variable expressivity. *Hum Genet*. 120:211–226.
- Engelmann TW. 1880. Zur anatomie und physiologie der flimmerzellen. *Arch Gel Physiol*. 23:505–535.
- Farbman AI, Brunjes PC, Rentfro L, Michas J, Ritz S. 1988. The effect of unilateral naris occlusion on cell dynamics in the developing rat olfactory epithelium. *J Neurosci*. 8:3290–3295.
- Farbman AI, Gesteland RC. 1974. Fine structure of olfactory epithelium in the mud puppy, *Necturus maculosus*. *Am J Anat*. 139:227–243.
- Flannery RJ, French DA, Kleene SJ. 2006. Clustering of cyclic-nucleotide-gated channels in olfactory cilia. *Biophys J*. 91:179–188.
- Flexner S. 1917. Mechanisms that defend the body from poliomyelitic infection, (a) external or extra-nervous, (b) internal or nervous. *Proc Natl Acad Sci USA*. 3:416–418.
- Fuchs JL, Schwark HD. 2004. Neuronal primary cilia: a review. *Cell Biol Int*. 28:111–118.
- Geng L, Okuhara D, Yu Z, Tian X, Cai Y, Shibasaki S, Somlo S. 2006. Polycystin-2 traffics to cilia independently of polycystin-1 by using an N-terminal RVxP motif. *J Cell Sci*. 119:1383–1395.
- Gerdes JM, Liu Y, Zaghoul NA, Leitch CC, Lawson SS, Kato M, Beachy PA, Beales PL, DeMartino GN, Fisher S, et al. 2007. Disruption of the basal body compromises proteasomal function and perturbs intracellular Wnt response. *Nat Genet*. 39:1350–1360.
- Gilula NB, Satir P. 1972. The ciliary necklace. A ciliary membrane specialization. *J Cell Biol*. 53:494–509.
- Goldstein BJ, Kulaga HM, Reed RR. 2003. Cloning and characterization of SLP3: a novel member of the stomatin family expressed by olfactory receptor neurons. *J Assoc Res Otolaryngol*. 4:74–82.
- Hagiwara H, Ohwada N, Takata K. 2004. Cell biology of normal and abnormal ciliogenesis in the ciliated epithelium. *Int Rev Cytol*. 234: 101–141.
- Hammond JW, Cai D, Verhey KJ. 2008. Tubulin modifications and their cellular functions. *Curr Opin Cell Biol*. 20:71–76.
- Hennessey TM, Andrews D, Nelson DL. 1983. Biochemical studies of the excitable membrane of *Paramecium tetraurelia*. VII. Sterols and other neutral lipids of cells and cilia. *J Lipid Res*. 24:575–587.
- Hichri H, Stoetzel C, Laurier V, Caron S, Sigaudy S, Sarda P, Hamel C, Martin-Coignard D, Gilles M, Leheup B, et al. 2005. Testing for triallelism: analysis of six BBS genes in a Bardet–Biedl syndrome family cohort. *Eur J Hum Genet*. 13:607–616.

- Iannaccone A, Mykytyn K, Persico AM, Searby CC, Baldi A, Jablonski MM, Sheffield VC. 2005. Clinical evidence of decreased olfaction in Bardet-Biedl syndrome caused by a deletion in the BBS4 gene. *Am J Med Genet A*. 132:343–346.
- Inglis PN, Borojevich KA, Leroux MR. 2006. Piecing together a cilium. *Trends Genet*. 22:491–500.
- Insinna C, Pathak N, Perkins B, Drummond I, Besharse JC. 2008. The homodimeric kinesin, Kif17, is essential for vertebrate photoreceptor sensory outer segment development. *Dev Biol*. 316:160–170.
- Jafek BW, Murrew B, Linschoten M. 2000. Evaluation and treatment of anosmia. *Curr Opin Otolaryngol Head N Surg*. 8:63–67.
- Jenkins PM, Hurd TW, Zhang L, McEwen DP, Brown RL, Margolis B, Verhey KJ, Martens JR. 2006. Ciliary targeting of olfactory CNG channels requires the CNGB1b subunit and the kinesin-2 motor protein, KIF17. *Curr Biol*. 16:1211–1216.
- Kim J, Krishnaswami SR, Gleeson JG. 2008. CEP290 interacts with the centriolar satellite component PCM-1 and is required for Rab8 localization to the primary cilium. *Hum Mol Genet*. 17:3796–3805.
- Kim JC, Badano JL, Sibold S, Esmail MA, Hill J, Hoskins BE, Leitch CC, Venner K, Ansley SJ, Ross AJ, et al. 2004. The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression. *Nat Genet*. 36:462–470.
- Klimmeck D, Mayer U, Ungerer N, Warnken U, Schnolzer M, Frings S, Mohrlen F. 2008. Calcium-signaling networks in olfactory receptor neurons. *Neuroscience*. 151:901–912.
- Klysik M. 2008. Ciliary syndromes and treatment. *Pathol Res Pract*. 204:77–88.
- Kobayakawa K, Hayashi R, Morita K, Miyamichi K, Oka Y, Tsuboi A, Sakano H. 2002. Stomatin-related olfactory protein, SRO, specifically expressed in the murine olfactory sensory neurons. *J Neurosci*. 22:5931–5937.
- Koenekoop RK. 2004. An overview of Leber congenital amaurosis: a model to understand human retinal development. *Surv Ophthalmol*. 49:379–398.
- Kozminski KG, Johnson KA, Forscher P, Rosenbaum JL. 1993. A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proc Natl Acad Sci USA*. 90:5519–5523.
- Kulaga HM, Leitch CC, Eichers ER, Badano JL, Lesemann A, Hoskins BE, Lupski JR, Beales PL, Reed RR, Katsanis N. 2004. Loss of BBS proteins causes anosmia in humans and defects in olfactory cilia structure and function in the mouse. *Nat Genet*. 36:994–998.
- Leber T. 1869. *Über retinitis pigmentosa und angeborene amaurose*. *Graefes Arch Clin Exp Ophthalmol*. 15:1–25.
- Li JB, Gerdes JM, Haycraft CJ, Fan Y, Teslovich TM, May-Simera H, Li H, Blacque OE, Li L, et al. 2004. Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. *Cell*. 117:541–552.
- Lidow MS, Menco BP. 1984. Observations on axonemes and membranes of olfactory and respiratory cilia in frogs and rats using tannic acid-supplemented fixation and photographic rotation. *J Ultrastruct Res*. 86:18–30.
- Lin F, Hiesberger T, Cordes K, Sinclair AM, Goldstein LS, Somlo S, Igarashi P. 2003. Kidney-specific inactivation of the KIF3A subunit of kinesin-II inhibits renal ciliogenesis and produces polycystic kidney disease. *Proc Natl Acad Sci USA*. 100:5286–5291.
- Mair RG, Gesteland RC, Blank DL. 1982. Changes in morphology and physiology of olfactory receptor cilia during development. *Neuroscience*. 7:3091–3103.
- Margalit T, Lancet D. 1993. Expression of olfactory receptor and transduction genes during rat development. *Brain Res Dev Brain Res*. 73:7–16.
- Mashukova A, Spehr M, Hatt H, Neuhaus EM. 2006. Beta-arrestin2-mediated internalization of mammalian odorant receptors. *J Neurosci*. 26:9902–9912.
- Matsuzaki O, Bakin RE, Cai X, Menco BP, Ronnett GV. 1999. Localization of the olfactory cyclic nucleotide-gated channel subunit 1 in normal, embryonic and regenerating olfactory epithelium. *Neuroscience*. 94:131–140.
- Mayer U, Kuller A, Daiber PC, Neudorf I, Warnken U, Schnolzer M, Frings S, Mohrlen F. 2009. The proteome of rat olfactory sensory cilia. *Proteomics*. 9:322–334.
- Mayer U, Ungerer N, Klimmeck D, Warnken U, Schnolzer M, Frings S, Mohrlen F. 2009. Proteomic analysis of a membrane preparation from rat olfactory sensory cilia. *Chem Senses*. 33:145–162.
- Mazelova J, Astuto-Gribble L, Inoue H, Tam BM, Schonteich E, Prekeris R, Moritz OL, Randazzo PA, Deretic D. 2009. Ciliary targeting motif VxPx directs assembly of a trafficking module through Arf4. *EMBO J*. 28:183–19.
- McClintock TS, Glasser CE, Bose SC, Bergman DA. 2008. Tissue expression patterns identify mouse cilia genes. *Physiol Genomics*. 32:198–206.
- McEwen DP, Koenekoop RK, Khanna H, Jenkins PM, Lopez I, Swaroop A, Martens JR. 2007. Hypomorphic CEP290/NPHP6 mutations result in anosmia caused by the selective loss of G proteins in cilia of olfactory sensory neurons. *Proc Natl Acad Sci USA*. 104:15917–15922.
- Menco B. 1992. Ultrastructural studies on membrane, cytoskeletal, mucous, and protective compartments in olfaction. *Microsc Res Tech*. 22:215–224.
- Menco BP. 1980a. Qualitative and quantitative freeze-fracture studies on olfactory and nasal respiratory epithelial surfaces of frog, ox, rat, and dog. II. Cell apices, cilia, and microvilli. *Cell Tissue Res*. 211:5–29.
- Menco BP. 1980b. Qualitative and quantitative freeze-fracture studies on olfactory and nasal respiratory epithelial surfaces of frog, ox, rat, and dog. III. Tight-junctions. *Cell Tissue Res*. 211:361–373.
- Menco BP. 1980c. Qualitative and quantitative freeze-fracture studies on olfactory and nasal respiratory structures of frog, ox, rat, and dog. I. A general survey. *Cell Tissue Res*. 207:183–209.
- Menco BP. 1984. Ciliated and microvillous structures of rat olfactory and nasal respiratory epithelia. A study using ultra-rapid cryo-fixation followed by freeze-substitution or freeze-etching. *Cell Tissue Res*. 235:225–241.
- Menco BP. 1997. Ultrastructural aspects of olfactory signaling. *Chem Senses*. 22:295–311.
- Menco BP, Farbman AI. 1985. Genesis of cilia and microvilli of rat nasal epithelia during pre-natal development. II. Olfactory epithelium, a morphometric analysis. *J Cell Sci*. 78:311–336.
- Menco BP, Morrison EE. 2003. Morphology of the mammalian olfactory epithelium: form, fine structure, function, and pathology. In: Doty RL, editor. *Handbook on olfaction and gustation*. New York: Informa Health Care. p. 17–49.
- Menco M. 1980d. Qualitative and quantitative freeze-fracture studies on olfactory and respiratory epithelial surfaces of frog, ox, rat, and dog. IV. Ciliogenesis and ciliary necklaces (including high-voltage observations). *Cell Tissue Res*. 212:1–16.
- Michalakakis S, Reiser J, Geiger H, Wetzel C, Zong X, Bradley J, Spehr M, Huttel S, Gerstner A, Pfeifer A, et al. 2006. Loss of CNGB1 protein leads to olfactory dysfunction and subciliary cyclic nucleotide-gated channel trapping. *J Biol Chem*. 281:35156–35166.



- Mokrzan EM, Lewis JS, Mykytyn K. 2007. Differences in renal tubule primary cilia length in a mouse model of Bardet-Biedl syndrome. *Nephron Exp Nephrol.* 106:e88–e96.
- Mukhopadhyay S, Lu Y, Shaham S, Sengupta P. 2008. Sensory signaling-dependent remodeling of olfactory cilia architecture in *C. elegans*. *Dev Cell.* 14:762–774.
- Murdoch B, Roskams AJ. 2007. Olfactory epithelium progenitors: insights from transgenic mice and in vitro biology. *J Mol Histol.* 38:581–599.
- Murphy C, Schubert CR, Cruickshanks KJ, Klein BE, Klein R, Nondahl DM. 2002. Prevalence of olfactory impairment in older adults. *J Am Med Assoc.* 288:2307–2312.
- Mykytyn K, Mullins RF, Andrews M, Chiang AP, Swiderski RE, Yang B, Braun T, Casavant T, Stone EM, Sheffield VC. 2004. Bardet-Biedl syndrome type 4 (BBS4)-null mice implicate Bbs4 in flagella formation but not global cilia assembly. *Proc Natl Acad Sci USA.* 101:8664–8669.
- Mykytyn K, Nishimura DY, Searby CC, Beck G, Bugge K, Haines HL, Cornier AS, Cox GF, Fulton AB, Carmi R, et al. 2003. Evaluation of complex inheritance involving the most common Bardet-Biedl syndrome locus (BBS1). *Am J Hum Genet.* 72:429–437.
- Naguro T, Iwashita K. 1992. Olfactory epithelium in young adult and aging rats as seen with high-resolution scanning electron microscopy. *Microsc Res Tech.* 23:62–75.
- Nguyen-Khoa BA, Goehring EL, Jr., Vendiola RM, Pezzullo JC, Jones JK. 2007. Epidemiologic study of smell disturbance in 2 medical insurance claims populations. *Arch Otolaryngol Head Neck Surg.* 133:748–757.
- Nishimura DY, Fath M, Mullins RF, Searby C, Andrews M, Davis R, Andorf JL, Mykytyn K, Swiderski RE, Yang B, et al. 2004. Bbs2-null mice have neurosensory deficits, a defect in social dominance, and retinopathy associated with mislocalization of rhodopsin. *Proc Natl Acad Sci USA.* 101:16588–16593.
- Northcutt RG, Gans C. 1983. The genesis of neural crest and epidermal placodes: a reinterpretation of vertebrate origins. *Q Rev Biol.* 58:1–28.
- Ostrowski LE, Blackburn K, Radde KM, Moyer MB, Schlatzer DM, Moseley A, Boucher RC. 2002. A proteomic analysis of human cilia: identification of novel components. *Mol Cell Proteomics.* 1:451–465.
- Ou G, Blacque OE, Snow JJ, Leroux MR, Scholey JM. 2005. Functional coordination of intraflagellar transport motors. *Nature.* 436:583–587.
- Pathak N, Obara T, Mangos S, Liu Y, Drummond IA. 2007. The zebrafish fleer gene encodes an essential regulator of cilia tubulin polyglutamylation. *Mol Biol Cell.* 18:4353–4364.
- Pazour GJ, Agrin N, Leszyk J, Witman GB. 2005. Proteomic analysis of a eukaryotic cilium. *J Cell Biol.* 170:103–113.
- Pazour GJ, Wilkerson CG, Witman GB. 1998. A dynein light chain is essential for the retrograde particle movement of intraflagellar transport (IFT). *J Cell Biol.* 141:979–992.
- Qin H, Burnette DT, Bae YK, Forscher P, Barr MM, Rosenbaum JL. 2005. Intraflagellar transport is required for the vectorial movement of TRPV channels in the ciliary membrane. *Curr Biol.* 15:1695–1699.
- Reese TS. 1965. Olfactory cilia in the frog. *J Cell Biol.* 25:209–230.
- Roayaie K, Crump JG, Sagasti A, Bargmann CI. 1998. The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. *Neuron.* 20:55–67.
- Rosenbaum JL, Witman GB. 2002. Intraflagellar Transport. *Nat Rev Mol Cell Biol.* 3:813–825.
- Saito H, Mimmack M, Kishimoto J, Keverne EB, Emson PC. 1998. Expression of olfactory receptors, G-proteins and AxCAMs during the development and maturation of olfactory sensory neurons in the mouse. *Brain Res Dev Brain Res.* 110:69–81.
- Sammata N, Yu TT, Bose SC, McClintock TS. 2007. Mouse olfactory sensory neurons express 10,000 genes. *J Comp Neurol.* 502:1138–1156.
- Satir P, Christensen ST. 2007. Overview of structure and function of mammalian cilia. *Annu Rev Physiol.* 69:377–400.
- Schermer B, Höpker K, Omran H, Ghenoiu C, Fliegau M, Fekete A, Horvath J, Köttgen M, Hackl M, Zschiedrich S, et al. 2005. Phosphorylation by casein kinase 2 induces PACS-1 binding of nephrocystin and targeting to cilia. *EMBO J.* 24:4415–4424.
- Scholey JM. 2003. Intraflagellar transport. *Annu Rev Cell Dev Biol.* 19:423–443.
- Scholey JM. 2008. Intraflagellar transport motors in cilia: moving along the cell's antenna. *J Cell Biol.* 180:23–29.
- Schreiber S, Fleischer J, Breer H, Boekhoff I. 2000. A possible role for caveolin as a signaling organizer in olfactory sensory membranes. *J Biol Chem.* 275:24115–24123.
- Schultz EW, Gebhardt LP. 1936. Chemoprophylaxis of poliomyelitis: a progress report. *Cal West Med.* 45:138–140.
- Schwarzenbacher K, Fleischer J, Breer H. 2005. Formation and maturation of olfactory cilia monitored by odorant receptor-specific antibodies. *Histochem Cell Biol.* 123:419–428.
- Schwarzenbacher K, Fleischer J, Breer H, Conzelmann S. 2004. Expression of olfactory receptors in the cribriform mesenchyme during prenatal development. *Gene Expr Patterns.* 4:543–552.
- Seifert K. 1971. [Light and electron microscopic studies on the organ of Jacobson (vomero-nasal organ) of cats]. *Arch Klin Exp Ohren Nasen Kehlkopfheilkd.* 200:223–251.
- Smith JC, Northey JG, Garg J, Pearlman RE, Siu KW. 2005. Robust method for proteome analysis by MS/MS using an entire translated genome: demonstration on the ciliome of *Tetrahymena thermophila*. *J Proteome Res.* 4:909–919.
- Snow JJ, Ou G, Gunnarson AL, Walker MR, Zhou HM, Brust-Mascher I, Scholey JM. 2004. Two anterograde intraflagellar transport motors cooperate to build sensory cilia on *C. elegans* neurons. *Nat Cell Biol.* 6:1109–1113.
- Stoetzel C, Laurier V, Davis EE, Muller J, Rix S, Badano JL, Leitch CC, Salem N, Chouery E, Corbani S, et al. 2006. BBS10 encodes a vertebrate-specific chaperonin-like protein and is a major BBS locus. *Nat Genet.* 38:521–524.
- Stolc V, Samanta MP, Tongprasit W, Marshall WF. 2005. Genome-wide transcriptional analysis of flagellar regeneration in *Chlamydomonas reinhardtii* identifies orthologs of ciliary disease genes. *Proc Natl Acad Sci USA.* 102:3703–3707.
- Strotmann J, Wanner I, Helfrich T, Breer H. 1995. Receptor expression in olfactory neurons during rat development: in situ hybridization studies. *Eur J Neurosci.* 7:492–500.
- Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D, Zhang J, Soden R, Hayakawa M, Kreiman G, et al. 2004. A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci USA.* 101:6062–6067.
- Tabaton M, Monaco S, Cordone MP, Colucci M, Giaccone G, Tagliavini F, Zanusso G. 2004. Prion deposition in olfactory biopsy of sporadic Creutzfeldt-Jakob disease. *Ann Neurol.* 55:294–296.
- Tillman TS, Cascio M. 2003. Effects of membrane lipids on ion channel structure and function. *Cell Biochem Biophys.* 38:161–190.

- Toller SV. 1999. Assessing the impact of anosmia: review of a questionnaire's findings. *Chem Senses*. 24:705–712.
- Tsujikawa M, Malicki J. 2004. Intraflagellar transport genes are essential for differentiation and survival of vertebrate sensory neurons. *Neuron*. 42:703–716.
- Wheatley DN, Wang AM, Strugnell GE. 1996. Expression of primary cilia in mammalian cells. *Cell Biol Int*. 20:73–81.
- Wigley WC, Fabunmi RP, Lee MG, Marino CR, Muallem S, DeMartino GN, Thomas PJ. 1999. Dynamic association of proteasomal machinery with the centrosome. *J Cell Biol*. 145:481–490.
- Yamamoto M. 1976. An electron microscopic study of the olfactory mucosa in the bat and rabbit. *Arch Histol Japon*. 38:359–412.
- Yang J, Liu X, Yue G, Adamian M, Bulgakov O, Li T. 2002. Rootletin, a novel coiled-coil protein, is a structural component of the ciliary rootlet. *J Cell Biol*. 159:431–440.
- Youker RT, Shinde U, Day R, Thomas G. 2009. At the Crossroads of Homeostasis and Disease: Roles of the PACS proteins in Membrane Traffic and Apoptosis. *Biophys J*. In Press.

*Accepted March 26, 2009*