# Making sense of cilia and flagella

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Data reported at an international meeting on the sensory and motile functions of cilia, including the primary cilium found on most cells in the human body, have thrust this organelle to the forefront of studies on the cell biology of human disease.

## Introduction

The most comprehensive meeting ever assembled on the Biology of Cilia and Flagella was sponsored by FASEB and held recently at Vermont Academy in Saxtons River, from August 4–9, 2007. The meeting was organized by Joel Rosenbaum (Yale University), George Witman (University of Massachusetts Medical School), and Romano Dallai (University of Siena). Approximately 175 scientists representing over 70 laboratories from the USA, Canada, Japan, and Europe attended and presented their latest data. It will surely be remembered as a pivotal meeting for the field, as it brought together a heretofore disparate group of laboratories and focused their attention on the role of the cilium in development, motility, health, and disease. This was an appropriate time for such a meeting because there has been a recent deluge of research reports on cilia and their role in many diseases where the relationship to cilia was previously unknown. The fires of this research interest were stoked by the identification of the genes and polypeptides of the intraflagellar transport (IFT) motility system that is responsible for the assembly and maintenance of almost all eukaryotic cilia. Mutations in certain IFT genes led directly to studies on the role of cilia in polycystic kidney disease (PKD) and *situs inversus*, as well as a host of other diseases in which the sensory and/or motor functions of cilia are required. To understand the role of cilia in health and disease, the meeting was organized to provide a thorough background into the basic cell biology of cilia and flagella: their structure and biochemistry and the IFT process that maintains them. This in turn provided the background for sessions on the role of cilia in some major human genetic diseases and syndromes, including PKD, primary cilia dyskinesia, retinal degenerative disease, planar cell polarity, and developmental diseases grouped within Bardet-Biedl syndrome, whose characteristic phenotypes include obesity and diabetes.

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Abbreviations used in this paper: ARPKD, autosomal recessive polycystic disease; BBS, Bardet-Biedl syndrome; IFT, intraflagellar transport; OFD1, orofaciodigital syndrome type I; PKD, polycystic kidney disease.

## Background

The meeting began, appropriately, with a dedication to Bjorn Afzelius, presented by Romano Dallai, a close friend and colleague. In the 1930's, a Swiss physician, Manes Kartagener, observed the characteristic triad phenotype that came to be known as Kartagener's Syndrome (*situs inversus*, sinusitis, and bronchiectasis) (Kartagener, 1968). Afzelius was the first to observe that a number of male patients with Kartagener's Syndrome also were sterile. He determined that the sperm and airway cilia from these patients had abnormal axonemal ultrastructure; some patients were missing, for example, the dynein arms required for flagellar motility (Afzelius, 1976). The relationship between these ultrastructural observations on cilia and flagella and the pathology of *situs inversus*, however, remained enigmatic for over 20 years after Afzelius' study.

Afzelius, the slated keynote speaker at the meeting, was unable to attend. Instead, George Witman presented a comprehensive review of the role of cilia in disease, starting with Afzelius' investigations on Kartagener's syndrome patients. He reviewed recent breakthroughs on flagellar assembly and the discovery of IFT, first described in the Rosenbaum laboratory (Kozminski et al., 1993), and the seminal observations of Nobutaka Hirokawa and colleagues from the University of Tokyo. The latter group showed that the "9+0" cilia of the embryonic node have a unique motility and are required for the development of normal left/right organ asymmetry (Nonaka et al., 1998). Thus, a definitive link to ciliary motility was finally made, explaining the developmental and cell biological basis for *situs inversus* in Kartagener's patients. Note also that some nodal cilia have recently been reported to be 9+2 (Caspary et al., 2007); for more discussion of this point, see the section below on cilia as signal receivers.

Witman then described how studies on IFT in *Chlamydomonas* led to the discovery that defects in primary cilia caused PKD in mammals (Pazour et al., 2000). The connection between cilia and PKD was soon clarified by the discovery that the polycystins—products of the genes that are defective in PKD in humans—are displayed on the primary cilia of kidney tubule cells (Pazour et al., 2002; Yoder et al., 2002). Thus, mutations in ciliary proteins (the polycystins) or a complete loss of cilia (due to defects in IFT) result in the pathology of PKD. This initial correlation between primary cilia and a specific disease was then followed by a multitude of research papers linking a variety of cystic and developmental diseases to normal cilia function.

## The intraflagellar transport process

The construction of a cilium or a flagellum (Fig. 1) is dependent on proper functioning


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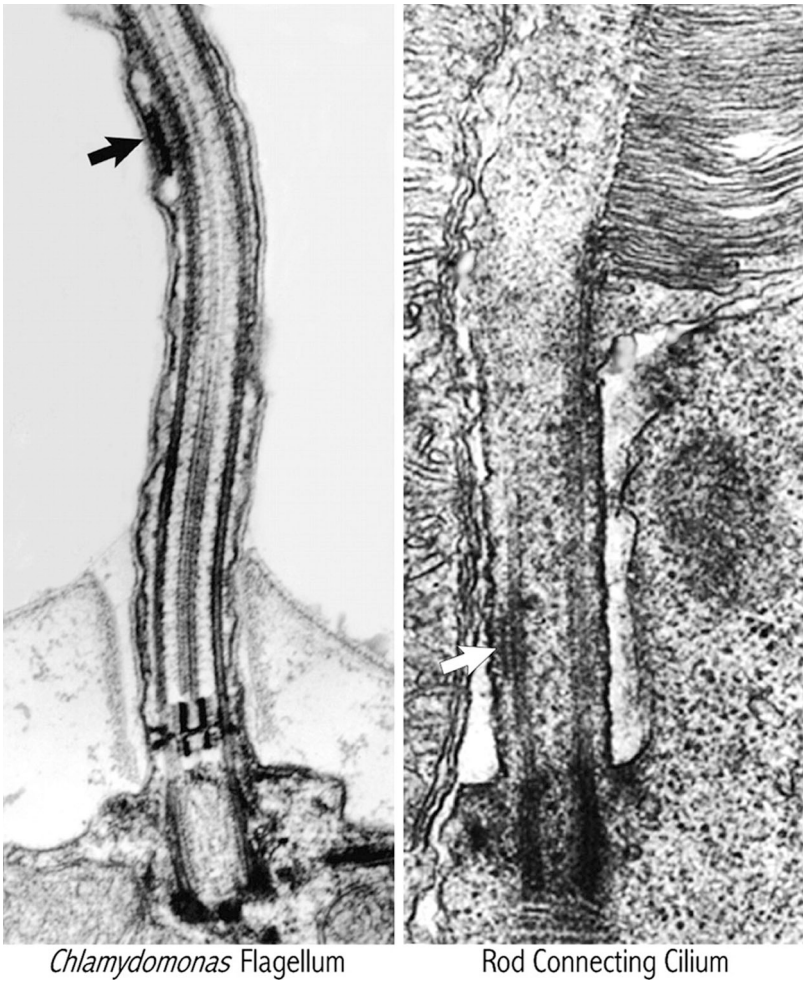


Figure 1. **Transmission electron micrographs of (left) a *Chlamydomonas* flagellum and (right) the connecting cilum from a vertebrate rod cell.** IFT particles in the flagellum and connecting cilium are shown by arrows. Flagellum micrograph courtesy of Karl A. Johnson and vertebrate rod reprinted with permission from Elsevier (Sandborn, E.B. 1970. Cells and Tissues by Light and Electron Microscopy. Vol. 1. Academic Press, New York. 366 pp.).

of the process of intraflagellar transport. IFT particles, which are not membrane bound, move proteins to their site of assembly at the flagellar tip and return used proteins to the cell body for degradation or recycling (Fig. 2). The particles can be separated biochemically into two fractions, called IFT Complex A (6 proteins) and Complex B (~11 proteins). The conference was initiated with a report by Douglas Cole (University of Idaho) on how the polypeptides of Complex B are associated with one another. Work on purified Complex B has indicated there is a core particle comprised of three polypeptides (IFT46, 52, and 88) on which the remaining Complex B polypeptides assemble. Maurice Kernan (SUNY at Stony Brook) and Che-Chia Tsao (University of Rochester) followed with reports showing that IFT Complex B is required for anterograde movement of cargo and normal flagellar assembly in *Drosophila* and *Tetrahymena* cilia, respectively, but that Complex A is required only in retrograde transport.

## Structure and biochemistry of cilia and flagella

The technique of cryo-electron microscopy/tomography coupled to computer enhancement has contributed new insights into the high resolution structure of the axoneme and the complex inner and outer dynein arms which are composed of multiple polypeptides. Reports by both Daniela Nicastro (Brandeis University) on the structure of ciliary axonemes and dynein, and Takashi Ishikawa (ETH, Zurich) on the dynein complex of wild-type and mutant *Chlamydomonas*, provided new three-dimensional information on the dynein motor complex and how it may be functioning to cause microtubule outer doublet sliding. However, these two groups differed in their reports with respect to the angle of orientation the outer arm dynein heads have relative to the doublet microtubules, which has important implications for the mechanism of force generation by the multi-headed dynein. Both reports were founded on the elegant negative stain images of the dynein motor by Stan Burgess (University of Leeds) who presented single-particle analysis of the circular motor head of the dynein heavy chain and how it might be moving. Using replicas obtained by quick freeze-deep etching, Pietro Lupetti (University of Siena) reported that the two dynein heads are positioned at different distances from the A-tubule. Furthermore, the stalk and the stem domains do not reside in the same plane as the head, which is contrary to what had been previously reported for isolated dynein adsorbed to a flat surface. In the first use of tomography to study the structure of IFT particles, Gaia Pigino (University of Siena) showed that these are complex structures, but that periodicities of 8.5 nm or 25 nm could be observed in a given group of IFT particles depending on location within the particles.

Reports on dynein structure were complemented by biochemical analyses of the dynein motor complex by Stephen King (University of Connecticut Health Center) and continued in reports on the control of dynein activity by the dynein regulatory complex (DRC) by Mary Porter (University of Minnesota), the role of calmodulin and the radial spokes in the control of flagellar beating by Elizabeth Smith (Dartmouth College), and the function of kinases and phosphatases, some associated with the flagellar radial spokes, in the control of dynein activity by Winfield Sale (Emory University) and by Avanti Gokhale (Emory University). Additional important analyses of the diverse, functional capabilities of dynein subforms and domains were described by Ritsu Kamiya (University of Tokyo), and new advances on dynein regulation were presented by Chikako Shingyoji (University of Tokyo). Part of the dynein control mechanism involves the orientation of the central pair microtubules, and David Mitchell (SUNY Upstate Medical University)

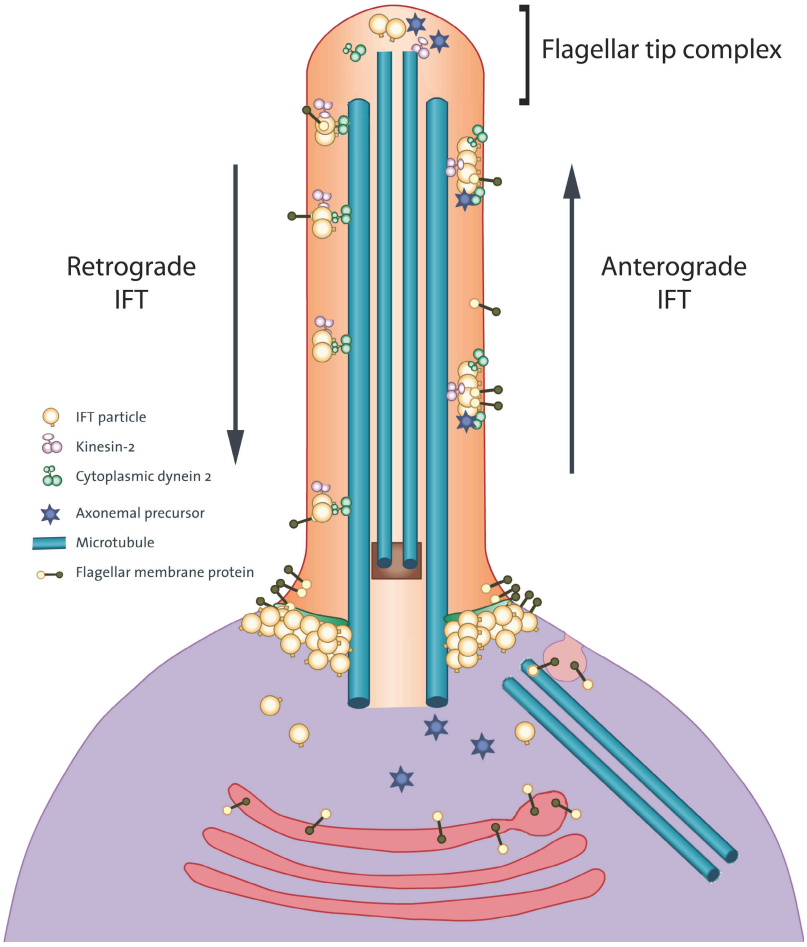


Figure 2. **Diagram showing the main features of intraflagellar transport (IFT).** IFT particles and the associated cargo required for flagellar assembly and maintenance gather around the basal body region at the base of the flagellum where they associate with each other for their trip past the transition zone (flagellar pore) and into the flagellum. They are transported by anterograde IFT to the flagellar tip, the axonemal assembly site, powered by the motor protein kinesin-2. Flagellar membrane proteins, components of vesicles that have budded from the Golgi and subsequently fused with the plasma membrane adjacent to the basal body, are picked up by IFT particles for their transport past the transition zone and onto the flagellar membrane. The membrane proteins are then moved within the plane of the ciliary membrane bilayer by IFT (Qin et al., 2005). At the flagellar tip, anterograde cargo is unloaded, turnover cargo is picked up, the kinesin-2 motor is inactivated for transport back to the cytoplasm, and cytoplasmic dynein 2, itself an anterograde cargo protein, is activated to power the retrograde trip back to the cytoplasm. Adapted from Rosenbaum and Witman [2002].

provided an update on how this complex central pair apparatus rotates, modulating the radial-spoke based activity of the dynein arms in different halves of the doublet circle. This central pair complex also contains kinesin (Bernstein et al., 1994), whose function is required for motility but in a manner yet to be determined.

### Cilia length and cell cycle control

One of the highlights of the meeting was a group of talks on the control of ciliary length and stability, and ciliary resorption, in particular as a prelude to cell division. Although IFT is involved in length control because it delivers the required axonemal and membrane precursors to the flagellum (Qin et al., 2004), length is, in turn, controlled by a kinase–phosphatase signaling system. Paul Lefebvre (University of Minnesota) has defined this system in an elegant fashion using the long-flagella (lf) mutants of *Chlamydomonas* that grow flagella to two or three times their normal length. Two of these mutants encode protein kinases: LF4, a MAP kinase, and LF2, a protein kinase of the CDK family. The LF4 and LF2 kinases, which localize to the flagella and the cytoplasm, respectively, may act either as length sensors, perhaps by monitoring "time of flight" on moving IFT particles, or as enforcers of length control by activating disassembly of flagella that have grown in length beyond a predetermined set point. In addition, William Dentler (University of Kansas) reported that disruption by Brefeldin A of membrane delivery to the flagellum from the Golgi induced flagellar disassembly and inhibited flagellar assembly without affecting IFT.

A related series of presentations addressed the relationship between resorption of the primary cilium and the ability of the cell to exit $G_o$ and reenter the cell cycle. This cilia–cell cycle control hypothesis has been discussed for decades, but other than an observed correlation between cilia resorption and cell cycle progression no direct cause-and-effect data have been presented. This has been remedied by several sets of data addressing cell cycle control. First, Lynne Quarmby (Simon Fraser University) showed how mutations in the Cnk and Nek kinases (of which there are many different ones in ciliated organisms) of *Chlamydomonas* affected flagellar resorption and the cell cycle. Second, Erica Golemis (Fox Chase Cancer Center) reported an interaction between the cell cycle kinase Aurora A and the pro-metastatic scaffolding protein HEF1 that occurs at the basal body in response to external cues. This interaction activates Aurora A kinase activity, the target of which is HDAC6, a deacetylase that removes the acetate group from axonemal tubulin, thus destabilizing the axoneme as a prelude to disassembly of the primary cilium. Tubulin deacetylation by HDAC6 is necessary and sufficient for cilia destabilization and resorption, and this is required for entry into the cell cycle from Go (Pugacheva et al., 2007). Interestingly, Pan et al. (2004) previously identified an Aurora-like kinase (CALK) as a key component in flagellar resorption in *Chlamydomonas*. Third, the coregulation of the cilia cycle and the cell cycle was nicely documented by Zhaohui Wang (Yale University), who presented evidence that IFT27, a small G protein and a component of IFT Complex B, also plays a role in cell division. The amount of IFT27 is constant per cell, and thus as the cell grows, the concentration of IFT27 falls. When the concentration of IFT27 decreases below a certain level, resorption of the

Table I. **Diseases related to ciliary defects**

| Defects in motile cilia | Defects in primary cilia |
|---|---|
| **Symptoms** | **Symptoms** |
| Respiratory tract infections, bronchiectasis, sinusitis, male infertility, *situs inversus*, hydrocephalus | Cysts in kidney, liver, pancreas; polycystic kidney disease; nephronophthisis; type 2 diabetes |
| | **Sensory defects** |
| | Retinal degeneration, anosmia (loss of smell), hearing impairment |
| **Syndromes** | **Neurological disorders** |
| Primary ciliary dyskinesia, Kartagener's syndrome, Siewert syndrome | Neural tube closure and neural patterning defects, hydrocephalus, juvenile myoclonic epilepsy, cognitive disorders |
| | **Other** |
| | Polydactyly, obesity, urological dysfunction, *situs inversus* |
| | **Syndromes** |
| | Bardet-Biedl syndrome, Meckel-Gruber syndrome, Von Hippel Lindau disease, Loken-Senior syndrome, Alstrom syndrome |

flagella is induced, and the cell enters S-phase. A rise in IFT27, due to new transcription, is then required for the completion of cell division. If IFT27 does not rise (i.e., in a knocked-down cell), the cell cycle is blocked at cytokinesis.

## The flagellar tip

IFT particles moving via the activity of kinesin-2 in the anterograde direction (toward the tip of the flagellum) carry inactive cytoplasmic dynein as cargo; the particles turn around at the tip and head back to the cytoplasm after releasing their cargo and picking up turnover products at the tip. During this process, activation of cytoplasmic dynein 2 and inactivation of kinesin-2 occur. This complex series of activities takes place with hardly a pause, and Lotte Pedersen (University of Copenhagen) presented an analysis of EB1 (microtubule end binding protein 1), which localizes to the flagellar tip (Pedersen et al., 2003) and that might be controlling the exchanges taking place. Pedersen also reported that EB1 is required for primary cilia formation in fibroblasts. In another approach to flagellar tip analysis, Roger Sloboda (Dartmouth College) reported data derived from analysis of flagella by difference gel electrophoresis, showing that flagella contain elements of a protein methylation pathway. Three specific but as-yet-unidentified proteins of the axoneme contain methyl-arginine residues. Analysis of the events occurring at the tip is further complicated by the presence there of kinesin-13, a motor protein known to mediate tubulin dimer removal from the plus ends of microtubules. Scott Dawson (University of California, Davis) showed that a kinesin-13 mutation (S280N) caused elongation of flagella in Giardia, and this has also been recently demonstrated in *Leishmania* by Blaineau et al. (2007).

## Targeting proteins to the cilium

The flagellum is a separate cellular compartment, not unlike the nucleus, separated from the bulk of the cytoplasm by a pore-like structure composed of the transition fibers, which hold the basal body/centriole to the membrane and which delineate the flagellar membrane from the plasma membrane. It was already known from work on targeting glucose receptors to the trypanosome membrane that specific sequences are required for entry into the flagellar compartment (Piper et al., 1995). New work presented by Dusanka Deretic (University of New Mexico) showed that a sequence motif (VxPx) in rhodopsin is required for the interaction of rhodopsin with accessory transport proteins and entry of rhodopsin into the connecting cilium and rod outer segment (Fig. 1). Similarly, Stefan Somlo (Yale University) reported that polycystin 2 cannot enter the cilium if a particular motif (RVxP) is mutated. Rather, the protein accumulates around the base of the cilium. In the former study, the VxPx motif was located at the C terminus, whereas in the latter study, the RVxP motif was at the N terminus. Gregory Pazour (University of Massachusetts Medical School) reported a C-terminal, 18-residue motif that targets fibrocystin to the cilium. Pazour had shown earlier that IFT20 could be found at the Golgi, and was, at least in part, responsible for shepherding vesicles which would become part of the ciliary membrane to their point of exocytosis adjacent to the basal body. It is thought that at this point IFT moves membrane-associated cargo to the ciliary membrane. Hence, it is here that the targeting sequences on ciliary membrane proteins are presumably recognized, i.e., in the region of the transition fibers, and indeed, this is where IFT particles are known to accumulate (Deane et al., 2001). Uwe Wolfrum (Johannes Gutenberg University of Mainz) described a protein network related to human Usher syndrome (combined blindness and deafness) that defines a specialized membrane domain in the apical, pericilliary region of the inner segment that serves as a target for vesicles destined for movement through the connecting cilium to the outer segment of photoreceptor cells. Christine Insinna (Medical College of Wisconsin) demonstrated a requirement for Kif17 (the mammalian homologue of worm Osm-3) for rod outer segment development (but not for kidney cilia development), perhaps to move along singlet microtubules as occurs in *Caenorhabditis elegans* (Snow et al., 2004). Philipp Trojan (Johannes Gutenberg University of Mainz) reported that the light dependent phosphorylation of centrin regulates the movement of transducin through the connecting cilium and into the outer segment. Maureen Barr (Rutgers University), working on the nephrocystins in the worm, proposed that these proteins function at the transition zone to dock and organize IFT particles, thus regulating access to the cilium.

## Bardet-Biedl syndrome

An exciting new development in ciliary assembly and function in relation to disease (Table I) concerns the Bardet-Biedl

syndrome (BBS) proteins. BBS is a disease syndrome resulting in a large array of pathologies, including obesity, defective bone development, diabetes, and other developmental disorders; there are at least 12 different BBS genes and the BBS proteins are located primarily in the centrosome–ciliary complex (Nachury et al., 2007). Max Nachury (from the Peter Jackson group, Genentech and Stanford) reported that many of the BBS proteins sediment together on gradients as a complex, called the BBSome, providing some of the first evidence that the diverse pathologies resulting from mutations in the different BBS genes are related. Alexander Loktev (Genentech/Stanford), also of this group, reported that the BBSome might be membrane associated, both in the cytoplasm and in the cilium itself. Unfortunately, primary cilia cannot (yet) be isolated in sufficient quantities from tissue culture cells, or from *C. elegans*, so the ciliary membrane association cannot be directly tested biochemically in these cells. The Genentech group, collaborating with Val Sheffield (University of Iowa), hypothesized that the BBSome is involved in the movement of ciliary membrane vesicles from the cytoplasm to the cilia surface. Membrane trafficking to and within the cilium occurs through the interaction of the BBSome with Rabin8, a GEF for the small GTPase Rab8, as inhibition of the production of active (i.e., GTP-bound) Rab8 blocks ciliation and induces BBS phenotypes in zebrafish. Related data from Gregory Pazour (University of Massachusetts Medical School) showed that RAb8 is important for opsin transport to the OS; a mutation in Rab8 causes opsin to remain in the inner segment in photoreceptor cells. Pazour also showed that the T22N mutation in Rab8 blocks ciliary targeting of PKHD1-GFP (i.e., fibrocystin-GFP).

Bradley Yoder (University of Alabama) used conditional alleles of two genes (Kif3A and Tg737/IFT88) required for ciliogenesis to demonstrate the connection between primary cilia and obesity (one of the phenotypes in Bardet-Biedl syndrome). Systemic loss of cilia in adult mice results in animals that eat too much (hyperphagia) and are thus obese and have related defects, such as problems with glucose homeostasis. By disrupting cilia only in the arcuate nucleus of the hypothalamus, Yoder could show that this mimicked the hyperphagic effect caused by a systemic loss of cilia, thus strongly suggesting that primary cilia in the CNS mediate the reception and/or processing of satiety signals (Davenport et al., 2007).

### Cilia and cell polarity

It is now relatively clear that the primary cilia of cells in developing tissues such as mammary gland and bone are absolutely essential for normal tissue morphogenesis. Investigations of bone development by Courtney Haycraft (University of South Carolina) and mammary gland development by Kimberly McDermott (University of California, San Francisco) from the Tlsty lab showed that these tissues do not develop properly in the Tg737/IFT88 mouse in which the primary cilia on most cells are either not present or greatly reduced in size (Pazour et al., 2000). Tubule branching in the mammary gland is greatly inhibited, and bone development likewise is highly abnormal, including polydactyly and improper bone length. The exact role of the primary cilia in the morphogenesis of these tissues is not yet known, but it almost certainly is going to involve a role in cell polarity during embryogenesis and subsequent postnatal development. With respect to cell polarity, Chonnettia Jones (Emory University) reported use of a conditional allele for Tg737/IFT88 in the cochlea. This resulted in a loss of the primary cilium (the kinocilium), and a defect in the organization of the stereocilia (which are actin-based microvilli). The alignment of the basal bodies is also disrupted, indicating that Tg737/IFT88 interacts with the planar cell polarity pathway (a noncanonical Wnt signaling pathway) and has a distinct role in placement of the centrioles. Using genetics combined with image analysis, Wallace Marshall (University of California, San Francisco) has begun to identify the basal body components required for positioning, while Susan Dutcher (Washington University) reviewed the basal body mutants in *Chlamydomonas* that assemble morphologically abnormal basal bodies. These mutants all have consistent phenotypes: flagellar assembly defects, errors in cleavage furrow placement, and supersensitivity to taxol.

In interesting work on the role of primary cilia in wound healing reported by Christensen (University of Copenhagen), it is clear that the cells which are moving to close the wound all have their primary cilia pointing in the direction of cell movement, whereas the nonmotile cells behind the wound do not. The possibility here is that the primary cilia on the wound edge first point in the proper direction because of external signals, thus reorienting the ciliary basal body/centriole, which in turn orients the associated cytoplasmic microtubules whose repositioning finally activates the dendritic assembly of actin to protrude the leading membrane edge, resulting in directed cell motility.

### Cilia as signal receivers

There are now many reported examples of how primary cilia sense the environment either by mechano-, chemo-, or photoreceptors on the membrane of the primary cilium. William Snell (University of Texas Southwest Medical Center) reported a role for several different kinases in the mating reaction in *Chlamydomonas*; this event depends on interaction of the flagella of plus and minus mating types and requires a functional IFT system. Søren Christensen (University of Copenhagen) reported the presence of a variety of receptors on the ciliary membrane including PDGFαα (Schneider et al., 2005), which could coordinate both growth control and directional cell migration in development and wound healing. Work reported by Kathryn Anderson (Memorial Sloan-Kettering Cancer Center), Bradley Yoder (University of Alabama), and Søren Christensen showed that components of the Hedgehog (Hh) signaling pathway, including the Hh receptor (patched) and its interacting protein (smoothened), are present in the primary cilium as well. Two years ago Haycraft et al. (2005) showed that the transcription factor Gli 3, which is inhibited by smoothened upon Hh activation of patched, is localized to the tips of primary cilia. Perhaps the most detailed work on receptors associated with cilia has been performed on polycystins 1 and 2 (PC1 and 2, the gene products of PKD1 and 2, respectively), which are found on the ciliary membrane, in addition to other areas in the cell such as the ER and the apical cell surface. The primary cilium has been shown by Helle Praetorius (University of Aarhus) to be able to sense flow;

Jing Zhou (Harvard University), Michael Caplan (Yale University), and others have proposed that movement of fluid in the kidney tubules causes ciliary bending and the consequent opening of $Ca^{2+}$ channels encoded by PKD2. The resultant calcium flow into the cell keeps the cell in the nondividing state. Lack of fluid flow and/or inhibition of calcium influx due to the loss/malfunction of PC2 allow cleavage of the cytoplasmic C terminus of PC1. Michael Caplan (Yale University) showed that the C terminus then finds its way into the nucleus, activating signaling pathways that modulate the uncontrolled cell division characteristic of PKD.

This hypothesis of PKD pathogenesis via regulated intramembrane proteolysis (Brown et al., 2000) controlled by fluid flow (Chauvet et al., 2004) may, however, be oversimplified because Gregory Germino (Johns Hopkins University) and colleagues identified a two-day postnatal interval in mice that dramatically determines the kidney's response to PKD1 inactivation. Loss of PC1 at any point before this time resulted in severely cystic kidneys within three weeks, while loss of PC1 after this critical two-day period did not result in a cystic pathology for as long as five months. Bradley Yoder (University of Alabama) noted a similar pattern of late onset cystic disease in his systemic model of Tg737/IFT88 inactivation. So, although cilia are indeed important in normal kidney development and maintenance (Pazour et al., 2000; Qin et al., 2001; Yoder et al., 2002), it would appear that they are doing more than merely sensing fluid flow via polycystin-mediated calcium channel activation. For example, Stefan Somlo (Yale University) also presented data on the flow hypothesis of PKD, suggesting that calcium flux could occur in the kidney tubule cells when PC2 is not present in the cilia, by use of a mutant form of PC2 that retains its ability to act as a calcium channel but that does not localize to the primary cilium. Thus, PC2 is required for the calcium response, but it appears that ciliary PC2 is not required. However, the most direct positive evidence for the mechanosensory activity of kidney cilia continues to be the work of Praetorius and Spring (2001), who showed that moving a single cilium caused calcium entry into that cell. Continuing this work using freshly isolated renal tubules, Helle Praetorius (University of Aarhus) reported that renal flow induces activation of purinergic P2 receptors, resulting in a calcium increase that could be inhibited by externally applied ATP scavengers such as apyrase. Thus, flow may be inducing an ATP release that in turn activates purinergic P2 receptors leading to the calcium increase. If specific (P2Y2) purinergic receptors are knocked out, the flow response is significantly lower ($P < 0.05$). Note that the matter is far from settled, however, because one class of P2 receptors (P2YR) has the ability to release calcium from internal stores (Song et al., 2007).

The activity of receptors and calcium in the cilium requires now a return to the discussion of *situs inversus* introduced at the beginning of this review. Two hypotheses were proposed to explain how leftward flow of extraembryonic fluid in the node, caused by the motile nodal cilia, produces left/right body asymmetry. Nobutaka Hirokawa (University of Tokyo) proposed that the flow moves vesicles filled with morphogens (sonic hedgehog and retinoic acid) from right to left (Tanaka et al., 2005), activating signaling pathways in the receiving cells on the left side of the node, resulting in asymmetric folding. Alternatively, Martina Brueckner (Yale University) proposed that the two populations of cilia present in the node (recall Caspary et al., 2007) work in concert to set up the body plan. Those in the center of the node are motile and are responsible for the leftward fluid flow, which in turn bends the nonmotile cilia on the left side of the node, inducing calcium flow through ciliary PKD channels (McGrath et al., 2003). Brueckner reported that inversin, which has an essential function in left/right development, relocalizes in the cytoplasm of cultured IMCD3 cells in response to elevated calcium. Asymmetry of inversin localization in cells on the left side of the node also occurs, and this is thought to lead to activation of signaling pathways that ultimately results in asymmetric morphogenesis of the body organs.

## Cilia and diseases other than PKD and BBS

A very important series of results were reported linking cilia abnormalities to a range of human diseases beyond those (PKD, BBS) already mentioned. Karl Lechtreck (University of Massachusetts Medical School) showed that the protein hydin—first identified as a component of one of the central pair structures in *Chlamydomonas* that were so elegantly detailed by David Mitchell (SUNY Upstate Medical University)—is involved in the development of hydrocephalus (cerebrospinal fluid accumulation in brain ventricles). In stunningly beautiful videos of ciliary motility on cells lining the brain ventricles, hydin-deficient ependymal cilia were observed to be stiff and unable to form the beat pattern characteristic of normal cilia. Hydrocephalus develops presumably because a lack of proper motility results in decreased fluid transport. In a related presentation, Heymut Omran (University Children's Hospital, Freiburg) reported for the first time that a mutation in the DNAI2 gene encoding outer arm dynein intermediate chain 2 results in primary ciliary dyskinesia (characterized by defects in motile cilia of the respiratory tract, embryonic node, and sperm) in some patients.

Meckel-Gruber syndrome is a rare recessive disorder characterized by cystic kidneys, polydactyly, and incomplete skull closure. Helen Dawe (University of Oxford) showed that Meckel-Gruber syndrome proteins Mks1 (localized to the centrosome) and MKS3 (meckelin, localized to the primary cilium) are required for centrosome migration and subsequent ciliogenesis, perhaps through interactions with Rho kinase and myosin II. Veena Singla (University of California, San Francisco) reported that the OFD1 (orofaciodigital syndrome type I) gene product localizes to the basal body, and cells lacking the OFD1 gene product cannot generate cilia. Thus, in the absence of cilia due to the loss of a key basal body component, malformations of the face, oral cavity, and digits, which together with PKD are characteristics of OFD syndrome, occur. Surprisingly, loss of OFD1 does not affect the cell cycle in embryonic stem cells in culture. Cholangiocytes (epithelial cells lining the bile duct) in the PCK rat, a model for autosomal recessive polycystic disease (ARPKD), do not express fibrocystin due to a mutation in the PKHD1 gene. Anatoliy Masyuk (Mayo Clinic) showed that the basal bodies in this rat model are heterogeneous in size, contain extra appendages, and are positioned incorrectly in the cell.

These structural and functional abnormalities cause hepatic cystogenesis.

The protein cystin, ~5% the size of fibrocystin, is disrupted in the cpk mouse model of ARPKD. Cystin has an N-terminal myristoylation signal, a ciliary localization signal, and two nuclear localization signals. Often, but not always, cystin resides at the ciliary tip. Lisa Guay-Woodford (University of Alabama) showed that a myristoyl-electrostatic switch (McLaughlin and Aderem, 1995) controls the cycling of cystin on and off the membrane and hence the localization of cystin to the cilium or the nucleus. The S17A mutation enhances the association of cystin with the ciliary membrane, preventing its nuclear localization; when in the nucleus, however, cystin interacts with necdin and affects the expression of renal cell genes. Among these are cdc2 and c-myc, the former explaining why inhibitors of cyclin-dependent kinases are effective in treating cystic disease in the mouse kidney.

In a report clearly showing that tubulin polyglutamylation is required for cilia development in vertebrates, Iain Drummond (Massachusetts General Hospital) showed that in zebrafish, the fleer (flr) genes have a pleiotropic affect; flr mutants have kidney cysts, cannot form rod outer segments, have hydrocephalus, and left/right asymmetry defects. In addition, flr mutant cilia are short, and lack the outer side of the B-tubule of the axoneme, which is where polyglutamylated tubulin is located (Redeker et al., 2005). Thus, flr protein is involved, perhaps as a cofactor, in tubulin glutamylation. The *C. elegans* gene dyf-1 is a homologue of flr and is required for tubulin glutamylation in sensory neuron cilia as well (Pathak et al., 2007).

Finally, Uwe Wolfrum (Johannes Gutenberg University of Mainz) presented clear electron microscopic evidence showing that IFT proteins can be found on vesicles near the synapse in rod inner segments before vesicle docking and fusion with the periciliary membrane. These data are consistent with the hypothesis of Jekely and Arendt (2006), who have proposed that the cilium originally derived from a specialized membrane patch to which coated vesicles were transported by primordial IFT machinery. Their hypothesis is supported by the homology of certain IFT proteins to current day COPI and clathrin-coated vesicle proteins. Furthermore, the work of Wolfrum points out the importance of this transport network for cilia assembly and should caution investigators interpreting, for example, knock-down experiments with cilia or IFT-related proteins. A knock-down phenotype may not have anything to do directly with the ciliary apparatus itself, but perhaps to some component of the steps of vesicle budding, transport, and fusion that are required in the cytoplasm before the actual formation of a cilium or flagellum.

### Conclusions

So many significant and outstanding advances were reported at this meeting that it is impossible to single out one or two key results to highlight here. Those readers interested in obtaining further information on the topics discussed at the meeting should consult some recent reviews (Rosenbaum and Witman 2002; Sloboda 2005; Scholey and Anderson 2006; Singla and Reiter 2006; Pan and Snell 2007; Yoder 2007). It is clear from the new data presented and the excitement evident in Saxtons River, Vermont that much more will be learned, quite rapidly, about the unifying role cilia play in the biology of cell division, signaling, development, and the maintenance of normal cell functions. Comparatively speaking, the data summarized in this report represent primarily only the tip of the cilium.

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