Published in final edited form as:
Traffic. 2014 June ; 15(6): 717–726. doi:10.1111/tra.12166.

New insights into an old organelle: Meeting report on Biology of Cilia and Flagella

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Introduction

The rising interest of the scientific community in cilia biology was evident from the fact that registration for the 3rd FASEB conference on "The Biology of Cilia and Flagella" closed out before the early bird deadline. Cilia and flagella are organelles of profound medical importance; defects in their structure or function result in a plethora of human diseases called ciliopathies. 240 clinicians and basic scientists from around the world gathered from June 23 to June 28, 2013 at Sheraton at the Falls, Niagara Falls, NY to present and discuss their research on this intensely studied subcellular structure. The meeting was organized by Gregory Pazour (University of Massachusetts Medical School), Bradley Yoder (University of Alabama-Birmingham), and Maureen Barr (Rutgers University) and was sponsored by the Federation of American Societies for Experimental Biology (FASEB). Here, we report highlights, points of discussion, and emerging themes from this exciting meeting.

Ciliary length and structural regulation

A major unresolved issue in cilia biology is an understanding of how the ciliary length is regulated. Keynote speaker Wallace Marshall (UCSF) opened the meeting with a discussion of the mechanisms by which flagellar length control is mediated in the blue-green algae *Chlamydomonas*. He found that flagellar length is influenced by cytoplasmic components including actin and microtubules (MTs), with the latter being severed by cytoplasmically located katanin. Actin and the long flagella gene 4 (*lf4*) act in parallel to regulate ciliary length via modulation of IFT injection rate. Using PDMS (Polydimethylsiloxane)-based devices to stretch cilia, Marshall also demonstrated that mechanical stress modulates ciliary length perhaps via regulation of actin dynamics. Junmin Pan (Tsinghua University) demonstrated that *Chlamydomonas* possesses a length sensing system that converts length information into *Chlamydomonas* aurora-like protein kinase (CALK) phosphorylation at a key residue in the kinase activation loop (1). Thus, CALK/Aurora A phosphorylation levels serve as an excellent reporter of flagellar length. Gert Jansen (Erasmus) found that the ubiquitin-conjugating enzyme variant UEV-3 and the p38 MAP kinase PMK-3 regulate cilium length and IFT in *C. elegans*. Continuing the theme of identification of molecules

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required for regulation of ciliary length, Jonathan Eggenschwiler (University of Georgia) reported that mice lacking *ccrk* (cell cycle related kinase), the vertebrate homolog of *Chlamydomonas* LF2 (2), exhibit variable ciliary lengths and altered Sonic hedgehog (Shh) signaling. His group had previously reported that the TBC (Tre-2, BUB2p, and Cdc16p) domain protein Broad-minded (Bromi) interacts with CCRK, and *bromi* mutants exhibit similar phenotypes, suggesting that Bromi and CCRK act in the same pathway. Magdalena Cardenas-Rodriguez (Badano Lab, Institute Pasteur de Montevideo) reported that CCDC28, a second site modifier of Bardet-Biedl Syndrome (BBS) that interacts with multiple BBS proteins, also interacts with SIN1 (MAPKAP1), a structural member of the mTORC2 complex. Modulation of Sin1 but not Rictor (another component of the mTORC2 complex) function affects cilia length in hTERT-RPE cells and in zebrafish embryos (3).

Correct ciliary structure and length control requires post-translational modifications of both basal body (BB) and axonemal MTs. Renata Basto (Institut Curie, Paris France) showed that Bug22 is a BB and ciliary protein that regulates the timely deposition of axonemal post-translation modifications in *Drosophila* and mammalian cells. Bug22 also regulates the length and shape of primary spermatocyte centrioles in *Drosophila* and the length of primary cilia in mammalian RPE cells (4). Robert O'Hagan (Barr lab, Rutgers) found that CCPP-1 deglutamylase (5) and the TTLL tubulin glutamylases regulate MT A–B doublet stability, the affinity and velocity of certain ciliary motors, and the localization of ciliary receptors. O'Hagan proposed that tight regulation of glutamylation of MTs is important for ciliary function and structure. Narendra Pathak (Drummond lab, Massachusetts General Hospital) showed that Ccp5 is the key deglutamylase necessary for normal cilia function in zebrafish. Pathak previously showed that tubulin glutamylation is reduced in *fleer* mutants (6) and showed here that *ccp5* knockdown in *fleer* mutants increases glutamylation in mutant cilia. He proposed a model whereby Fleer may inhibit a CCP deglutamylase or activate a TTLL glutamylase to main tubulin glutamylation at optimal levels for normal ciliogenesis.

The basal body (BB) and transition zone (TZ)

The cilium nucleates from the BB that is derived from a mother centriole. Swadhin Chandra Jana (Bettencourt-Dias Lab, Instituto Gulbenkian de Ciência) examined how the TZ is acquired during the centriole-to-BB conversion using super resolution microscopy in *Drosophila*. He showed that BBs display distinct cell-type specific morphologies and differential localization of some BB/TZ components in different ciliated cell types, and suggested that this diversity may be correlated with function. Exploring the mechanisms by which the centriole matures to the BB and initiates ciliogenesis, Joon Kim (Korea Advanced Institute of Science and Technology) showed that CCDC41 localizes to the mother centriole distal appendages and may act with IFT20 to dock the primary ciliary vesicle to the membrane at the onset of ciliogenesis. Using immuno-EM and STORM super-resolution microscopy, Ken-Ichi Takemaru (Stony Brook University) reported that the BB protein Chibby localizes as rings to distal appendages in a CEP164-dependent manner. Chibby coordinates the timely recruitment of Rab8 to the basal body prior to docking to the apical membrane. Using quantitative proteomics and super-resolution microscopy, Barbara Tanos (Tsou Lab, Memorial Sloan-Kettering Cancer Center) identified 4 novel distal appendage proteins CEP83 (CCDC41), SCLT1, FBF1, CEP89 (CCDC123) and one known protein

Cep164. She showed that distal appendages are required for membrane docking of the maturing BB, TTBK2 recruitment to mother centrioles, CEP110 removal specifically from the mature (mother) centriole, and cilia initiation (7). Sarah Goetz (Anderson Lab, Memorial Sloan-Kettering Cancer Center) showed that the spinocerebellar ataxia-associated gene

Tau tubulin kinase 2 (TTBK2) controls initiation of ciliogenesis in part via removing CEP110 from mother centrioles. In cell culture, TTBK2::GFP localizes to the TZ, becoming enriched at the mother centriole upon serum withdrawal and initiating ciliogenesis. Anne-Marie Tassin (Institut Curie) discovered that the deubiquitinating enzyme CYLD regulates ciliogenesis. CYLD localizes to the centrosome/basal bodies via its interaction with CAP350. While the CYLD knockout mouse has no developmental phenotype, catalytically inactive CYLD mice display perinatal defects. CYLD centrosome localization and catalytic activity is required to promote ciliogenesis.

Transitioning from the BB to the formation of the transition zone (TZ), the so-called ciliary gate at the ciliary base, several speakers discussed the molecular mechanisms of TZ assembly in a range of model organisms. Mature *C. elegans* sensory cilia lack BBs. Alex Dammermann (Max F. Perutz Laboratories) showed that while *C. elegans* centrioles degenerate early in ciliogenesis, transition fiber-like structures (TFs) may persist. HYLS-1 is the only centriolar protein that remains at the ciliary base and is required for TZ assembly. He also reported the identification of a potential CEP290 homolog in *C. elegans* and showed that TZ components assemble via the MKS and NPHP modules (8), as well as the CEP290 TZ module. Piali Sengupta (Brandeis) discovered a role for the conserved actin-binding and Akt substrate protein Girdin in ciliogenesis in *C. elegans* and mammalian RPE cells. Girdin localizes to the TZ region and is important for localization of TZ components, ARL-13, and specific ciliary GPCRs.

The molecular composition and organization of the TZ was discussed by Francesc Garcia-Gonzalo (Reiter Lab, UCSF). The Reiter lab and others previous found that proteins involved in Meckel (MKS) and Joubert (JBTS) syndromes form a large complex at the TZ (9). Using structured illumination microscopy, Garcia-Gonzalo showed that MKS complex members localize distally from the TF marker CEP164. STORM microscopy reveals that MKS complex members Tctn1 and Mks1 accumulate in 8–9 foci that together form a discontinuous ring at the TZ. Both results are consistent with Y-link localization of these proteins. Y-links are present in Tctn1-null MEF and *C elegans tctn-1* mutant cilia, but are absent in *C. elegans* mutant for both *nphp-4* and *tctn-1*. Genetic interactions are also observed in mice, with Tctn1/Nphp4 double mutant embryos exhibiting a much higher incidence of exencephaly and forelimb polydactyly, and fewer cilia. The work of Jung-Chi Liao (Columbia University) indicates that the TZ complex organization may be visualized used stimulated emission depletion (STED) super resolution microscopy. He described the TZ protein complex in RPE-1 cells at 50–60 nm resolution. Using *Chlamydomonas* and mouse models, Branch Craige (Witman lab, University of Massachusetts Medical School) showed that the NPHP4 and CEP290 TZ proteins assemble independently at the TZ, with NPHP4 localized more distally and in close association to the TZ membrane, while CEP290 is found between doublet microtubules and the membrane in the proximal region. In *Chlamydomonas, cep290* mutants exhibit defective Y-links while *nphp4* mutants display

wild-type length flagella, wild-type IFT, and wild-type TZ structure. In cells doubly mutant for NPHP4 and CEP290, ultrastructural analysis revealed electron-dense material and coated vesicles in the flagellum. Based on this and biochemical analyses of isolated mutant flagella, Craige proposed a model whereby the TZ has different layers that are necessary for establishing or retaining normal levels of membrane-associated proteins in the flagellum and excluding cytosolic housekeeping proteins.

Consistent with this model, Michel Leroux (Simon Fraser University) found that *C. elegans* MKS-5 localized to both the proximal and distal region of the TZ, the NPHP module to the TFs and TZ, and the MKS module to the TZ only, suggesting that MKS-5 may act as a TZ assembly factor. Once the TZ is formed, it forms a PtdIns(4,5)P2-enriched ciliary zone of exclusion in MKS-5-dependent manner. Further adding to our compendium of TZ proteins, Dennis Diener (Rosenbaum lab, Yale) reported a proteomic study of the TZ in *Chlamydomonas* flagella. He showed that the TZ proteome contains ESCRT system components that may play a role in flagellar abscission, release of vesicles from the flagella, and removal of ubiquitinated proteins from the flagellar membrane.

Ciliary signal transduction

The major role of cilia is to facilitate signal transduction in a range of cell types. A number of talks addressed the mechanisms by which ligand-receptor interaction in the cilium modulates signaling. In the context of wound healing, Peter Satir (AECOM) discussed the role of the receptor tyrosine kinase PDGFRaa, which localizes to the primary cilium in growth-arrested fibroblasts. Upon wounding, interaction of the receptor with the PDGF ligand activates AKT and MEK1/2-ERK1/2-p90RSK signaling; both proteins are localized at the cilia base. This ciliary signaling is essential for chemotactic migration of the cell to the wound site and is dependent on NHE1 alkalinization and translocation to the leading edge. This translocation in turn is dependent on MEK1/2-mediated phosphorylation of NHE1 and cytoskeletal microtubule organization and function (10). In the context of the well-described cilium-based Shh signaling, Saikat Mukhopadhyay (UT Southwestern) identified the orphan GPCR Gpr161 that acts as a negative regulator of the Sonic hedgehog (Shh) pathway and whose ciliary localization is IFT-A and Tulp3-dependent (11). He showed that GPR161 is internalized upon Shh signaling and inhibits the PKA signaling cascade, suggesting that Gpr161 may mediate dosage-appropriate Shh responses. Further underscoring the critical importance of cilia in Hh signaling, Pamela Tran (University of Kansas Medical Center) found that genetic and pharmacological inhibition of Hh signaling reduces renal cystogenic potential in the Thm1/IFT139 mouse model, and that small molecule Hh inhibitors reduce cystogenic potential of *jck* and *Pkd1* mutant kidney explants (12). Thibaut Eguether (Pazour Lab, University of Massachusetts Medical School) showed that IFT25/27 mutant mice have strong Hh defective phenotypes (such as polydactyly, cleft palate, lung isomerisms, heart septal defects). IFT25/27 are not required for ciliogenesis but are important for signal dependent transport of multiple Hh components. Hence, IFT25/27 are necessary to couple Hh components to IFT machinery, which may explain their absence in *C. elegans* and *Drosophila* that lack ciliary Hh signaling components.

Critical to effective signaling in cilia is the regulation of signaling protein composition in the ciliary membrane. Kasey Christopher (Weatherbee Lab, Yale University) showed that mouse schlei/Tmem107 regulates embryonic patterning, cilia number and morphology, as well as ciliary protein localization (PC2, Arl13, adenylyl cyclase AC3). Andrew Koemeter-Cox Mykytyn lab, Ohio State) showed that that the Kisspeptin neuropeptide receptor Kiss1r localizes to the primary cilia of gonadotropin-releasing hormone (GnRH) neurons. While cilia on GnRH neurons are not required for reproductive phenotypes in mice, cilia do regulate neuronal firing rate in response to kisspeptin, outlining a modulatory role for ciliary signaling in the regulation of neuronal functions.

To determine the ionic composition inside cilia and determine what ion channels are located in the ciliary membrane, Markus Delling (Clapham Lab, Children's Hospital Boston) employed genetically encoded calcium indicators and direct patch clamping of primary cilia (13, 14). Smoothened-mCherry-GCaMP3 imaging and perforated patch revealed very high intraciliary calcium levels. Ciliary currents are inhibited by calcium and attenuated by PKD1-L1 and PKD2-L1 siRNA in hRPE-2 cells. Changes in intraciliary calcium result in small delayed changes in cytoplasmic calcium. Natalia Peunova (Cold Spring Harbor Laboratory) showed that nitric oxide, produced by the neuronal isoform of NO synthase, controls cilia polarity, growth and directional flow in two models of mucociliary epithelium, Xenopus embryonic skin and mouse trachea. She described separate nNOS-mediated signaling pathways controlling distinct features of motile cilia, proposing a mechanistic explanation of the link between low levels of exhaled NO and primary ciliary dyskinesia.

Specific ciliary signaling proteins can be localized to defined ciliary membrane compartments and effective signaling requires effective protein turnover. Oliver Blacque (University College Dublin) showed that the Arl13 small GTPase is restricted to the proximal region of the cilium in a cell-type specific manner in mammalian cells, and in a developmental stage-specific manner in *C. elegans* neurons. He proposed that the TZ and distal segments act as proximal and distal barriers, respectively, and restrict ARL13 localization to the proximal ciliary membrane domain (15). Blacque also discussed clathrin mediated endocytosis (CME) and proposed that balance of exocytosis vs endocytosis at the periciliary membrane regulates ciliary membrane volume and protein localization (16). Consistent with an important role for CME in cilia biology, Soren Christensen (University of Copenhagen) showed that TGFβ signaling is associated with endocytosis at the pocket region of primary cilia. In an unstimulated state, the TGFβ receptor localizes to the tip of the cilium and the SMAD2/3 transcription factors are not activated (as measured by phospho-SMAD antibodies). Upon TGFb stimulation, the receptor redistributes to the ciliary pocket, and SMAD2/3 are activated and translocate to the nucleus to activate target genes in fibroblasts and during cardiomyogenesis. Christensen proposed that the ciliary pocket serves as a region for cross talk between signaling pathways to control development and tissue homeostasis (17). Bill Snell (UT Southwestern) showed that regulated trafficking of a membrane protein to *Chlamydomonas* flagella does not require IFT and is directed by cytoplasmic microtubules (18). He showed that the SAG1 integral membrane protein on plus gametes is important for flagellar adhesion/signaling. In resting gametes, the majority of SAG1 proteins is excluded from flagellum. Upon cAMP induction, there is dramatic

enrichment of SAG1 in flagellum and apical surface within 5–10 minutes. In this context, Esben Lorentzen (Max Planck Institute, Martinsried) presented structural and biochemical studies of the IFT-B core complex. High resolution crystal structures of sub-complexes and domains of purified IFT-B proteins revealed that IFT74/81 forms a tubulin-binding module required for ciliogenesis (19). The IFT81 and IFT74 N-terminal domains bind tubulin via a conserved calponin homology domain and highly basic (Pi>12) regions, respectively.

Finally, a report from the Rosenbaum lab indicated that the cilia not only receive signals, but also may themselves signal to nearby cells. Christopher Wood (Rosenbaum lab, Yale) reported that the cilium secretes bioactive ectosomes that carry a protease necessary to liberate daughter cells after mitosis and proposes that cilia may be an underappreciated source of active, extracellular vesicles (20).

Active and passive mechanisms of ciliary transport

Many pathways have been implicated in ciliary transport including the BBSome and the exocyst complex. However, it remains unclear whether soluble proteins enter the cilium through an active (such as in nuclear transport) or passive process. Using an in vitro assay and single molecule imaging, Maxence Nachury (Stanford School of Medicine) showed that a diffusion barrier passively regulates access of soluble proteins to the ciliary compartment in a size-dependent manner (21). He also developed an *in vitro* system to study passive entry into cilia and demonstrated that receptors can passively diffuse within the ciliary membrane and associate only stochastically with IFT trains (21, 22). Karl Lechtreck (University of Georgia) showed that the axonemal proteins DRC4, DRC2, and PF16 are IFT cargoes in *Chlamydomonas* flagella, and that DRC4 requires IFT for ciliary entry and distribution. DRC4 unloading appears to be a spatially unregulated process. DRC4 transport events were significantly increased in conditions of flagellar regeneration versus steady state, indicating that IFT cargo loading is a regulated process (23).

Several speakers addressed the functions of the BBSome. Xuefeng Su (Zhou Lab, Harvard Medical School) showed that PC1 C-tail binds BBS1, 5, 8, and 8. YFP-tagged PC1 in IMCD3 cells is targeted to primary cilia, and this localization is reduced upon knockdown of Bbs1. Norann Zaghloul (University of Maryland) showed that loss of BBS1, 3, or 4 or ALMS1 results in ligand-independent enhanced Notch signaling in zebrafish embryos and cultured human cells. Increased signaling in BBS/ALMS-depleted cells is due to accumulation of Notch receptor in late endosomes, accompanied by loss of degradation and decreased trafficking to lysosomes. Zaghloul proposes that BBS and Alstrom proteins regulate Notch receptor trafficking through endosomes. Gerasimos Langousis (Hill lab, UCLA) investigated BBSome function in *T. brucei* and showed the importance of flagellum biology in infectious diseases. Calvin Carter (Sheffield Lab, Univ. of Iowa) found that BBS genes are crucial for the development of a subpopulation ($NG2^{+}PDGFR\alpha^{+}$) of periventricular progenitors. An imbalance in the survival and proliferation of these periventricular progenitors results in hydrocephalus. Lithium restores proliferation and partially rescues hydrocephalus defects in a mouse model.

Finally, Joshua Lipschutz (University of Pennsylvania) presented a model for the role of Cdc42 in the delivery of ciliary proteins (24). Cdc42 localizes the exocyst to primary cilia, whereupon the exocyst targets and docks vesicles carrying ciliary proteins. In the absence of exocyst function, there is increased expression of phosphorylated ERK, which results in abnormal ciliogenesis and PKD.

Ciliary and flagellar motility

Several speakers addressed mechanisms of ciliary motility. Daniela Nicastro (Brandeis) used cryo-electron tomography to resolve the 3D structure of axonemal dynein with all important domains resolved in three conformational states: post-powerstroke (for which previous structures by X-ray and cryo-EM exist), pre-powerstroke I (not attached to MT), pre-powerstroke II (attached to MT). Her data support a head-swing and winch model of dynein movement. In collaboration with George Witman, Nicastro localized the DRC3 subunit within the nexin-dynein regulatory complex using cryo-ET. Nicastro also reported the development and use of TYGRESS (TomographY Guided REconstruction of Subcellular Structure), a novel cryo-EM technique for high-resolution 3D reconstruction of macromolecular complexes maintained in their cellular context. To address the issue of the mechanisms by which the 24 nm periodicity of outer arm dynein is maintained, using rotary shadowing EM, Mikito Owa (University of Tokyo) showed that purified outer dynein arm-docking complex (ODA-DC) has a 24 nm ellipsoid shape. The ODA-DC cooperatively binds to the outer doublet A tubule from the proximal part to the distal part. Rebecca Burdine (Princeton University) reported that the evolutionarily conserved *kurly* gene is required for outer dynein arm localization and genetically interacts with several components in the planar cell polarity pathway in zebrafish. Niki Loges (University Hospital Muenster) showed that dyslexia susceptibility 1 candidate 1 (DYX1C1) deficiency causes primary ciliary dyskinesia and proposed that DYX1C1 is a newly identified dynein axonemal assembly factor (25). On a different note, Ahmet Yildiz (University of California Berkeley) asked the question: how does transport of trains transmit force to components outside the flagellar membrane? IFT and flagellar surface motilities differ significantly. Yildiz showed that IFT transports flagellar membrane glycoproteins (FMGs); that IFT and bead motilities are similar; that during flagellar gliding, some IFT trains pause in the leading flagellum; and that ciliary dynein-1b is the motor responsible for gliding (26).

The Obesity Ciliopathies

Many ciliopathies are associated with obesity, indicating a critical role for cilia in regulating the complex hormonal signaling pathways underlying energy metabolism. SNPs in FTO, the human fat mass and obesity-associated gene, have the strongest association with obesity. Rudolph Leibel (Columbia University) previously showed that an intronic SNP controls the expression of FTO and the nearby gene RPGRIP1L/MKS5. Leibel finds that Rpgrip1l(+/−) heterozygous mice are hyperphagic, weigh more, and have more body fat compared to littermates. RPGRIP1L and the leptin receptor coimmunoprecipate and colocalize in cilia. He went on to show that ciliogenesis and leptin-R ciliary localization are impaired in the hypothalamus of Rpgrip1l(+/−) animals.

Nicholas Berbari (Yoder lab, UAB) showed that ciliopathy mutant mice are resistant to the anorectic actions of leptin only when obese, and that mutations in the melanocortin receptor MchR1 do not result in obesity phenotypes. Alex Loktev (Jackson Lab, Stanford) identified several new GPCRs enriched in the hypothalamus, and identified the NPY2R neuropeptide Y receptor that augments ligand-dependent cAMP signaling in the POMC neurons of the arcuate hypothalamus (27). The NPY2R ciliary trafficking sequence is different from that in somatostatin receptor SSTR3, and NPY2R ciliary localization is dependent on BBS and Tubby proteins, suggesting a link between the ciliary localization of this protein and obesity phenotypes

Speakers also addressed the connection between ciliopathies and diabetes. Agata Jurczyk (University of Massachusetts Medical School) found that cilia in pancreatic islets regulate insulin secretion. Bbs2 knockout mice have fewer and shorter primary cilia, are glucose intolerant, and have dysregulated insulin secretion. Indeed, isolated islets from Bbs2 knockout animals show insulin hypersecretion in response to glucose and lose somatostatin inhibition of insulin secretion. Sukanya Lodh (Zaghloul lab, University of Maryland) showed that *bbs* zebrafish morphants have fewer pancreatic progenitor cells while *alms1* morphants have fewer beta cells, decreased secretion of insulin and are less sensitive to glucose stimulated beta cell expansion. However the beta cell mass was either enhanced or unchanged in *bbs* morphants.

Cilia, the heart, and embryonic development

Cilia are critical for embryonic development and the correct differentiation and morphogenesis of multiple tissues. To study architecture of the developing heart, Sigolène Meilhac (Institut Pasteur) developed a novel approach for quantifying tissue polarity in 3D and was able to trace back polarity of myocardium to embryonic states (28). She demonstrated that cadherin Fat4 is important to restrict heart growth rate but not polarity. Cecilia Lo (University of Pittsburgh) performed ENU mutagenesis screens conducted with noninvasive fetal ultrasound imaging to identify genes important in mammalian heart development and congenital heart disease. Whole exome sequencing revealed an enrichment of ciliopathy genes. Based on analysis of a Dnah5 mutant called Dakshi, Lo concluded that cilia function in the embryonic node is not essential for breaking symmetry or establishing the left-right axis in the mouse. However, motile cilia function in the embryonic node is required for “robustness” in the specification of the left-right axis.

In *Xenopus*, asymmetric ATP4 and serotonin mRNAs are observed before ciliogenesis at the 2–4 cell stage and 32–64 cell stage, arguing against a role for cilia in left-right asymmetry determination (29). Martin Blum (University of Hohenheim) presented work showing that serotonin is required for specification of the superficial mesoderm (SM) and that ATP4 is required for SM specification and cilia polarization. Blum concluded that cilia and leftward flow are necessary and sufficient to break symmetry in *Xenopus*.

Dominic Norris (MRC Harwell) previously showed that the PKD1-like locus Pkd1l1 establishes left-right asymmetry and physically interacts with Pkd2 (30). Here he showed that PKD1L1 acts downstream of flow but upstream of early gene expression asymmetry,

proposing that the extracellular domain of Pkd1L1 may be an important flow sensor. Tamara Caspary (Emory University) addressed the role of cilia in the developing brain. She showed that Arl13b plays critical roles in polarity of developing radial glia scaffold, radial progenitor migration, and interneuron migration in the developing cerebral cortex (31).

Cilia and Kidneys

Polycystic kidney disease is an archetypical ciliopathy. Rebekah Rasooly (Division of Kidney, Urologic, and Hematologic Diseases, NIDDK/NIH) discussed NIH funding for kidney research and NIDDK support of basic PKD-related research. Dr. Rasooly also emphasized that public access is required for publications resulting from NIH funded research.

Similar to models of oncogenesis, a second hit hypothesis has been proposed to explain the cellular recessive phenotype of autosomal dominant polycystic kidney disease (ADPKD). Peter Harris (Mayo Clinic) presented mouse genetic studies suggesting that cystogenesis results from PKD gene dosage reduction and stochastic events such as kidney damage. Harris also showed that the PKD1 gene product polycystin-1 (PC1) has different glycoforms. PC1 requires PC2 for maturation, and each of these proteins exhibits distinct subcellular localization. Thus, PC2 localizes along the cilium, while PC1 is near the TZ or proximal cilium (inversin compartment).

In contrast to ADPKD patients who show a proliferative renal phenotype, nephronophthisis patients display a degenerative renal phenotype, Bernhard Schermer (University of Cologne) showed that NPHP9/Nek8 acts similarly to NPHP4 (32) by interacting with and cooperatively interfering with the Hippo kinase signaling cascade. NPHP4 promotes nuclear accumulation and interaction of NPHP9 with TAZ/YAP transcriptional regulators. David Beier (Seattle Children's Research Institute) further discussed a role for NPHP9/Nek8 in cilium-based signaling. Intriguingly, he showed that loss of Nek8 increases histone H2AX phosphorylation, a measure of DNA damage. In response to DNA damage, NEK8 also translocates from the ciliary inversin compartment to the nucleus, and associates with nascent DNA and proteins involved in DNA damage. Jagesh Shah (Harvard Medical School) further addressed the role of Nek8 in the inversin compartment. He found that NEK8 interacts with ANKS6 (also called SAM-Cystin), a protein mutated in dominant forms of PKD and located in the Inversin compartment. Thus ANKS6 is a new component of the Inversin compartment. ANKS6 does not interact with Inversin or NPHP3, and is phosphorylated by NEK8.

Identification of genes implicated in human ciliopathies

Given the importance of dysregulated cilia function in broad spectrum ciliopathies, a session was devoted to progress in identifying new genes involved in human ciliopathies. Friedhelm Hildebrandt (Boston Children's Hospital/Harvard) presented his work on the identification of novel genes of nephronophthisis-related ciliopathies (NPHP-RC) that implicate developmental and degenerative disease mechanisms. NPHP may cause dysplasia rather than degeneration due to DNA damage response signaling defects in ciliopathies (33). By sequencing all 20 known JS genes in the genomes of the entire University of Washington

Joubert Syndrome cohort (n= 416), Ruxandra Bachmann-Gagescu (University of Zurich) concluded that there is minimal evidence that true oligogenicity (i.e. combined heterozygous variants in ≥2 different Joubert genes) could cause Joubert Syndrome. Meral Gunay-Aygun (NHGRI, NIH) presented data from an ongoing NIH Study initiated in 2002 on clinical and molecular investigations into human ciliopathies. 263 patients with non-motile ciliopathies of ARPKD Joubert and Related, ADPKD, BBS, Alstrom, OFD, PKD-CHF (Congenital hepatic fibrosis) are enrolled. Sequence analysis of the genomes of 113 patients with NPH +/− syndromes by Sophie Saunier (Inserm, Paris France) identified 19% of new genes in 43% of cases, including IFT140 missense mutations (34). Heleen Arts (Radboud University Nijmegen Medical Centre) discussed the genetics and pathobiology of complex ciliopathies affecting the kidney (35). Defects in IFT genes are often associated with ciliopathies characterized by abnormal skeletal development and renal insufficiency and with phenotypic heterogeneity. Future challenges include implementation of next generation sequencing in diagnostics and understanding phenotypic variation within/between families with ciliopathies. Finally, Jeremy McIntyre (Martens lab, University of Michigan) demonstrated that gene therapy may rescue ciliary mutant phenotypes (36). IFT88/ORPK mutant mice lack olfactory cilia and are anosmic, and mutations in IFT88 are linked to human disease. McIntyre showed that adenoviral delivery of IFT88:GFP restored olfactory cilia and function to ORPK olfactory sensory neurons. Moreover, synaptic activity was restored in the olfactory bulb and body weight of treated mice increased.

New Techniques

Interspersed in different sessions were talks on the development of new techniques to monitor aspects of ciliogenesis and ciliary function. To visualize intra-ciliary calcium dynamics, Shiaulou Yuan (Brueckner and Sun Labs, Yale University) targeted the genetically encoded calcium indicator GCaMP5 to the cilium using Arl13b. To define interaction networks at mother centriole appendages and the TZ, João Gonçalves (Pelletier Lab, Mount Sinai Hospital, Toronto), used BioID (proximity-dependent biotin *id*entification). Biotinylation occurs before lysis, so weak or transient interactions can be detected. To study ciliary cAMP signaling, Vera Jansen (Kaupp Lab, Caesar Research Center) engineered a bacterial photoactivated adenylyl cyclase (bPAC), enabling precise spatio-temporal control over cAMP levels in sperm. To study the TZ permeability barrier and visualize diffusion processes in intact living cells, Takanari Inoue (Johns Hopkins University, Baltimore, MD) developed a chemically induced dimerization trap technique that relies on the rapamycin-inducible interaction of FK506-rapamycin-binding domain (FRB) and immunophilin FK506-binding protein-12 (FKBP)(37). Inoue demonstrated that molecules of Stokes radius 3–8 nm can diffuse into primary cilia, and that the diffusity of each probe depends on its size. Quantitative modeling supported molecular sieving with a pore radius of 8 nm. It should be noted that Inoue and Nachury disagree about the size of the molecules that can freely diffuse in, with Nachury finding that molecules larger than 5nm fail to diffuse into cilia (21).

Conclusions

This dynamic meeting covered the depth and breadth of cilia/flagella biology, and addressed several questions that are of great interest. These include a resolution of the nature and function of a ciliary pore, whether IFT is required to traffic ciliary receptors, the role of the cilium in left-right axis determination, and the molecular composition of the Y-links at the TZ among others. Presented work further described the complexity of the transition zone and highlighted discrete ciliary compartments. Newly discovered and tantalizing roles of cilia as secretors of signaling vesicles and potential positioning devices on migrating cells, as well as their largely mysterious roles in neurons and other cells embedded deep in tissues and organs will no doubt continue to fascinate researchers. Given the rapid advances in the field, we look forward to more revelations at another exciting meeting on this topic July 19–24, 2015 in Snowmass, CO, which will be organized by Maureen Barr, Iain Drummond, and Jagesh Shah.

Acknowledgments

We thank the many researchers who granted permission to cite their work and apologize to those whose work was not included due to space limitations. This meeting was supported in part by FASEB SRC, NIH/National Institute of Diabetes (R13 DK098943), Digestive, and Kidney Diseases, the PKD Foundation, Amgen, The Porter Endowment, Nikon Instruments, University of Alabama at Birmingham, Primary Cilia Dyskinesia Foundation, March of Dimes, Company of Biologists Ltd, and Cytoskeleton.

Funding: Related work in the authors' labs is supported by grants DK059418 and DK074746 (M.M.B.) and GM56223 (P.S.)

List of abbreviations

ADPKD	autosomal dominant polycystic kidney disease
BB	basal body
BBS	Bardet-Biedl Syndrome
Hh	hedgehog
IFT	Intraflagellar transport
JBTS	Joubert Syndrome
MKS	Meckel Syndrome
MT	microtubule
NPHP	nephronophthisis
PC	polycystin
TF	transition fibre
TZ	transition zone

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