Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

The complexity of the cilium: spatiotemporal diversityof an ancient organelle

Westley Heydeck[1,3], Lorraine Fievet[1,2,3], Erica E Davis[1] and Nicholas Katsanis[1]

Cilia are microtubule-based appendages present on almost all vertebrate cell types where they mediate a myriad of cellular processes critical for development and homeostasis. In humans, impaired ciliary function is associated with an ever-expanding repertoire of phenotypically-overlapping yet highly variable genetic disorders, the ciliopathies. Extensive work to elucidate the structure, function, and composition of the cilium is offering hints that the 'static' representation of the cilium is a gross oversimplification of a highly dynamic organelle whose functions are choreographed dynamically across cell types, developmental, and homeostatic contexts. Understanding this diversity will require discerning ciliary versus non-ciliary roles for classically-defined 'ciliary' proteins; defining ciliary protein–protein interaction networks within and beyond the cilium; and resolving the spatiotemporal diversity of ciliary structure and function. Here, focusing on one evolutionarily conserved ciliary module, the intraflagellar transport system, we explore these ideas and propose potential future studies that will improve our knowledge gaps of the oversimplified cilium and, by extension, inform the reasons that underscore the striking range of clinical pathologies associated with ciliary dysfunction.

Addresses
[1] Center for Human Disease Modeling, Duke University Medical Center, Durham, NC 27705, USA
[2] Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), INSERM U964, CNRS UMR 7104, Université de Strasbourg, 67404 Illkirch, France

Corresponding author: Katsanis, Nicholas (nicholas.katsanis@duke.edu)

[3] Authors contributed equally.

Current Opinion in Cell Biology 2018, **55**:139–149

This review comes from a themed issue on **Differentiation and disease**

Edited by **Katja Röper** and **Xosé R Bustelo**

https://doi.org/10.1016/j.ceb.2018.08.001

Introduction: celebrating two centuries of progress in the ciliary biology field

Cilia are conserved microtubule-based appendages residing at the apical surface of almost all terminally-differentiated cell types. Based predominantly on the past two decades of research, we now know that cilia function both during embryogenesis and also in differentiated tissues to regulate a multitude of cellular processes [1,2,3••]. From a historical perspective, cilia are among the oldest observed organelles; they were discovered in the 17th century by Leeuwenhoek and noted for their remarkable motile properties [4]. The appreciation for motile cilia intensified with the realization that multiciliated cells are present in diverse vertebrate tissue types and include cilia lining the respiratory tract, lungs, inner ear, and brain ventricles [5–7]. In 1998, a landmark study uncovered a link between a distinct form of motile cilia in the node and mammalian determination of left–right asymmetry during development [8]. While initial functional studies were focused on motile cilia because of their obvious functions in fluid or cell propulsion, the primary cilium was largely neglected and considered a vestigial structure, despite documentation in the 19th century [9]. Several key findings brought functional relevance to the primary cilium; these include but are not limited to: (1) the causal relationship between primary ciliary dysfunction and the cystic renal pathology in the Oak Ridge Polycystic Kidney mouse ($Tg737^{OPRK}$) [10,11]; (2) the discovery of a role for primary cilia in Hedgehog (Hh) signal transduction in mice [12]; and (3) the observation that polycystin-1 and polycystin-2, both associated with renal disease, mediate calcium mechanosensation in a primary cilia-dependent manner [13]. Subsequent *in vitro* and *in vivo* work associated the primary cilium to additional morphogenetic pathways including Notch, Wnt, Hippo, mTOR, and PDGFR signaling, redefining the cilium as a cellular antenna critical for development, homeostasis and regenerative processes [14,15•].

Consistent with the near-ubiquitous presence of cilia across tissues, and their critical role in organogenesis and maintenance, it is not surprising that perturbation in cilia structure or function causes a host of human genetic disorders. Primary Ciliary Dyskinesia, characterized by *situs inversus*, hydrocephalus and chronic airway infections [16], garnered initial attention as a clinical entity caused by impaired motile ciliary beating capacity. In the early 2000s, defects in the primary cilium were implicated as the molecular cause of additional rare human genetic disorders, including isolated renal cystic disease (nephronophthisis; NPHP [17,18]); and Bardet–Biedl syndrome (BBS), a genetically heterogeneous disorder characterized by retinitis pigmentosa, polydactyly, obesity, learning difficulties, and renal

anomalies [19]. These studies established a role for cilia in human disease; defined the ciliopathies as a clinical collection of organellar disorders; and led to the identification of a multitude of additional phenotypically-overlapping pathologies [20]. Ciliopathies manifest in a spectrum of hallmark phenotypes with variable penetrance and expressivity [3••,20,21]. Although individually rare, there are ~100 suspected or established cilia-related clinical synopses reported in the Online Mendelian Inheritance in Man database (https://omim.org/) with a collective incidence of ~1:1000, which is comparable to Down syndrome [22].

A multidisciplinary suite of approaches has been employed to investigate the molecular processes governing ciliogenesis, homeostasis, and pathology. Electron microscopy has helped characterize ciliary ultrastructure [23] and has provided evidence supporting clinical diagnosis for ciliopathies such as Primary Ciliary Dyskinesia [24]. Three main types of cilia have been described based on microtubule arrangements and biological functions: primary (9 + 0), motile (9 + 2) and nodal cilia (9 + 0 microtubule configurations, respectively) and their ultrastructural characteristics have been reviewed extensively elsewhere [25–27], including well-known exceptions to this trichotomy [25].

Given the importance of these organelles in human disease, considerable effort has been directed at cataloguing the protein composition of the cilium [28–38,39••]. Multiple groups have contributed to the assembly of the ciliary proteome (ciliome), an extensive list of ~1000 proteins that are found within the ciliary/flagellar axoneme and the underlying basal body/centriole. The core data used to construct the ciliary proteome leveraged the extensive evolutionary conservation of the organelle and integrated mass spectrometry, functional genomics, and comparative genomics data across phyla. Notably, a proteomic study on primary cilia from mouse renal cells showed that 25% of purified proteins were not shared with previously characterized proteomes from motile and specialized sensory cilia, suggesting a subpopulation that is primary cilia-specific [40]. A subset of ciliary proteins has also been grouped into distinct molecular modules identified through biochemical studies performed *in vitro*. For instance, the BBSome is composed of a subcomplex of eight BBS proteins [41] which has been shown to translocate between the cytoplasm and the transition zone [42] at the ciliary base, and to transport ciliary components within the cilium [43]. Since then, other stable macromolecular complexes have been defined, including the transition zone complex and the NPHP complex, while further evidence has also intimated the existence of a septin pore ring, to name but a few examples [44].

Challenges and opportunities

Although the characterization of ciliary ultrastructure; protein composition; and cellular functions have been heralded, appropriately, as significant progress, they still represent an overly simplified view of an organelle underpinned by substantial complexity and diversity of composition and function. Intersection of datasets from diverse *in vitro* models, ranging from renal to retinal epithelial cells, with that of *in vivo* ciliated models spanning eukaryotic taxa have led to the cartography of a 'generic' cilium [39••,40]. From a genetic standpoint, aggregate data suggest that allelism at a single causal locus can account for some clinical diversity. For example, recessive mutations in *TTC21B* are associated with a phenotypic spectrum ranging from isolated and syndromic NPHP, focal segmental glomerulosclerosis, to the skeletal ciliopathy Jeune Asphyxiating Thoracic Dystrophy [45–47]. To date, nonsense mutations have been observed exclusively in the latter clinical group, while a recurrent p.P209L variant has been associated with isolated renal disease, offering a partial explanation to phenotype diversity. However, there are other ciliopathies for which private missense alleles can cause divergent clinical presentations without a clear genotype–phenotype correlation, such as mutations in *IFT172*, which can cause isolated retinitis pigmentosa, BBS, or Jeune Asphyxiating Thoracic Dystrophy [48,49]. These differences can likely be explained either by stochastic reasons or by secondary genetic variation affecting either known ciliary proteins, or extra-ciliary processes required for ciliary function [22]. However, genetic information overlaid onto a generic ciliary map is an overly simplistic viewpoint, as evidenced, for example, by a greater susceptibility to retinal pathology in some cases *versus* skeletal phenotypes in other ciliopathies. A reasonable posit is that some of the clinical variability within ciliopathies can be explained further with: (1) a systems biology approach to understand ciliary and non-ciliary binding proteins; (2) an improved understanding of non-ciliary roles for ciliary proteins; and (3) elucidation of unique spatiotemporal functions of ciliary proteins. Leveraging existing -omics datasets can already offer some clues.

Unbiased systems-level studies to characterize ciliary networks

Recent high throughput approaches have enabled the identification of novel ciliary effectors using genome-wide screens performed *in vitro* (Table 1) [50–53,54••,55••]. Small interfering (si)RNA-based functional genomics screens conducted in ciliated mammalian cell lines have focused primarily on identifying regulators of ciliogenesis, which in turn, led to the identification of novel ciliopathy genes [50,52,53]. These include *INPP5E*, mutations in which cause Joubert syndrome; and *Agtpbp1*, mutated in mice exhibiting ciliopathy-like phenotypes [50]. Further, a combinatorial approach of cell-based genome-wide screening and whole exome sequencing identified a genetic cause for approximately 5% of unexplained Joubert syndrome cases by uncovering mutations in *KIAA0586*, a gene known to be important for