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Piecing together a ciliome

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Cilia are slender microtubule-based appendages that emanate from the surfaces of a large proportion of eukaryotic cells. The motile and non-motile forms of cilia represent *bona fide* organelles comprising distinct repertoires of proteins that serve specific roles in locomotion or fluid movement, and sense chemical or physical extracellular cues. Owing in part to the growing number of genes associated with ciliary disorders, such as polycystic kidney disease and Bardet–Biedl syndrome, there has been a recent profusion of studies aimed at unveiling the protein makeup of cilia. The approaches used are complementary, involving several different organisms and spanning the fields of bioinformatics, genomics and proteomics. Here we review these studies and assess the various data sets to help define a comprehensive ciliary proteome, or 'ciliome'. We have compiled a cilia protein database that includes known cilia-associated proteins and numerous putative ciliary proteins including RAB-like small GTPases, which might be implicated in vesicular trafficking, and the microtubule-binding protein MIP-T3, some of which might be associated with ciliopathies.

Introduction

Cilia are primordial and almost ubiquitous, versatile organelles

The cilium is an evolutionarily ancient subcellular structure that was probably present in the ancestral eukaryote and was subsequently lost in some lineages [1], although only a few well-studied model organisms lack cilia. Examples of non-ciliated organisms include the plant *Arabidopsis thaliana*, the fungus *Saccharomyces cerevisiae,* and the slime mold *Dictyostelium discoideum*. By contrast, most unicellular and multicellular eukaryotes have cilia, and in vertebrates, cilia are present on the surface of numerous cell types [2–4]. As appendages that are strategically positioned on cell surfaces, cilia have been advantageously adapted during evolution to perform a multitude of functions related to motility and sensory perception [2,3,5–7].

Structures and general functions of cilia

There are two basic types of cilia: motile and non-motile (Figure 1). Their axonemes consist of nine doublet microtubules, with all motile cilia – except those at the embryonic node – containing an additional central pair of microtubules. The distal part of motile or non-motile cilia can comprise singlet microtubules, for example, as observed in *Caenorhabditis elegans*, *Chlamydomonas* gametes undergoing mating or mammalian pancreatic cilia [8–11]. Motile cilia (alternatively named flagella) exhibit wave-like or beating motions that are powered by the molecular motor dynein and used either for locomotion, for example, in spermatozoa or unicellular organisms such as *Chlamydomonas reinhardtii*, or to generate mucosal or fluid flow, as exemplified by respiratory and nodal cilia [3,7,12]. Some cells have a single motile cilium (e.g. sperm), whereas many cells have up to hundreds of copies (e.g. respiratory epithelial cells or unicellular organisms such as *Tetrahymena*). Non-motile (or primary) cilia are usually associated with various sensory functions and are present singly on cells [2,3,5]. Their precise roles are cell-type dependent; for example, specialized cilia are responsible for vision, olfaction and mechanosensation.

Components required for the biogenesis, maintenance and function of cilia

The axonemal microtubules of all cilia nucleate and extend from a basal body, a centriolar structure most often composed of a radial array of nine triplet microtubules (Figure 1). In most cells, basal bodies associate with cell membranes and cilia are assembled as 'extracellular' membrane-enclosed compartments. Cilium biogenesis and maintenance almost invariably depends on an intraflagellar transport (IFT) machinery that moves cargo towards the ciliary tip by kinesin motor(s) and recycles components back to the base through a dynein retrograde motor (Figure 1; [3,6,12,13]). The motors associate with the IFT particle subcomplexes A and B, which together contain >17 subunits [13,14]. Not all motor-IFT machinery components are likely to have been identified; several novel IFT-associated proteins have been recently uncovered, including six different Bardet–Biedl syndrome (BBS) proteins and a previously uncharacterized kinesin activator, DYF-1 [15–18]. Interestingly, these and other newly identified proteins (e.g. DYF-3 and DYF-13) seem to have specialized roles in building the distal portions of *C. elegans cilia,* and they probably have similar roles in other organisms because they are evolutionarily conserved [15,19–21]. It is notable that in some species, for example, in *Drosophila* sperm cells and *Plasmodium*, cilia are pre-assembled in the cytosol independent of the IFT machinery [22,23].

The molecular makeup of motile and non-motile cilia is likely to overlap substantially, although each type will boast components required to accommodate their specific functions. For example, non-motile cilia lack motility-associated proteins (e.g. outer and inner dynein arm and radial spoke proteins, microtubule central pair-associated proteins) but can be enriched for proteins related to sensory perception

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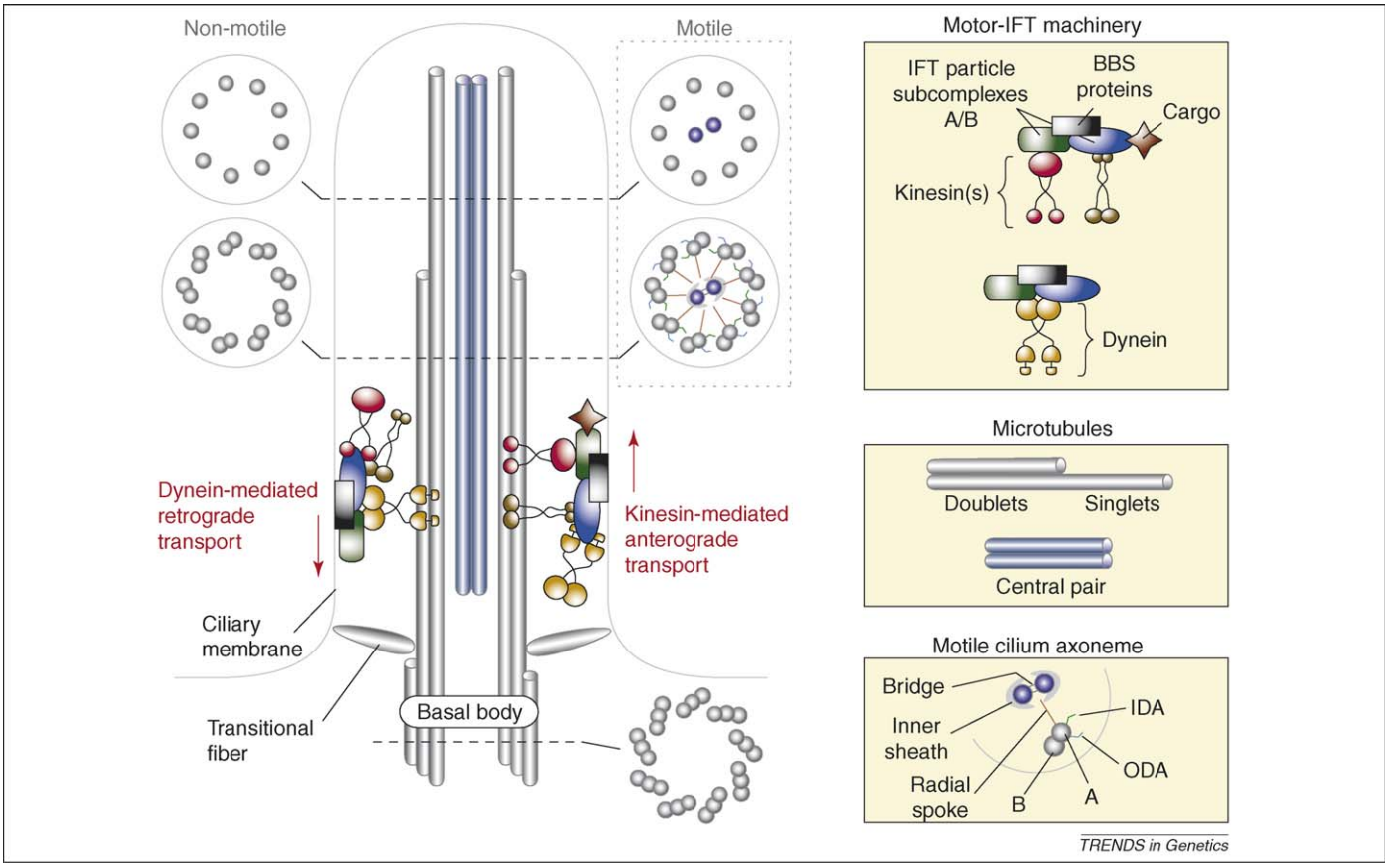

Figure 1. A working model of the components and organization of motile and non-motile cilia and of the intraflagellar transport (IFT) machinery. Ciliary axonemes, consisting of doublet microtubules and in some cases singlet microtubules at the distal end, nucleate and extend from a (usually) triplet-microtubule centriolar structure termed the basal body. For simplicity, both motile and non-motile ciliary structures are shown together; non-motile cilia lack the central pair of microtubules and associated motility structures, including the inner and outer dynein arms (IDA and ODA, respectively) and radial spokes. Cross-sections show the microtubule architectures at different regions of the motile and non-motile cilia. The IFT machinery consists of kinesin and dynein molecular motors, IFT particle subcomplexes (A and B) and various other components (e.g. BBS proteins). Motor-IFT particles probably dock near the basal body, presumably at the structures termed transitional fibers. From there, ciliary cargo (e.g. axonemal components, receptors) are moved towards the tip of the cilium by one or two IFT-kinesin(s) (heterotrimeric and homodimeric kinesin-II in *Caenorhabditis elegans*) and IFT components are recycled back to the base by IFT-dynein-mediated transport. Abbreviations: A, A-tubule; B, B-tubule.

(e.g. receptors and downstream signaling components). Indeed, non-motile cilia have been linked to several signaling processes that are crucial for development, including the Hedgehog, PDGFRαα and Wnt signaling pathways [24–28].

Ciliary disorders

Aberrant motile and non-motile cilia functions are known to result in an astonishing number of different ailments (Box 1). A non-exhaustive list of 'classical' ciliopathies, discussed in detail in several reviews [7,28–30], includes polycystic kidney disease (PKD), retinal degeneration, laterality defects, chronic respiratory problems, *situs inversus*, hydrocephalus and infertility. More recently, the discovery of other cilia-associated genetic disorders, including Bardet–Biedl and Alström syndromes, has extended the list of cilia-related phenotypes to include obesity, diabetes, hypertension, heart defects, sensory deficits (anosmia, hearing impairment), skeletal, neurological and developmental anomalies [3,28,31–36]. Mutations in several ciliary genes (e.g. *BBS* and dynein component genes) cause pleiotropic phenotypes affecting numerous tissues and cells, although in some cases the defects can be restricted (e.g. hydrocephalus in the mouse *hydin* knockout; discussed in the next section).

Bioinformatic, genomic and proteomic studies identify ciliary proteins

Bioinformatic search for X boxes in C. elegans promoters

A landmark study by Swoboda *et al.* [37] revealed that the expression of *C. elegans* genes required for cilium biogenesis, normally restricted to ciliated sensory neurons (60 of the 302 neurons in total), is controlled by the regulatory factor X (RFX)-type transcription factor, DAF-19. Several genes encoding IFT proteins are regulated by DAF-19, which binds to a conserved promoter element termed the 'X box'. Other *C. elegans* ciliogenic genes, including six *bbs* genes and novel IFT genes such as *dyf-1*, *dyf-3* and *dyf-13*, were later shown to also be regulated by the DAF-19/X boxes [15,16,18–20,33]. This indicated that *C. elegans* X-box-containing genes expressed exclusively in ciliated cells are likely to encode proteins with important ciliary functions and, notably, human RFX3 and at least one of the two *Drosophila* RFX transcription factors also regulate proper cilium assembly and function [38,39]. These findings prompted genome-wide searches for such genes in *C. elegans*.

In one study, Efimenko *et al.* [40] scanned *C. elegans* promoters for a 14-bp 'average' (degenerate) X-box

Box 1. Syndromes and ailments associated with basal body and ciliary dysfunction

There are numerous disorders linked to basal body and/or cilia dysfunction, including (but not restricted to): PKD, PCD (immotile cilia syndrome), nephronophthisis, Senior-Loken syndrome, Joubert syndrome, Meckel syndrome, oral-facial-digital syndrome, Alström syndrome and Bardet–Biedl syndrome. These syndromes are typically associated with one or more of the symptoms described below.

Cystic kidneys
Autosomal dominant polycystic kidney disease (ADPKD), autosomal recessive PKD (ARPKD) and nephronophthisis (NPHP) represent the major cystic kidney diseases. Proteins implicated in these cystic diseases localize mainly to renal cilia, basal bodies and centrosomes, where they are likely to have important roles in mechanosensation of fluid flow, cell cycle control and modulating signaling pathways [48].

Retinal degeneration and retinitis pigmentosa
The light-receiving outer segment (OS) of vertebrate photoreceptor rods and cones are derived from specialized primary cilia. IFT within the connecting cilium is responsible for maintaining the integrity of the OS, which undergoes a rapid turnover of proteins and lipids, by driving the movement of components from the inner segment to the OS [3,30]. Compromised IFT results in degeneration of the OS and accumulation of key OS molecules (e.g. opsins) in the inner segment.

Situs Inversus
Motile primary cilia projecting from the embryonic node generate a flow of morphogens that is sensed by another class of (non-motile) cilia. The morphogen flow is crucial in establishing correct left–right axis asymmetry in vertebrates, and defects in either motile or non-motile cilia function results in randomized or reversed organ laterality (*situs inversus*) [2,29].

Anosmia
The olfactory epithelium is rich in non-motile cilia that express a wide variety of olfactory receptors required for odorant reception. Abrogated primary cilia function, for example, in Bardet–Biedl syndrome, is associated with a decreased sense of smell [35,36].

Respiratory problems
Motile cilia line the respiratory airways, where they serve to clear foreign particles. Sinusitis, rhinitis, bronchitis and otitis have all been associated with motile cilia dysfunction [29].

Infertility
Motile cilia are implicated in both male and female infertility [29]. Deficiencies in the whip-like flagella of sperm cells result in male infertility, and the movement of immature sperm in efferent ductules of the testes also depends on motile cilia. Female fertility is at least partially dependent on the transport of eggs by cilia lining the fallopian tubes.

Hydrocephalus
Epithelial cells of brain ventricle ependyma have motile cilia responsible for generating a cerebrospinal fluid flow. Defects in these cilia can result in the accumulation of fluid in the ventricles, a condition termed hydrocephalus [61].

Other ailments
Additional ailments associated with some disorders, such as the Bardet–Biedl, Alström and Meckel syndromes, include obesity, diabetes, liver fibrosis, hypertension, heart malformations, skeletal anomalies (e.g. polydactyly), cognitive impairment and developmental defects such as exencephaly [2,5,28–31,34,48,71].

consensus sequence. A set of 758 genes having an occurrence of the motif with up to three mismatches (when sequences were compared with a refined consensus sequence) and within 1000-bp upstream of the start codon were identified. Because *bona fide C. elegans* X boxes are typically ~100-bp upstream of start codons and tend to be conserved [33,37], more stringent criteria (within 250 bp and $\leq$1 mismatch) could be considered to uncover novel ciliogenic and ciliary genes with greater confidence. In all, 164 genes fulfill these criteria, including six previously uncharacterized *xbx* genes whose expression in ciliated cells was shown to be reduced in a *daf-19* mutant background. One of the genes, *xbx-2*, encodes a Tctex-1 domain-containing dynein light chain that was visualized to undergo IFT when fused to green fluorescent protein (GFP). Another protein identified, TUB-1, was recently shown to localize to cilia and undergo IFT [41]; its disruption in *C. elegans* causes ciliary phenotypes (chemosensory defect and increased life span) and, intriguingly, an increase in lipid accumulation similar to that seen in a mouse knockout of the gene homolog, *tubby* [42–44].

In a similar study, Blacque *et al.* [19] used a profile Hidden Markov Model created with 22 X-box sequences to query all *C. elegans* promoters. The training set included several X boxes from genes previously identified by Fan *et al.* [16] that were shown to be expressed strictly in ciliated cells. The search yielded 1572 genes with X-box sequences within 1.5-Kb of start codons and with significant human homologs. Canonically positioned X boxes (<250-bp from start codon) were found in 293 of these gene promoters. From this data set, the expression of 14 uncharacterized but evolutionarily conserved genes was shown to be restricted to ciliated neurons. One of these (*dyf-13*) encodes a novel IFT protein required for proper cilium formation.

Together, the two bioinformatic studies identified numerous putative novel ciliary genes, including kinases, receptors and transcription factors, many of which seem to be unique to sensory (non-motile) cilia. Although the two data sets probably include a significant proportion of false positives and might miss more divergent but functional X boxes, they are complementary because they only overlap partially. The predictive abilities of these data sets to identify ciliary and ciliopathy-associated genes is substantial; for example, C48B6.8 was initially confirmed by Blacque *et al.* [19] to be expressed exclusively in ciliated cells, and the human homolog was later shown to be mutated in some patients with Bardet–Biedl syndrome [45], identifying it as *BBS9*. Similarly, the human homologs of two X-box-containing *C. elegans* genes (R148.1/*xbx-7* and F35D2.4) are now associated with Meckel syndrome [46,47], a putative basal body and ciliary disorder characterized by polydactyly, renal and hepatic cystic dysplasia and developmental defects in the central nervous system (occipital encephalocele).

Comparative genomic analyses

The availability of numerous sequenced genomes from ciliated and non-ciliated eukaryotes has recently set the stage for powerful comparative genomic studies. *In silico* subtraction of homologous genes found in non-ciliated organisms from a ciliated genomic data set should enrich for genes that have unique, cilium-specific functions. Li *et al.* [18] successfully applied such an approach by using a panel of genomes from different organisms. One

study involved finding the intersection between the ciliated human and *Chlamydomonas* genomes and subtracting the non-ciliated *Arabidopsis* genome. This yielded a so-called flagellar apparatus-basal body (FABB) proteome containing 688 proteins. The data set included a large percentage of known ciliary proteins (52 of 58 queried), several known basal body proteins (four of six examined) and a large proportion (~50%) of proteins with no known function or link to cilia. Similar genomic arithmetic was performed by obtaining the intersection between the *Chlamydomonas* and other ciliated organism genomes (*Mus musculus, Ciona intestinalis, C. elegans* and *Drosophila*) and excluding *Arabidopsis* gene homologs. This produced data sets that overlapped to a large degree with the FABB proteome. The smaller amount of overlap with the *C. elegans* comparison could be partly explained by the lack of motile cilia in the nematode. Notable disease-associated proteins present in the FABB proteome included most known BBS proteins in addition to NPHP4 and Fibrocystin, which are linked to kidney disorders [48], and Hydin, which is implicated in hydrocephalus [49].

The authors then used two different approaches to determine the likelihood that genes within the FABB data set have basal body or ciliary functions. Approximately a third of the 103 genes examined (e.g. BBS or uncharacterized genes) were found by RT–PCR to be transcriptionally upregulated three- to 13-fold during the process of *Chlamydomonas* flagellar regeneration, an indication that they are required for cilium biogenesis and/or function. Notably, the human homolog of an upregulated gene mapped to the *BBS5* critical gene interval and was confirmed by sequencing to be disrupted in some BBS patients. In the second approach, six genes that were not upregulated during flagellar assembly and therefore are predicted to have basal body functions were chosen for knockdown by RNA interference (RNAi). Remarkably, five of six tested showed flagellar phenotypes such as paralyzed flagella or slow swimming, or a cleavage furrow defect often implicated in basal body function. Directed RNAi of the *Chlamydomonas BBS5* gene resulted in several transformants lacking flagella, implicating BBS as a ciliary disorder.

A similar comparative genomic study by Avidor-Reiss *et al.* [23] comprised six ciliated organisms (*Drosophila melanogaster*, *Homo sapiens, C. elegans*, *C. reinhardtii*, *Plasmodium falciparum* and *Trypanosoma. brucei*) and three non-ciliated organisms (*A. thaliana*, *S. cerevisiae* and *D. discoideum*). Using *Drosophila* as an anchor, genome subtractions were employed to reveal four classes of genes: (i) those conserved in all ciliated organisms (16 genes); (ii) those conserved in organisms with motile cilia (i.e. in all ciliated organisms except *C. elegans*; 18 genes); (iii) those found in organisms with compartmentalized (but not cytosol-assembled) cilia (i.e. all ciliated organisms except *P. falciparum*; 103 genes); and (iv) those specifically present in organisms with compartmentalized and motile cilia (i.e. humans, *Chlamydomonas*, *Trypanosoma* and *Drosophila* but not *C. elegans* or *Plasmodium*; 50 genes).

Of the 187 genes identified in total, the authors observed that 30 out of 36 known ciliogenic genes examined were present, including those encoding evolutionarily conserved IFT and motor proteins. This indicated that the bioinformatic analyses enriched significantly for cilia-specific genes. Nearly a third (52) of the 187 *Drosophila* genes identified were found to have an X-box sequence in their promoters, implicating RFX transcription factor(s) as important regulator(s) of ciliogenesis, as in *C. elegans* [38]. Seventeen genes were chosen for expression analysis; remarkably, all transgenes were found to be expressed exclusively in mechanosensory and chemosensory ciliated neurons, with the exception of one (a dynein light chain), which is also expressed in sperm cells. Interestingly, two of the genes included small GTP-binding proteins of the ARF-like family, ARL3 and ARL6. The first has been associated with cilium biogenesis in *Leishmania* [50] and the second was subsequently shown to encode a BBS protein implicated in IFT, BBS3 [16,51]. These two ARL proteins were both identified in the bioinformatic search for *C. elegans* X boxes [16,19], and a third ARL protein – ARL2L1/ARL-13b/scorpion – was also uncovered in the X-box studies and results in cystic kidneys when disrupted in zebrafish [52].

One advantage of comparative genomic studies is that cell body or basal body proteins that support cilia function but are not specifically localized to the organelle (e.g. cannot be detected in proteomic analysis of isolated cilia) can be identified. However, the approach used relies almost exclusively on identifying gene counterparts (orthologs) by homology searching. Consequently, *bona fide* ciliary genes might be excluded because they are members of conserved multigene families with similar but non-redundant functions (e.g. tubulins and molecular motor components), or they are too divergent, small or sequence information-poor (e.g. contain repetitive sequences such as coiled coils) to be identified reliably. Performing additional comparative genomic analyses with new organisms will help to uncover a larger complement of ciliary genes, some of which might be organism-specific.

Flagellar regeneration transcriptome analyses

In unicellular organisms, cilia are normally resorbed before cell division and can be shed during certain environmental conditions. Cilia regeneration would be expected to require substantial upregulation of genes necessary for building a functional organelle. Using a DNA oligonucleotide microarray, Stolc *et al.* [53] monitored the genome-wide expression of genes from *Chlamydomonas* undergoing reflagellation following acid shock-induced flagellar severing. The authors identified 220 genes that had more than twofold induction during flagellar regeneration, including 85 genes previously known to encode cilia or basal body components. Using a less stringent criterion of >1.75-fold induction, 35 of 56 genes known to be transcriptionally induced during reflagellation (as shown by Li *et al.* [18]) were identified, whereas none of 54 uninduced genes and 20 non-flagellar genes had such an increase. These data confirm the low false-positive rate of the approach, but present a relatively high false-negative rate because of the stringent threshold criterion used for selecting upregulated genes.

Interestingly, genes encoding the actin- and tubulin-folding chaperone CCT (chaperonin-containing TCP-1) were identified, confirming its role in cilium biogenesis [54] although it is not a cilium-specific chaperone *per se* and

so would have been missed by genomic subtraction [55]. Another interesting finding to emerge from this study is that the homologs of two nuclear DNA helicase components, reptin and pontin, which are implicated in cystic kidney formation in zebrafish and are therefore likely to have a ciliary role [52], were both strongly upregulated during reflagellation. Thus, obtaining transcriptional profiles can serve to uncover components that are not necessarily present within cilia but can have important roles in regulating ciliary biogenesis or function. A third cystic kidney-associated gene, *dyf-3* (also known as qilin), was also found to be transcriptionally upregulated during flagellar regeneration and is now known to be associated with intraflagellar transport [18,21,53].

Ciliated cell-specific transcriptome analyses

Another means of obtaining a transcriptome enriched for ciliary genes is to compare, in a multicellular animal, the transcription profiles of ciliated cells with those lacking cilia. Such an approach was employed by Blacque *et al.* [19] by using a panel of embryonic ciliated or non-ciliated cells from *C. elegans* combined with serial analysis of gene expression (SAGE). All 60 ciliated sensory neurons were specifically marked using GFP expressed from a *bbs-1* promoter, isolated by disrupting the embryo and fluorescence-activated cell sorting, and subjected to SAGE expression profiling. Similarly, specific subsets of cells expressing pan-neuronal-, intestinal- or muscle-specific GFP markers were analyzed by SAGE. When a 1.5-fold or greater level of expression in ciliated cells versus each of the pan-neuronal (mostly non-ciliated), non-ciliated intestinal and muscle cell transcriptomes is considered, 1282 genes are represented. Many of the expected ciliary genes are found in this data set, including IFT and *bbs* genes, as are numerous other genes of unknown function. The prominin 1 membrane protein homolog, F08B12.1, represents an interesting example that deserves further attention given its strong enrichment (13-fold) in ciliated cells. It has not been associated with cilia function, but is found in membrane protrusions and the sperm tail, and its disruption in the mouse causes retinal degeneration [56].

The advantage of the microarray and SAGE over proteomic studies is that they can uncover any gene required for the formation or function of cilia, irrespective of the subcellular localization of the encoded protein. For example, in *C. elegans*, such studies might help identify specialized genes required for the function or differentiation of all or specific ciliated sensory neurons. A study by Colosimo *et al.* [57] employed microarrays to assess the difference in expression profiles between *C. elegans* unsorted embryonic cells and two isolated ciliated sensory neurons, the olfactory AWB neuron and the thermosensory AFD neuron. Overall, however, these approaches can have both high false-positive and negative rates because of low enrichment factors (particularly for poorly expressed genes), emphasizing the need for additional complementary studies (e.g. proteomic analyses) to define genes important for cilia function.

Proteomic studies of motile cilia

A powerful means to identify ciliary proteins is simply to perform proteomic analyses on isolated cilia. The first attempt to identify a ciliary axoneme proteome, using cilia released from human bronchial epithelial tissue culture cells as starting material, was described by Ostrowski *et al.* [58]. The cilia were extracted with detergent to yield ciliary axonemes, and peptides were generated by proteolytic digestion either directly or following 1D or 2D gel electrophoresis separations of the proteins. Liquid chromatography (LC)-mass spectrometric (MS) analyses of the peptides revealed a total of 214 proteins identifiable on the basis of one or more peptide match(es). Several known ciliary axonemal proteins were identified, including tubulins, dynein components and radial spoke proteins, in addition to flagellar or sperm-specific components, such as the sperm-associated antigen 6 (SPAG6)/*Chlamydomonas* PF16 protein. Many of the proteins identified were initially listed as uncharacterized, but as pointed out by Marshall [59], several of these are now known to have an ortholog in other organisms, including the cystic kidney-associated DYF-3/qilin IFT protein [21,52].

More recently, Smith *et al.* [60] performed 2D LC separations on tryptic peptides isolated from two independently obtained, membrane-free preparations of *Tetrahymena thermophila* ciliary axonemes, and analyzed the eluted peptides by MS. A total of 223 proteins were deemed *bona fide* ciliary components. Of these, 139 matched entries from non-redundant databases. Many of the evolutionarily conserved proteins are components previously localized to motile cilia, such as structural (e.g. tubulins, radial spokes proteins, PF16) and motor (kinesin and dynein subunits) proteins. Among the interesting ciliary protein candidates identified is Hydin (Hydrocephalus-inducing), so-named because its disruption in the mouse mutant *hy3* results in a net accumulation of cerebrospinal fluid within the ventricular system of the brain. Although the function of the protein remains to be discovered, its presence in ciliated epithelial tissues (including the ependymal layer of brain ventricles) [49], observation in motile cilia data sets and the link between cilia dysfunction and hydrocephalus (e.g. see Ref. [61]) suggests that it supports the function of motile cilia.

A significant advantage of using isolated cilia is that they can be fractionated biochemically to obtain different sub-organellar protein extracts for proteomic studies. Pazour *et al.* [62] used such a strategy to reveal a *Chlamydomonas* ciliary proteome enriched for proteins in the membrane plus axoneme, in the membrane plus matrix (the matrix contains components such as IFT proteins that are not tightly associated with either the axoneme or membrane) or in two differentially extracted axoneme preparations. The complete data set of proteins identified in the preparations included 360 proteins represented by five or more peptides, deemed to represent ciliary proteins with a high level of confidence, and 292 additional proteins with two-to-four peptides that probably includes many *bona fide* ciliary components but also many false positives. An additional 492 proteins were identified on the basis of a single peptide. Remarkably, 97 of 101 known *Chlamydomonas* ciliary proteins were present in the complete data set, providing strong evidence for the completeness of the identified ciliome. Several of the genes encoding proteins found in the proteome were chosen for RT–PCR expression

analysis during flagellar regeneration. Most of the 87 genes that had an uncharacterized human homolog (60 of 69 tested) were induced by deflagellation, indicating their potential involvement in ciliary functions.

Several proteins – including tRNA synthethases, ribosomal proteins and histones – were highlighted as being probable contaminants. A key discovery by the authors is that >90 signal transduction proteins (e.g. kinases, phosphatases, many potential Ca^{2+}-binding EF hand-containing proteins, receptors, channels and small GTPases) were uncovered, underlining the capacity for sensory perception by motile cilia. The discovery of multiple ciliary proteins associated with cystic kidney disease as also notable, including polycystin 2, fibrocystin, ARL2L1/ARL13b/scorpion, DYF-3/qilin and the NIMA kinase NEK1 [48,63].

In the most recent study, Broadhead *et al.* [64] performed a proteomic analysis on the unique flagellum of the bloodstream parasite *T. brucei*. Both within its vector (the African tsetse fly) and host, *T. brucei* has a flagellum that is associated with a paraflagellar rod (PFR) structure not found in other cilia discussed earlier. The ciliary axoneme, basal body and PFR were isolated using a standard procedure of treatment with detergent and high salt concentration, and used to derive a ciliary proteome by LC-MS. Of the 331 proteins identified, many (208) are specific to trypanosomatids and although some of these could be attributed to the inclusion of the paraflagellar rod in the proteome, most novel axonemal components might be unique to this group of organisms. Not surprisingly, most of the remaining 123 proteins are motile and cilia-specific, showing a large degree of overlap with the *Chlamydomonas* and *Tetrahymena* ciliomes, whereas only a few are homologous to *C. elegans* and *Drosophila* proteins.

As seen in other studies, several proteins identified by Broadhead *et al.* [64] are implicated in human or mouse diseases, including Hydin and PACRG. Notably, the requirement of both of these proteins for cilia function (motility) was validated in this study. Another interesting observation made by the authors is that 34 of the *T. brucei* genes uncovered have homologs in humans that map to the critical intervals of a diverse set of genetically mapped diseases with potential ciliary involvement. These include primary ciliary dyskinesia (PCD), PKD, retinal dystrophies and BRESEK syndrome (which is characterized by cystic kidneys and polydactyly, among other ailments; http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id = 300404) [64].

One drawback of proteomic studies is their limited sensitivity. Thus, true ciliary proteins of low abundance might not be identified, or might be represented with only one peptide and thus cannot be considered *bona fide* ciliary proteins because the subset of proteins represented with few peptides is more likely to contain contaminating proteins. Another potential difficulty is that biochemical fractionation might remove ciliary components. For example, although all studied *C. elegans* BBS proteins have been shown to be components of the IFT machinery, it is intriguing that none of the homologous proteins from humans, *Chlamydomonas* or *Tetrahymena* were identified in the proteomic studies. Such shortcomings, as with those specific to the bioinformatic and genomic studies, again highlight the need to combine the results of different complementary studies to help in the identification of proteins that have ciliary roles.

Compilation of a ciliary proteome

Understanding the varied physiological functions of motile and non-motile cilia and identifying novel genes linked to ciliopathies requires a thorough understanding of the components present within these complex organelles. Using the known and candidate ciliary genes identified in the studies outlined here, we have compiled a ciliary proteome database that is accessible online at http://www.ciliome.com (see Figure 1 in the supplementary material online). The website can accept queries with defined search criteria, including keyword, choice of ciliary data sets to include or exclude, and organism(s) to display for cross-species comparisons of the best reciprocal Blast hits. The output can be viewed online as raw text or as html with links to the respective gene entries in databases, or it can be saved and imported into a spreadsheet for searching or further manipulations. To facilitate the discovery of genes associated with ciliopathies, we have included an option similar to that presented by Broadhead *et al.* [64] to show all cloned or mapped disease loci from OMIM (http://www.ncbi.nlm.nih.gov/omim) that coincide with the candidate human genes.

To uncover novel putative ciliary proteins, we focused our attention on those found in three or more of the ciliary data sets presented earlier. Of these (157 in total), 50 are either uncharacterized or not previously associated with ciliary functions, but do have a human homolog (Table 1). We suspect that this list will include numerous novel proteins important for the biogenesis or function of cilia. The remaining 107 proteins are listed in supplementary Table 1 along with 36 other known ciliary proteins not necessarily represented in the data sets. Relevant references, where applicable, are also included in this supplementary table.

Among the top candidates from Table 1 is an evolutionarily conserved protein, TNF receptor-associated factor 3 interacting protein 1 (TRAFIP1 or MIP-T3), found in seven different studies. Although it is not known to function within cilia, its expression pattern is restricted to ciliated cells in *C. elegans* [19] and intriguingly, it interacts with microtubules and modulates the function of STAT6, a protein that localizes to cilia and binds to the autosomal dominant PKD-associated ciliary protein, polycystin 1 [65,66]. Another interesting candidate (RABL5), identified in five of the nine studies, is a member of the RAB family of small GTPases that are known to have diverse roles in vesicle-associated intracellular trafficking [67] but have not been implicated in ciliary functions. This finding is of interest in light of the recent discovery that the related ARL small GTPase ARL6/BBS3 participates in intraflagellar transport [16], and that another RAB protein (RABL4) was found in three studies (Table 1). Several other proteins were uncovered in four studies, including the WD-repeat protein 35, which is expressed specifically in ciliated cells of both *C. elegans* and *Drosophila* [19,23]. Finally, proteins containing WD40, TPR or coiled-coil

Table 1. Proteins with mammalian orthologs identified in three or more studies with no previously associated ciliary role[a,b]

C. elegans	*C. intestinalis*	*C. reinhardtii*	*D. melanogaster*	*H. sapiens*	*Mus musculus*	1	2	3	4	5	6	7	8	9	Annotation
C02H7.1	004318	C_140070	CG3259	Traf3ip1	Traf3ip1	✓	✓	✓	✓	✓		✓		✓	MIP-T3
	006655	C_2130007	CG15161	NP_064538.2	1500035H01Rik	✓				✓	✓	✓		✓	Uncharacterized conserved protein
T28F3.6		C_250115		RABL5	Rabl5		✓		✓	✓	✓	✓			Rab-like GTPase 5
	006720	C_220104	CG15429	NP_653208.2	Q5NCY3_mouse	✓				✓		✓		✓	Uncharacterized conserved protein; cyt-b5 domain
C54G7.4	008946	C_1970008	Oseg4	WDR35	Wdr35	✓		✓		✓		✓			WD-repeat protein 35
C32E8.3	004525	C_150171	CG4893	CG38_human	2700055K07Rik	✓		✓		✓	✓				Uncharacterized conserved protein
Y38F2AL.2		C_30026	CG9227	NP_085055.1	BC028440	✓	✓	✓		✓					B9 domain-containing protein
K03E6.4		C_530031		NP_056496.1	Eppb9		✓	✓	✓	✓					Endothelial precursor protein B9
C54C6.6	009514	C_100132	CG5343	NP_037374.1	Gtl3			✓			✓	✓	✓		Uncharacterized transcription factor
M04C9.5	002836	C_60170	CG31711	112144	021363			✓	✓			✓		✓	Male germ cell-associated kinase (MAK)
gst-1		C_650070		GSTP1	038155				✓	✓	✓	✓			Glutathione S-transferase
	003205	C_110027	CG31784	173013	4921513E08Rik					✓		✓	✓	✓	Uncharacterized conserved protein
	009031	C_660055		SPAT4_human	031518					✓		✓	✓	✓	Testis spermatocyte apoptosis-related
		C_170175		185055	4930504H06Rik					✓	✓	✓		✓	Uncharacterized conserved protein
	001121	C_990023		NP_060835.1	032221					✓	✓	✓		✓	Meiosis-specific nuclear structural protein
		C_20048		MYCBP							✓	✓	✓	✓	Gap junction α-10 protein (connexin 59)
T03G11.3		C_30206	CG10999	NP_057094.1	2810002I04Rik	✓	✓			✓					Uncharacterized conserved protein
ZK520.3	004990	C_210077	Oseg6	WDR19	Wdr19	✓	✓			✓					WD-40 repeat-containing protein
R10F2.5	005104	C_1080047	CG13178	NP_996668.1	1810007P19Rik	✓		✓		✓					Oxidored-nitro domain-containing protein
pde-1	006658	C_1270001	Pde1c	PDE1C	Pde1c	✓	✓			✓					Ca/CaM-dependent phosphodiesterase
F09G8.5		C_10266	CG14995	CU002_human	1810043G02Rik	✓				✓		✓			Uncharacterized conserved protein
K07G5.3	003261		CG18631	Q9H8A7_human	5730509K17Rik	✓	✓	✓							Uncharacterized calcium-binding protein
B0464.2	008994	C_30110	CG2469	SH2BP1	Sh2bp1		✓	✓		✓					SH2-binding protein with TPR repeats
B0495.7		C_20025	CG11961	KIAA1815	D19Wsu12e		✓	✓	✓						Aminopeptidase of the M20 family
C42C1.5	000596	C_530010	CG1129	GMPPB	Amigo3		✓	✓				✓			GDP-mannose pyrophosphorylase
C50B6.2	009649	C_600061		NASP	Nasp		✓	✓		✓					Cell-cycle regulated, histone-binding protein
cca-1		C_10217	Ca-lpha1T	CACNA1I	022416		✓		✓	✓					Voltage-dependent T-type calcium channel
F13H8.2	005068	C_150183	CG8064	WDR3	Wdr3		✓	✓				✓			WD-repeat protein 3
bas-2	006533	C_410115	Hn	PAH	Pah		✓	✓		✓					Phenylalanine-4-hydroxylase
Y37E11AR.3		C_760027		NP_945341.2	AB112350		✓	✓		✓					Phospholipase D
kin-1	006522	C_50062	Pka-C1	PRKACA	Prkaca		✓	✓				✓			cAMP-dependent protein kinase
hpd-1	005482	C_1530029	CG11796	158104	029445			✓	✓			✓			4-hydroxy-phenylpyruvate dioxygenase

Table 1 (*Continued*)

C. elegans	*C. intestinalis*	*C. reinhardtii*	*D. melanogaster*	*H. sapiens*	*Mus musculus*	1	2	3	4	5	6	7	8	9	Annotation
ZK328.7	000589	C_270146		123607	032514			✓		✓		✓			TPR-domain-containing protein
		C_30061		158023	029442					✓		✓		✓	Uncharacterized WD40 repeat-containing protein
	005655	C_1160041		NP_149115.1	4933417K04Rik					✓		✓		✓	Uncharacterized conserved protein
		C_340102	CG30268	CT026_human	048201					✓		✓	✓		Uncharacterized conserved protein
	005129	C_370121	CG13855	164675	046192					✓		✓		✓	Uncharacterized conserved protein
	002323	C_10057	CG13693	103021	036598					✓		✓		✓	Uncharacterized conserved protein
	003918	C_650031		161973	045915					✓		✓		✓	Uncharacterized conserved protein
	003219	C_290056	CG16719	CT028_human	4931426K16Rik					✓	✓		✓		Uncharacterized conserved protein
		C_1020054		GSTP1	060803					✓	✓	✓			Glutathione S-transferase
	001158	C_460082		184154	064307					✓		✓		✓	Leucine-rich repeat-containing protein
	005086	C_450119		AAT1_human	022805					✓		✓	✓		AMY-1(c-myc-binding protein)-associating protein
	006719	C_80172		151023	026679					✓		✓		✓	Enkurin; CaM/TRPC-channel-binding protein
	007810	C_30068		ZMYND12						✓	✓			✓	Zn-finger MYND-domain-containing protein
		C_2010002	CG16984	124074	013155					✓		✓		✓	Uncharacterized conserved protein
	009579	C_180190		RABL4	016637						✓	✓		✓	Rab-like GTPase 4
	002836	C_60170		MAK	021363						✓	✓		✓	Serine–threonine protein kinase MAK
	002829	C_1090038	CG8362	NME7	Nme7						✓		✓	✓	Nucleoside-diphosphate kinase
	005629	C_630058	CG10064	NP_659491.2	1700019F09Rik	✓						✓		✓	Novel WD-repeat-containing protein

[a]Ensembl accession numbers of the genes from the corresponding organism are shown for *Caenorhabditis elegans*, *Ciona intestinalis*, *Chlamydomonas reinhardtii* and *Drosophila melanogaster*.
[b]The genomic and bioinformatic studies are as follows: 1, Avidor-Reiss *et al.* [23]; 2–3, Blacque et al. SAGE and X box, respectively [19]; 4, Efimenko *et al.* [40]; 5, Li *et al.* [18], and the proteomic studies include 6, Ostrowski *et al.* [58]; 7, Pazour *et al.* [62]; 8, Smith *et al.* [60]; 9, Stolc *et al.* [53].

motifs – which are highly represented in known IFT proteins [12,18] – were uncovered in three separate studies.

Although there is a distinct advantage to using different organisms and experimental approaches to identify interesting ciliary genes, even genes uncovered in one or two studies or in a single organism could be of utmost interest; for example, the Bardet–Biedl syndrome *BBS9* gene is conserved in ciliated organisms [45], however, it was only identified in the *C. elegans* SAGE and X-box studies. It is also important to note that the ciliary proteome, or ciliome, represents a compilation of many different putative ciliary proteomes, representing several species and, as such, is probably enriched for common ciliary proteins but is incomplete owing to the limitations of each experimental approach used and the types of cilia represented.

We note that although basal bodies have important roles in the biogenesis of cilia and seem to be a common resting place for many ciliary proteins, we did not include the basal body proteomic analysis performed by Keller *et al.* [68] as one of our ciliary data sets because it is not specifically enriched for proteins that reside within the ciliary organelle. For example, none of the motor components, IFT subcomplex A/B proteins, and BBS proteins was uncovered. One potential reason is that IFT machinery components concentrate at the base of the cilium on the transitional fibers (Figure 1; [12]), which might not have been co-purified with basal bodies before proteomic analysis. Nevertheless, the basal body proteome (and other centrosomal proteome studies; [69]) uncovered several proteins seemingly important for the function of cilia that are associated with probable basal body and/or ciliary disorders, such as the oral-facial-digital type 1 (OFD1) syndrome, the nephronophthisis (NPHP)-associated disorders Joubert and Senior-Loken syndromes, and Alstrom syndrome (ALMS1 protein) (Box 1; reviewed in Refs [48,70]). These ciliopathy-associated proteins are listed in Table 1 in the supplementary material.

Concluding remarks

Historically, the most recognizable and well-studied cilia were involved in motility processes, and they have been associated for ~30 years with several disorders including infertility, respiratory problems and hydrocephalus. Non-motile (sensory) cilia are now capturing an increasing amount of attention given their near ubiquity, participation in a variety of physiological functions and links to many different ailments, including obesity, kidney disease, retinal degeneration, neurosensory defects and developmental anomalies. Our compilation and cross-comparison of several ciliary proteome data sets can now be used to focus on previously unrecognized, putative ciliary proteins for functional characterization. In addition, our compilation represents a useful resource for researchers interested in identifying and studying new genes implicated in known or suspected ciliopathies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.tig.2006.07.006.

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