Review

Cell PRESS

Intraflagellar transport and the generation of dynamic, structurally and functionally diverse cilia

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Cilia are organelles that project from most eukaryotic organisms and cell types. Their pervasiveness stems from having remarkably versatile propulsive and sensory functions, which in humans are recognized to have essential roles in physiology and development. Underappreciated, however, are their diverse ultrastructures and typically bipartite organization consisting of doublet and singlet microtubules. Moreover, the overall shapes of the membrane-ensheathed cilia are varied, as exemplified by differences between hair-like olfactory cilia and rod- or cone-shaped photoreceptor connecting cilia-outer segments. Although cell-specific transcriptional programs are evidently crucial in establishing ciliary morphological specialization, few players directly involved in generating such diversity are known. Recent findings suggest that at least two molecular motors (kinesin-II and OSM-3/KIF17) can differentially mobilize the intraflagellar transport machinery required for ciliogenesis and, presumably, different cargo to help generate dynamic, structurally and functionally distinct cilia.

Evolution, prevalence and functions of eukaryotic cilia

A remarkable feature that distinguishes eukaryotes from prokaryotes is the presence of a ubiquitous intracellular trafficking system consisting of actin and tubulin cytoskeletal tracks and associated myosin, kinesin and dynein molecular motors. This transport system has empowered eukaryotic cells with the ability to move proteins, organelles and vesicles to their sites of action in a specific and regulated fashion. Other key players, including an expansive superfamily of small GTPases (e.g. Rab, Arf and Arl proteins), emerged early to control membrane transport events [1]. Together, this ensemble of proteins is postulated to have central roles in the origin of eukaryotic endomembranes, the nucleus, mitosis, endocytosis and phagocytosis [2].

The ancestral trafficking system paved the way for an equally important eukaryotic cell invention, the cilium. This microtubule-based organelle, endowed with propulsive and/or sensory functions, is usually built and maintained by intraflagellar transport (IFT; see Glossary) – a process that uses kinesin anterograde molecular motor(s) to mobilize ciliary cargo (e.g. structural components, membrane receptors) within cilia and cytoplasmic Dynein 1b to recycle components back to the base [3–7]. The usefulness of the motility and sensory functions of cilia might well have accelerated the divergence of eukaryotes from prokaryotes and subsequently facilitated diversification in metazoan lineages. Indeed, the functional plasticity of cilia is phenomenal and is exploited by numerous cell types in animals. Motile cilia (flagella) propel gametes and generate flow across various surfaces; non-motile (primary) cilia are implicated in virtually all known sensory processes, including chemosensation, mechanosensation and photosensation [8–10]. The signaling (e.g. Hedgehog, Wnt, platelet-derived growth factor receptor [PDGFR] alpha) roles of cilia are crucial for vertebrate development and anomalies in these functions engender

Glossary

Ciliary axoneme: the axoneme represents the microtubule-based structure and associated paraxonemal components that emanates from the basal body. It is surrounded by a ciliary matrix (or 'cytosol') and fully enclosed within a ciliary membrane.

Ciliary microtubule nomenclature: the tubulin-based ciliary axoneme consists of an axial array of microtubules (e.g. 9) occurring as doublets (×2) or singlets (×1), and characterized by the presence (+2) or absence (+0) of a central pair of microtubules. For example, the proximal ciliary segment (closest to the basal body) of a canonical motile cilium is normally described as 9×2+2, whereas a non-motile cilium is most often $9 \times 2 + 0$. This segment is often followed by a distal segment that displays singlet microtubules (e.g. $9 \times 1 + 2$ or $9 \times 1 + 0$). The basal body typically consists of triplet microtubules (9×3).

Ciliopathies: a class of human disorders characterized by defects in basal body and/or ciliary function. A growing list of ciliopathies with considerable genetic and phenotypic overlap includes BBS, Alström syndrome (ALMS), Meckel syndrome (MKS), Joubert syndrome (JBTS), Jeune asphyxiating thoracic dystrophy (JATD), Oral-facial-digital type I syndrome (OFD1), Senior-Løken syndrome (SLSN), Ciliary diskenesia, primary (PCD), nephronophthisis (NPHP), and polycystic kidney disease (PKD) [8–15].

Intraflagellar transport (IFT): IFT represents the movement of 'IFT trains' that simultaneously contact the microtubule axoneme and ciliary membrane. Each 'train' is made up of several 'IFT units' that consist of at least one active kinesin or dynein motor associated with large multi-protein assemblies termed IFT subcomplexes A and B. The IFT subcomplexes collectively contain ~20 different protein subunits [4,5,7]. Other IFT-associated components include BBS proteins and ciliary cargo [14].

Molecular motors: the two microtubule-based classes of motors that mobilize cargo along the ciliary axoneme are the ATPases kinesin and dynein [4–7]. Heterotrimeric kinesin-II consists of two kinesin subunits (KIF3A and KIF3B in humans) and a kinesin-associated protein (KAP), whereas OSM-3/KIF17 is a homodimeric kinesin. These kinesins move in the anterograde direction (from basal body to the tip of the cilium). The primary ciliary retrograde motor (which recycles components back to the base of cilia) is cytoplasmic dynein 1b, a dynein heavy chain subunit that assembles with at least two other components, namely a dynein light intermediate chain and a dynein light chain.

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0962-8924/$ – see front matter doi:10.1016/j.tcb.2009.04.002 Available online xxxxxx

several human disorders (ciliopathies), such as Bardet–Biedl syndrome (BBS), which collectively affect nearly all tissues and organs [8–15].

Jékely and Arendt [2] have speculated that a ciliary precusor initially arose in the ancestral eukaryote as a means to concentrate receptors and signaling components at a membrane patch or small bud, a process requiring cytoskeleton-associated vesicle trafficking. Eventually, elongation of this cellular microdomain, supported by IFT and a centriole-based microtubule-organizing center (basal body) positioned at the plasma membrane enabled true compartmentalization of sensory-signaling machinery. During or soon after the emergence of the ciliary axoneme, motility was achieved by incorporation of dynein and other components along its length. Hence, the ancestral eukaryote is surmised to have possessed a single motile, sensory cilium and few eukaryotic lineages, such as most land plants and fungi, secondarily lost their cilia [16].

Bioinformatic, genomic and proteomic studies jointly point to the existence of several hundred basal body and ciliary proteins [17,18], providing a fairly complete molecular parts list for understanding the motility, sensory and signaling functions of cilia. However, the key players specifically responsible for assembling different cilia types remain largely unknown. Based on recent evidence that we discuss here, it seems as if the conserved IFT machinery itself, assisted by regulatory factors, could be largely responsible for selectively transporting the cargo needed to generate dynamic, structurally and functionally distinct cilia.

The 'canonical' basal body – cilium ultrastructure includes microtubule singlets

Cilia exhibit two immutable commonalities; they nucleate from a basal body, a centriole-based structure also found in centrosomes and typically characterized by a ring of nine triplet microtubules (9×3), and they possess a microtubule-based axoneme (Figure 1a; see also ciliary microtubule nomenclature). Two omnipresent features are the transitional fibers (alar sheets), which emanate from the distal end of the basal body and contact the ciliary membrane, and a region adjoining the basal body termed the transition zone (Figure 1a). Also ubiquitous are doublet microtubules that initiate at the transition zone, extending from basal body A–B tubules (Figure 1a). In most motile cilia, a central pair of microtubules arise from the transition zone to give rise to the textbook example $9 \times 2 + 2$ microtubule arrangement; these are normally absent in non-motile cilia, which usually display a $9 \times 2 + 0$ microtubule structure (Figure 1a). Motile cilia microtubules also possess accessory structures – outer and inner dynein arms, radial spokes and central pair projections – that enable movement.

In some cilia, doublet microtubules form the majority of the axoneme. However, in a large proportion of motile and non-motile cilia, the distal end is built solely from A-tubule extensions (Figure 1a). These distal singlet microtubules (9×1) occur in unicellular organisms, such as *Tetrahymena* and *Chlamydomonas* [19,20], and in invertebrates, including *C. elegans* [21,22] and *Drosophila* [23]. In vertebrates, distal microtubule singlets are observed in cilia from most cell types, including liver, pancreas, kidney, olfactory epithelia, oviduct, respiratory tissue, and in rod or cone photoreceptors [24–29]. A bipartite axoneme consisting of microtubule doublets followed by singlets therefore represents an evolutionarily conserved feature of motile and non-motile cilia.

Diversity in cilia morphologies and ultrastructures

The most common motile and non-motile ciliary structures resemble a whip or slender rod. A variation on this theme is length, a characteristic regulated by IFT [30,31]. In vertebrates, cilia typically range from ~3–10 μm for many non-motile (e.g. kidney) cilia and motile (e.g. respiratory) cilia, to 50–150 μm for sperm flagella and ~200 μm for olfactory cilia (Figure 1b). An astounding several centimeters is reached in spermatozoa tails of some *Drosophila* species [32]. Interestingly, this latter cilium is assembled in an IFT-independent manner within the cytosol [33,34], an idiosyncrasy that could conceivably be used to shed light on the very functions of IFT.

Released from the functional constraints of having to propel fluids, primary cilia from metazoans show the greatest variety of morphological specializations. A primary example of this diversity is found in vertebrate rod and cone photoreceptor cells. Here, the inner segment, in which protein synthesis takes place, is followed by a narrow constriction – the connecting cilium. This terminates in a highly differentiated outer segment in which the axoneme anchors an elongated cylinder-shaped network of stacks containing opsins and signaling molecules (Figure 1b). The evolutionarily related non-visual photoreceptor of the pineal organ exhibits a similar structure [35]. In *C. elegans*, 60 of its 302 neurons have ciliated dendritic endings with shapes that show remarkable variability (Figure 1b). These include monociliated and biciliated rod-shaped forms (channel cilia) and some with elaborate wing- or finger-like membraneous appendages (e.g. AWA/B/C and AFD neuron cilia). *Drosophila* also sports varied cilia that function in mechanosensation and olfaction [23,36]. Some cilia exist as higher-order structures that probably reflect functional specialization. For example, campaniform sensilla of insect legs consist of ~500 axoneme bundles that stem from a single basal body region and are attached at their distal ends to the cuticle, forming a funnel-shaped structure (Figure 1b); these cilia were shown to act as impulse-generating mechanoreceptors – possibly the first demonstration that cilia transduce sensory information [37].

Variations in axonemal ultrastructure are observed across species and within organisms that contain different cilia types. Permutations include the absence or presence of one or more central pairs that are not necessarily correlated with motility. For example, nodal cilia lacking central pairs ($9 \times 2 + 0$) are motile and non-motile inner ear kinocilia possess a central pair ($9 \times 2 + 2$) [10]. Remarkably, these two axonemal ultrastructures, along with an atypical $9 \times 2 + 4$ structure, are all simultaneously present on the notochordal plate of rabbit embryos [38]. In *C. elegans* and *Drosophila*, some axonemes deviate from a symmetric circular pattern, have variable numbers of

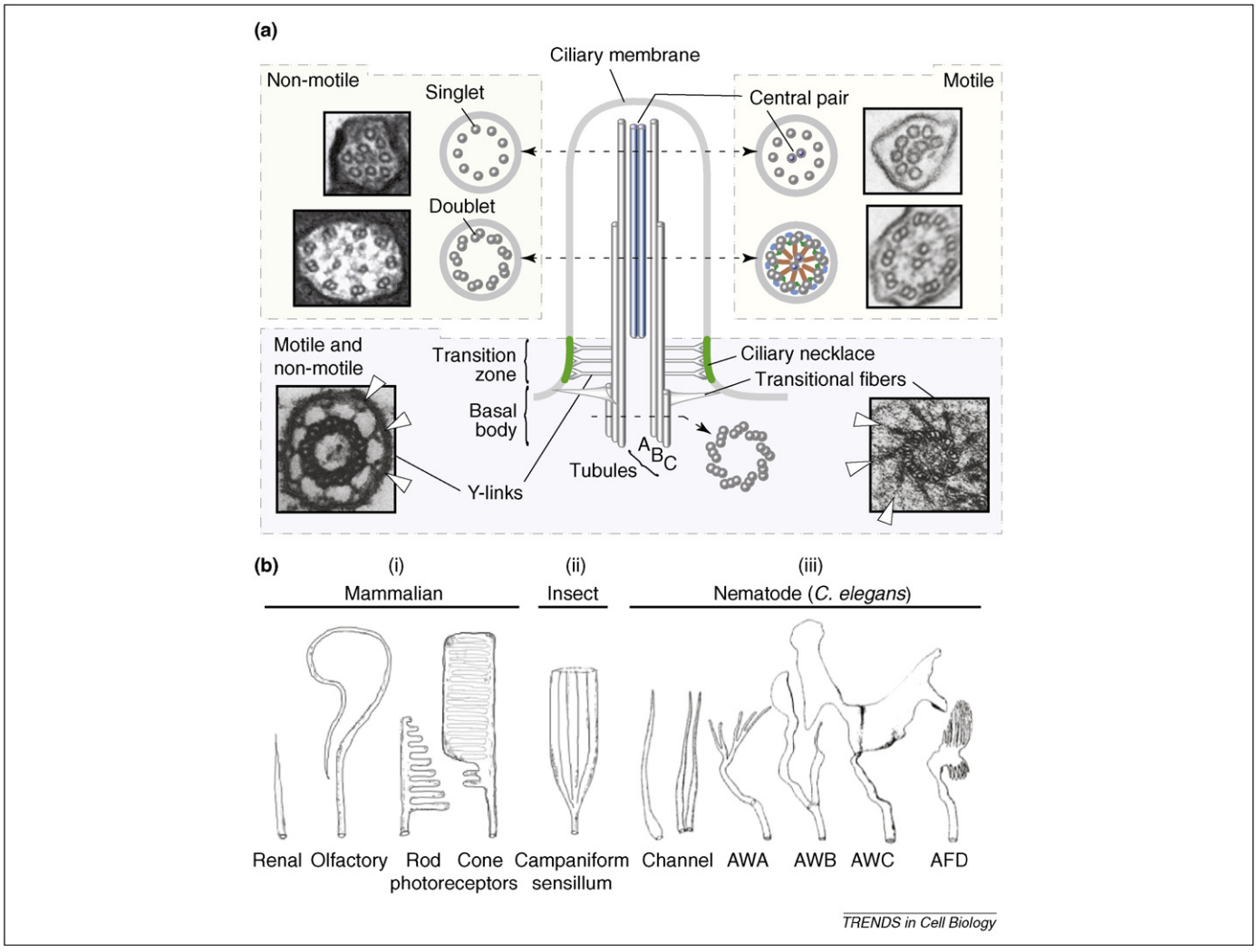

Figure 1. Canonical motile and non-motile ciliary ultrastructures and different cilia morphologies. **(a)** Ubiquitous basal body-ciliary ultrastructures. The basal body region is normally built from triplet microtubules, labeled A, B C. Transitional fibers, which emanate from the distal end of the basal body and contact the ciliary membrane, are shown schematically and in a transmission electron micrograph (TEM) cross-section of an *Elliptio* (mussel) cilium. Just above, the transition zone region is characterized by Y-shaped links that emanate from the microtubule axoneme and also contact the membrane. These are shown schematically and in a TEM cross-section of a monkey oviduct cilium. Some transitional fibers and Y-links are shown by arrowheads in the TEM micrographs. Together, these two membrane-contacting structures form a ciliary (flagellar) pore complex that restricts vesicle and perhaps protein entry into the organelle. Microtubule doublets in the proximal (middle) segment of cilia (stemming from A and B tubules) and singlets in the distal segment (A tubules) are depicted with or without central pair microtubules, respectively. Representative cross-section TEM micrographs from non-motile (*C. elegans*) or motile (*Tetrahymena*) cilia are also shown on the left and right, respectively. Ultrastructures typically found in non-motile, motile or both cilia types are indicated. TEM micrographs of motile cilia are adapted, with permission, from Ref. [19]; micrographs from *C. elegans* cilia are adapted, with permission, from Ref. [99]; micrographs showing Y-links are adapted, with permission, from Ref. [55]; micrographs of transition fibers are adapted, with permission, from Ref. [100]. **(b)** Examples of different ciliary morphologies. Schematics showing: (i) various mammalian cilia (renal, olfactory, photoreceptor rod and cone); (ii) insect campaniform sensillum mechanosensor; (iii) different *C. elegans* cilia types (representative rod-shaped channel cilia, AWA, AWB and AWC cilia with membraneous elaborations, and the thermosensory AFD cilium with finger-like projections). Basal bodies are at the very base of the different ciliary structures. *C. elegans* cilia drawings are adapted, with permission, from Ref. [22].

doublet and singlet microtubules (supernumerary microtubules are not attached to the basal body), possess cross-bridges between microtubules (e.g. as in *C. elegans* OLQ cilia) or are interrupted in their continuity with an elaboration (e.g. as with the *Drosophila* 'ciliary dilation' found in chordotonal cilia) [21,23]. As discussed later, evidence for the participation of IFT in generating some of the types of aforementioned ultrastructural and morphological variations is starting to accumulate.

Regulation of ciliogenic gene expression and establishment of ciliary diversity

The nature of the cellular programmes required to orchestrate the assembly of canonical cilia overlaid with cell-specific variations and functions are largely undiscovered. The most general ciliogenic instigator is the RFX transcription factor (TF), the role of which in directing ciliary gene expression and ciliogenesis was first discovered in *C. elegans* [39] and is now demonstrated in *Drosophila* [40], zebrafish [41] and mouse [42,43]. Although this is crucial in upregulating genes implicated in IFT (e.g. core IFT and BBS components), it is not present in many ciliated protists and probably cooperates with other factors to specify ciliary diversity. Several of these ciliary fate determinants have been uncovered at this point; they include the Forkhead domain TFs FoxJ1, also known as HFH-4 in vertebrates [44,45] and FKH-2 in *C. elegans* [46], the *C. elegans* zinc finger TFs CHE-1 and EGL-46 [47,48], and the murine homeobox protein *Noto* [49]. Their importance is exemplified by the finding that a single OTD/OTX-type

homeodomain transcription factor, TTX-1, is largely sufficient for specifying the conversion of a rod-type cilium into a highly differentiated AFD-like thermosensory cilium in *C. elegans* [21,50]. To what extent such factors contribute specifically to ciliary fate determination, as opposed to the general specification of cell fate, remains to be seen. Furthermore, although most targets of the RFX TF might be known [18], it will be important to identify the accessory TF factors that help to define ciliary fates, for example via transcriptional profiling of different ciliated cells (e.g. see Refs [51–53]). Once uncovered, the combined roles of the target proteins in ciliogenesis – and potential dependence on IFT for regulated entry into cilia – can be dissected.

A gated entry into the cilium tended by the IFT machinery?

The principle *raison d'être* for cilia is to concentrate, in an extracytoplasmic space, the machinery responsible for motility and/or the transduction of extracellular chemico-physico information. To achieve this, ciliary proteins (many of them vesicle-associated) must be targeted from the Golgi to, or near to, the basal body and then preferentially transported into the organelle. Concurrently, entry of non-ciliary proteins could also be curtailed. The existence of such a ciliary gate, or flagellar pore complex, is supported by detailed ultrastructural analyses of the basal body-transition zone region [54]. A probable element of the gate is the spiral array of transition fibers that connect the basal body to the ciliary membrane, in proximity to where vesicle fusion occurs (Figure 1a). Additional axoneme-to-membrane attachments (Y-shaped links) occur within the transition zone (Figure 1a). Both the transition fibers and Y-links collectively prevent vesicle entry and could in principle form a molecular sentinel for ciliary protein entry. Y-links also seem to organize annular membrane decorations, which together form the so-called ciliary necklace [55]. This 'necklace' could help demarcate the plasma membrane from the ciliary membrane, ostensibly forming a diffusion barrier for membrane proteins (Figure 1a). In neurons, the axon initial segment might serve a similar purpose [56]. A possible functional parallel between the ciliary gate and nuclear pore complex (NPC) is also immediately apparent; indeed, it should be explored given that a core NPC component which interacts with Ran to control nucleocytoplasmic shuttling, importin-β, is found at centrosomes and cilia and is required for ciliogenesis [57]. Perhaps most revealing, however, is that immunogold staining of *Chlamydomonas* IFT52, an IFT protein, supports the notion that IFT particles dock precisely at the tips of the transition fibers [58]. Similar IFT protein localization is also observed at *Xenopus* photoreceptor basal bodies [59]. Hence, the IFT machinery is ideally positioned to accept cargo and facilitate its entry into the cilium, a controlled process that could be specific for different ciliary types.

Mobilizing proteins to build and maintain diverse cilia: the IFT machinery

Discovered in 1993 by Kozminski and colleagues [3], IFT is largely believed to represent a general ciliary component transport mechanism that could adjust to the requirements of different cilia types. To date, however, few *bona fide* IFT-associated cargo components have been uncovered [4]. Several candidates are known based on their co-immunoprecipitation with core IFT components or failure to localize to cilia when IFT proteins or motors are disrupted. Examples of these include tubulin [36,60], motile cilium-specific radial spokes and axonemal proteins [60], *Chlamydomonas* cGMP-dependent protein kinase [61], mammalian cGMP-activated channel CNGB1b [62], *Chlamydomonas* blue-light sensitive receptor [63], photoreceptor opsin and arrestin [64], and Hedgehog signaling proteins [12]. Genuine IFT-like movement of even fewer proteins – namely the *C. elegans* TRPV channels OCR-2 and OSM-9, and *Chlamydomonas* PKD2 – have been observed [65,66]. BBS proteins, all of which undergo IFT in *C. elegans* [67–69], are unlikely to be 'cargo' deposited in cilia; rather, they probably function as adaptors for the transport of ciliary proteins, such as somatostatin receptor 3 (SSTR3) and melanin-concentrating hormone receptor 1 (MCHR1) [70]. The IFT-associated protein 2 (IFTA-2) RAB small GTPase could also be in such a category [71]. Of course, there is no *a priori* reason why IFT must be invoked for transporting most ciliary proteins; for example, movement of GFP-tagged *C. elegans* PKD-2 could not be detected [65]. However, the mammalian polycystic kidney disease (PKD)-associated protein interacts with kinesin-II and IFT protein subunits [72] and a small fraction of the *Chlamydomonas* PKD2 orthologue was subsequently shown to undergo IFT [66]. Elucidating whether IFT is required for transporting most or all ciliary components will prove to be a challenging, yet important, question.

Just as most IFT cargoes remain to be discovered, the mechanism(s) responsible for cargo transport by IFT trains (alternatively referred to as IFT rafts or barges), which contact the ciliary membrane directly [3], remains enigmatic. Cargo could in principle interact with motor(s), or the multi-subunit IFT subcomplexes A and B, or other IFT-associated components (e.g. BBS proteins) (Figure 2a); however, aside from the potential interaction of IFT46 with outer dynein arms [73], there is presently little molecular evidence of direct cargo attachment to any particular IFT-associated protein. In principle, some membrane proteins could be mobilized via bulk membrane transport by the IFT machinery. Very indirect evidence for this is the saltatory movement of the *C. elegans* TRPV channels OSM-9 and OCR-2 within cilia [65] – which hints at a loose association with IFT trains – and the similarly discontinuous movement of glycoprotein-associated microspheres on a ciliary surface [74]. Another possible indication of this phenomenon is provided by the IFT-like distribution of detergent-resistant membrane patches (i.e. lipid rafts) observed in Trypanosome cilia [75].

As first proposed by Avidor-Reiss and colleagues [36], it is probably evolutionarily and thus functionally meaningful that several IFT subcomplex A and B proteins possess the same apparently unique predicted domain structure – WD40 repeats (β-propeller) followed by TPR or solenoid repeats – found in α-COP, β′-COP and clathrin heavy chain proteins, which are indispensable coated vesicle (membrane) trafficking components. We surmise that BBS proteins, several of which are predicted to contain either a β-propeller domain (BBS1, BBS2 and BBS7) or TPR

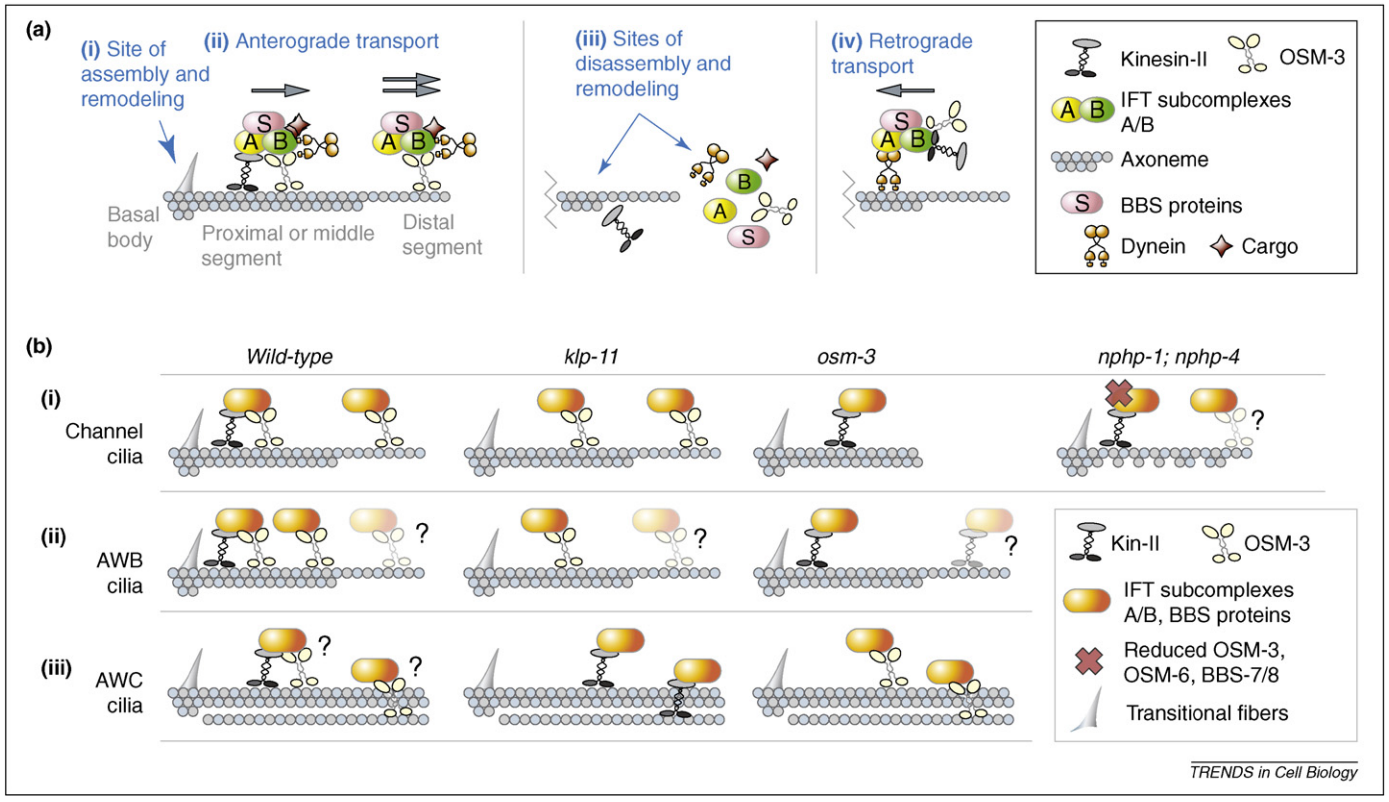

Figure 2. Distinct mechanisms of IFT in different cilia. **(a)** Model depicting a 'canonical' mechanism of IFT on a bipartite cilium consisting of a proximal (middle) segment with doublet microtubules, and a distal segment with singlet microtubules. (i) Assembly or remodeling of anterograde IFT particles occurs in the basal body region, perhaps specifically at the transitional fibers (tf). (ii) In *C. elegans* channel cilia, two kinesin motors (kinesin-II and OSM-3) function coordinately in the middle segment to transport the IFT subcomplexes A/B, BBS proteins and cargo; OSM-3 operates alone in the distal segment. Gray arrows represent direction of movement and relative speed; the kinesin-II/OSM-3 motors move together at ~0.5 μm/sec in the middle segment, whereas OSM-3 moves at ~1.3 μm/sec in the distal segment. In animals with disrupted BBS proteins (shown as 'S'), IFT subcomplex A and B components move separately in association with kinesin-II and OSM-3 motors, respectively [69,83]. (iii) Sites of kinesin motor deactivation occur between the middle and distal segments and the tip of the cilium; remodeling of the IFT machinery, probably through the disassembly of complexes, also includes activation of dynein in preparation for retrograde transport. (iv) Dynein-mediated retrograde transport recycles components back to the base of the cilium. In cilia lacking an extended stretch of singlet microtubules, the same principles apply except that remodeling occurs only at the end of the doublet microtubules and might not involve an OSM-3/KIF17 motor. **(b)** Variations in IFT mechanisms for different *C. elegans* cilia. (i) IFT in channel cilia. Although kinesin-II and OSM-3 cooperatively transport IFT subcomplexes A/B in the middle segment, only OSM-3 operates in the distal segment and its function is sufficient for building full-length cilia in the kinesin-II (*klp-11*) mutant. Cilia without OSM-3 function (*osm-3* mutant) lack distal segments. Disruption of nephrocystins (*nphp-1;nphp-4* double mutant) results in ciliary structure anomalies (shown by breaks in the microtubules), a shortened axoneme and reduced levels of OSM-3, OSM-6 and BBS proteins (BBS-7/8) in the cilia. (ii) In AWB cilia, kinesin-II and OSM-3 are partly decoupled, transporting a portion of subcomplexes A/B separately, and OSM-3 motility is infrequent in the distal segment. Full-length cilia can be assembled in the absence of either Kinesin-II (*klp-11* mutant) or OSM-3 (*osm-3* mutant). Ghosted particles indicate a possible reduced presence of a given IFT particle; question marks indicate uncertain transport profiles for the IFT particles. (iii) In AWC cilia, which contain doublet and singlet microtubules running the length of the ciliary axoneme (shown schematically with separate doublet and singlet microtubules), the *klp-11* or *osm-3* mutants both exhibit essentially full-length cilia. The transport profiles of the two kinesin motors within this type of cilium are not known (indicated by question marks).

repeats (BBS4, BBS8), could assemble to form comparable topologies – essentially as is observed for two interacting NPC components, Nup85 and Seh1 [76]. Indeed, these five BBS proteins are isolated as a complex in mammalian cells [77] and, consistent with this, several BBS proteins depend on each other for IFT in *C. elegans* [69]. The IFT–BBS machinery could therefore have evolved the ability to bind and traffic proteins embedded not within vesicles, but rather, in the nearly curve-free ciliary membrane environment.

Building diverse, dynamic cilia using IFT

Thus, it seems that the IFT machinery is ideally suited for not only providing the fundamental building blocks of cilia but potentially also the specific components required for generating their ultrastructural and morphological diversity. Results from studies in *C. elegans* in particular, but also zebrafish and *Chlamydomonas*, are providing excellent insight into how kinesin-2 motors (heterotrimeric kinesin-II and homodimeric OSM-3/KIF17) have important roles in this process and how it is regulated at many levels, including the basal body.

Roles of two C. elegans kinesin-2 motors in creating a 'canonical' bipartite cilium

In ciliated protists, such as *Chlamydomonas* and *Tetrahymena*, and in mouse kidney, disruption of kinesin-II abrogates ciliogenesis [5–7,79]. Intriguingly, ablation of *C. elegans* kinesin-II subunits was found to have little effect on ciliary structures or chemosensory capabilities of the organism, whereas disrupting the homodimeric OSM-3 kinesin, not previously implicated in ciliogenesis, caused channel cilia structure defects and chemosensory anomalies [5]. These surprising findings are now explained by the fact that in rod-shaped cilia, kinesin-II and OSM-3 act redundantly and coordinately in middle (proximal) segments to mobilize IFT particles, but only OSM-3 operates in distal segments (Figure 2a). Thus, the *osm-3*

mutant lacks distal segments and exhibit ciliary phenotypes, whereas removing kinesin-II (*klp-11* mutant) is tolerated when OSM-3 is present (Figure 2b). Importantly, as described later, variations on this dual-kinesin ciliogenic mechanism exist in different cilia types of *C. elegans* and metazoans.

Distinct mechanisms of IFT in different cilia

Most chemosensory cilia in *C. elegans* (channel cilia) display typical rod-like forms (Figure 1b). To shed light on how morphologically different cilia are assembled, the Sengupta laboratory [46] analysed the IFT mechanism in wing-shaped AWB neuron cilia (Figure 1b). Similar to channel cilia, both kinesin-II and OSM-3 kinesin movement is readily observed in the middle segment (doublet microtubules), and OSM-3 motility can be detected (albeit infrequently) in the distal segment (singlets) (Figure 2b). In contrast to the rod-shaped channel cilia, however, the AWB cilia kinesin-II and OSM-3 motors are partially uncoupled and can transport cargo separately at their own slow (0.5 μm/sec) and fast (1.3 μm/sec) velocities, respectively. Also unlike in rod cilia, kinesin-II or OSM-3 alone can assemble middle and distal segments. Given these observations, it is proposed that a subset of IFT particles in which motility relies exclusively on OSM-3 (or Kinesin-II) could conceivably transport distinct cargo proteins in AWB cilia [46]. Interestingly, an AWB neuron-specific target of the FKH-2 TF is KAP-1, a kinesin-II subunit, suggesting cell-specific modulation of the IFT machinery. Studying another morphologically unique cilium, from the AWC neuron (Figure 1b), the Scholey laboratory found similar redundancy in kinesin-II and OSM-3 function; disruption of either motor does not seemingly affect formation of doublet or singlet microtubules, which, unlike the bipartite rod cilia, run side-by-side the length of the organelle (Figure 2b) [80]. However, when AWC cilium function was specifically tested in assays for chemotaxis towards the odorant benzaldehyde, animals lacking OSM-3 had normal olfaction but those with mutations in either of two different kinesin-II subunits were impaired.

Together, the comparative studies of *C. elegans* IFT are beginning to suggest that modulating the activities of two anterograde motors might be required for the specific structural and/or functional requirements of distinct cilia, ostensibly because of their differential trafficking of sensory–signaling components. Similar conclusions were drawn by studying *Tetrahymena* Kin5 (OSM-3/KIF17); its function might specifically be required to build ciliary distal ends and is needed for the transport of a specific cargo molecule (guanine exchange factor Gef1) [81]. This principle probably also holds true in vertebrates, which typically possess bipartite cilia and have OSM-3/KIF17 in addition to kinesin-II (Box 1). A wide open question, with only a few tangible hints at an answer, is whether additional kinesin family members participate in IFT and help establish ciliary diversity by mobilizing discrete cargo (Box 2).

Regulation of IFT as a means to regulate cargo entry or movement in cilia

The assembly of anterograde IFT particles at or near to basal body transitional fibers and their subsequent movement onto the ciliary axoneme is probably coordinated with the selective loading of cargo. Hints of how this process could be regulated are starting to emerge. Recently, the Barr laboratory used *C. elegans* to uncover a potential role for two transition-zone-localized nephrocystin proteins, NPHP-1 and NPHP-4, in this process [82]. First, it was found that their disruption causes cilium-specific ultrastructural defects of widely differing severity, suggesting that the proteins perform distinct functions at the base of different types of cilia. Second, by observing the behaviors of several GFP-tagged ciliary proteins in wild-type and *nphp-1* or *nphp-4* mutants, the loss of these nephrocystins was observed to preferentially affect the proper assembly and ciliary motility of an IFT subcomplex B component (OSM-6/IFT52), OSM-3 kinesin, and the two BBS proteins

Box 1. The KIF17 (OSM-3) homodimeric kinesins: important roles in cilium development

The *C. elegans* kinesin OSM-3, part of the kinesin-2 family, has an essential role in assembling ciliary distal segments and, as discussed in this article, probably complements the functions of the other kinesin-2 family member (heterotrimeric kinesin-II) by assisting with the selective trafficking of cargo in distinct cilia [4,5]. Interestingly, most functions ascribed to the mammalian orthologue, KIF17, relate to dendritic trafficking. Hirokawa and colleagues first cloned and characterized KIF17 as a plus-end-directed, homodimeric, microtubule-based motor capable of selectively transporting the NR2B glutamate receptor subunit into the dendrites of pyramidal neurons [101]. Several other essential dendritic cargoes require KIF17 for proper localization, including the Kv4.2 K^+ channel, mRNA granules containing nuclear RNA export factor 2 (NXF2) and Staufen, and the developmentally regulated gene product Spatial [102–104]. Notably, targeting the glutamatergic GluR5 receptor to distal dendrites relies on KIF17 [105]. Together, the neuronal functions of KIF17 are important for animal behavior because mice overexpressing this motor show enhanced learning abilities [106].

Only recently has the role of KIF17 in vertebrate ciliary physiology started to emerge. The Martens laboratory showed that mammalian KIF17 is present not just in the brain as reported previously, but also in MDCK cells and olfactory sensory neurons (OSNs) [62]. By expressing a dominant-negative KIF17 construct, the kinesin was shown to be required for the ciliary localization of the CNG channel CNGB1b. Interestingly, a comparative functional study suggested that KIF17 is not essential for building full-length cilia, whereas KIF3A of heterotrimeric kinesin-II is. A similar conclusion was drawn by the Besharse laboratory, studying zebrafish [107]. The fish KIF17 orthologue co-precipitates and co-localizes with IFT proteins, and is present in many ciliated tissues, including the pronephros (kidney) and olfactory placodes; however, morpholino knockdown suggests that is not required for the formation of all cilia. Instead, it is required for the proper localization of opsin in ciliary photoreceptor outer segments, as well as for the development of the photosensory organelle, which has pronounced singlet microtubule extensions. A possible role in mouse spermatogenesis is also suggested for KIF17b, a testis-enriched isoform of the kinesin which interacts and co-localizes with the protein Spatial in mature sperm tails (flagella) [108]. Interestingly, Spatial contains a C-terminal RVxP motif that is known to be required for CNG channel and polycystin-2 ciliary localization [62,109]. Together, these findings suggest that similar to *C. elegans* OSM-3 and *Tetrahymena* Kin5, vertebrate KIF17 performs IFT-associated functions that probably supplement or complement the functions of kinesin-II in the transport of specific ciliary cargo.

Box 2. Involvement of additional kinesins: expanding the repertoire of ciliary cargo?

Several other kinesins from various species have been implicated in ciliary functions, but functional data for these motors are mostly lacking. Recently, a comprehensive phylogenetic analysis of kinesins from diverse eukaryotes defined two new families, kinesin-16 and kinesin-17. The family members are expressed in organisms that make cilia and flagella, but are absent from organisms devoid of these organelles [79]. The only suggestive finding for these new kinesins is for human KIF12, of the kinesin-16 family. A potential ciliary function is suggested by the discovery that it is a candidate modifier of polycystic kidney disease [110], a well-established ciliopathy. Coincident with this finding, the expression of KIF12 is regulated by the transcription factor HNF-1, the disruption of which results in cystic kidney disease [111].

In addition to the well-described kinesin-2 family IFT motors, there are other kinesins with clear ciliary functions. *C. elegans* KLP-6, of the kinesin-3 family, is a cilium-localized motor required for the proper ciliary localization of polycystins, which act as mechanosensory ion channels in male-specific ciliated sensory neurons and are implicated in polycystic kidney disease [112]. Consistent with such a function, disruption of KLP-6 leads to male mating behavioral defects, although the overall development of the sensory cilia seems normal. Whether KLP-6 has a direct role in IFT or has another function, for example anchoring of polycystins in the cilia, is undetermined. Another cilium-associated kinesin is Klp1 (kinesin-9 family), which in *Chlamydomonas* regulates the rate of flagellar beating in addition to contributing to the structural integrity of the central pair [113]. Finally, kinesin-13 family members characterized in *Giardia* and *Leishmania* contain non-processive motors that act to depolymerize microtubules, thereby regulating ciliary length [114,115]; interestingly, the *Chlamydomonas* orthologue crkinesin-13 requires the canonical IFT pathway for its ciliary transport, and thus activity [116].

It will be of interest to further characterize the probable or putative ciliary functions of some of the kinesins mentioned earlier because they might represent a means to expand the repertory of ciliary cargo beyond that carried by kinesin-2 family members. Also, a common theme for kinesins is their ability to multitask; for example, kinesin-II and OSM-3/KIF17 function in IFT and other types of cellular transport in vertebrates [78,79]. Thus, given the large number of kinesins found in ciliated organisms, for example 19 in *C. elegans*, 24 in *Drosophila* and 44 in humans, it could be that additional kinesins will be found to possess cilium-specific functions.

tested (BBS-7/8). Given the probable role of BBS proteins in trafficking specific ciliary cargo in association with IFT subcomplexes A and B and the ciliary membrane [14,69,77,83], nephrocystins could have a general role in modulating the assembly of IFT–BBS particles loaded with cell-type specific ciliary cargo. Indeed, a murine *NPHP1* mutant was recently shown to develop retinal degeneration, not as a result of impaired photoreceptor cilium formation, but probably because of inefficient IFT-mediated sorting and/or transport of cargo into the outer segment [84] – similar to that found for a *BBS* mouse knockout [85].

Regulation of IFT trains probably also occurs at the ciliary tip, potentially within the so-called flagellar tip complex [86] where remodeling of the IFT machinery needs to take place; this includes kinesin deactivation, dynein activation and release of cargo, especially in actively growing cilia in which assembly occurs robustly at the tip. Few components that localize specifically at the ciliary tip are known but one of them, the microtubule end-tracking protein EB1, is known to functionally associate with an IFT subcomplex B subunit (IFT172) [87]. Anterograde movement from doublet to singlet microtubules also seems to be regulated, as a *C. elegans* kinase (DYF-5) functions to prevent kinesin-II entry into the distal segment [88]. Finally, post-translational modifications on axonemal tubulin – which include acetylation, polyglutamylation and polyglycylation – might be important for ciliary stability and function. Such modification of the axoneme, which could affect IFT motors in a manner similar to its impact on mammalian KIF1A kinesin in neurites [89], depends at least in part on the IFT machinery. Specifically, an IFT protein (DYF-1/fleer) that modulates the OSM-3 kinesin and is required for building distal segments in *C. elegans* [83] was also found to affect ciliary polyglutamylation in zebrafish and *C. elegans*, perhaps by helping to transport tubulin tyrosine ligase-like (TTLL) protein(s) [90]. The discovery of DYF-1, and unveiling of IFT-associated functions for nephrocystins, suggest that many cellular factors which regulate ciliary trafficking remain to be revealed (Box 3).

Dynamic changes to cilia are modulated by IFT

Ciliary resorption is an important, dynamic event observed before cell-cycle entry because centrioles are reclaimed to function as microtubule-organizing centers (MTOCs) for cell division in virtually all organisms [91]. The resorption process and, indeed, cilium length control, seems largely dependent on the IFT machinery [30,31]. Pan and Snell [92] provided mechanistic evidence that during *Chlamydomonas* cilia shortening, cargo is prevented from undergoing IFT-dependent anterograde transport, whereas IFT trafficking is upregulated to facilitate axonemal component recycling. Ciliary resorption also occurs in at least some terminally differentiated cells; for example, the solitary cilium (kinocilium) present at the apex of the actin-based stereocilia resorbs in cochlear hair cell bundles during development but persists in vestibular hair cells [93]. Another dynamic change to ciliary structure – a pronounced increase in length through the extension of singlet microtubules (Figure 3a) – is observed in *Chlamydomonas* mating gametes and strictly depends on IFT [20,61]. Whether this involves an OSM-3/KIF17 motor is unknown, although there are tantalizing biochemical changes to the IFT particles during this event, suggesting possible changes in composition [61].

New findings by Mukhopadhyay and colleagues demonstrate that the *C. elegans* AWB cilium alters its morphology – shortening its axoneme and expanding its membraneous elaborations – in response to decreased environmental cues sensed by the cilium [94]. The striking changes, shown in Figure 3b, are revealed when signaling is abrogated in various mutant strains (i.e. in the guanylyl cyclase *odr-1*, cGMP-gated channel *tax-4*, and G protein coupled receptor kinase *grk-2* mutants), and in the absence of odorant-rich food (*E. coli*). This remodeling probably serves to optimize the distribution of receptors and signaling molecules for olfactory reception. Interestingly, the adaptation mechanism depends on the IFT machinery, but only selectively so, because the observed membrane expansion requires only one of two motors (kinesin-II) that otherwise act redundantly in ciliogenesis [46]. Furthermore, BBS proteins regulate this process (potentially at the level of IFT),

Box 3. Outstanding questions

With the exception of non-compartmentalized (cytosolic) ciliogenesis [36,117], there is substantive evidence from several different model systems that IFT is the principle mechanism driving cilium biogenesis [4,5,7]. Is there, however, compelling evidence that the same mechanism, probably augmented by cell-specific regulatory components, is the primary driving force for actively helping to generate the ultrastructural and morphological diversity observed in cilia? The answer is a tantalizing 'maybe' based on our current understanding. Several lines of research need to be taken to explore this question.

First, it will be necessary not only to define the conserved set of ciliary components through genetic, genomic and proteomic studies and comparative meta-analyses [17,18], but also to identify cilium-specific components by comparing the molecular parts lists of different ciliary types. Progress is being made on these fronts, for example with the first proteomic analysis of a non-motile cilium, the mouse photoreceptor [118], and transcriptome comparisons of morphologically different *C. elegans* ciliated cells responsible for chemosensation and thermosensation [52,53]. Together, such studies will collectively assist in the identification of proteins implicated in ciliogenesis and ciliary specialization. Second, to decipher the role and scope of IFT in building diverse cilia, it will be imperative to discover which of the hundreds of ciliary proteins require IFT to penetrate and localize correctly within the organelle. This important question could be tackled by direct identification of components that co-purify with the IFT machinery and, ultimately, *in vivo* observation of anterograde and retrograde movement for the corresponding GFP-tagged proteins. Many of the cargo proteins might not be abundant, however, so this strategy should be complemented by candidate protein approaches. Third, part of our attention should be turned to an ever-growing list of proteins – including several nephrocystins, and MKS1 and MKS1-related proteins containing B9 domains – that could cooperate with the IFT machinery to assist with the docking, sorting, and/or assembly of vesicle-associated ciliary cargo at the base of cilia [82,99,119]. Many of these proteins are conserved in essentially all ciliated organisms and are implicated in *bona fide* ciliopathies. Fourth, the importance of core IFT proteins (e.g. the Golgi-localized IFT20 [120]) and IFT-associated components (BBSome components, or the small GTPases ARL6/BBS3 and RAB8 [67,68,77]) in the selective trafficking of ciliary cargo from the Golgi apparatus to the base of cilia is certainly not well understood. Finally, the story will not be complete without further exploring the nature of different, functionally discrete ciliary compartments, and how these could be generated and maintained by the IFT machinery. Together, these combined approaches should clarify how functionally distinct cilia are created, providing fundamental insights into the mechanisms of motility and signal transduction.

As a final thought, we might be able to glean aspects of IFT function by experimentally comparing cytosolic versus compartmentalized (IFT-dependent) ciliogenesis. Could the IFT-independent cytosolic assembly of cilia in *Drosophila* spermatozoa, *Cryptosporidia*, *Plasmodia* and *Theileria* [117] point to important roles for IFT in enabling and potentially regulating the transport of specific ciliary cargo required for the sensory functions of the organelle?

as does the RAB8 small GTPase, the membrane trafficking role of which in mammalian ciliogenesis is associated with BBS proteins [77]. Hence, dynamically regulating ciliary length, morphology and function represents important IFT-dependent processes.

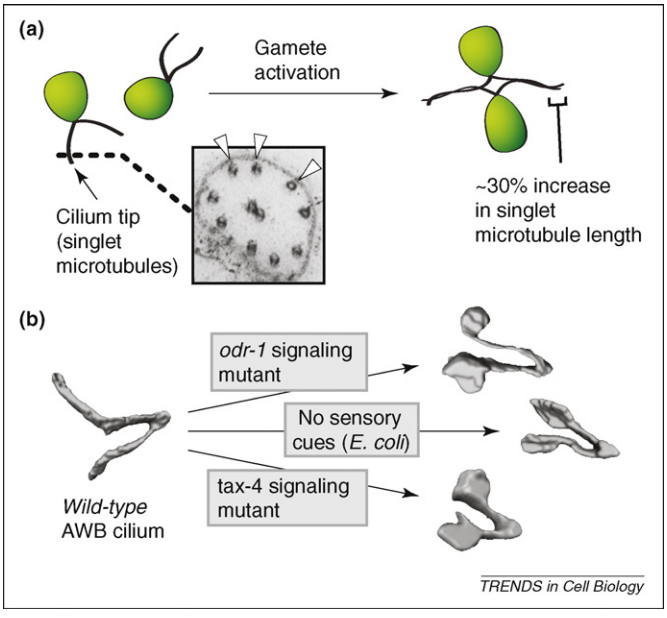

Figure 3. IFT-dependent dynamic changes in cilia. **(a)** *Chlamydomonas reinhardtii* gametes extend singlet microtubules (A-tubules) by ~30% during mating (gamete activation). Gametes of different mating types join via their cilia before cell adhesion and fusion. The TEM micrograph, showing a cross-section of microtubule singlets (individual arrowheads), is from Ref. [20]. **(b)** AWB cilia undergo changes in ultrastructure (shortened axoneme) and morphology (increased membrane area) to adapt to sensory deprivation. These changes, which depend on IFT, can be observed in two signaling mutants (guanylyl cyclase *odr-1* and cGMP-gated channel *tax-4*), and deprivation of *E. coli* (food) odorants in a synthetic growth medium. The figure, showing the 3D reconstructions of wild-type of sensory-deprived cilia, is adapted, with permission, from Ref. [94].

Functional compartmentalization of cilia established by IFT

The IFT-dependent creation of distinct ultrastructural zones at the level of ciliary axonemes – most prominently the proximal and distal segments – can be envisioned to establish unique functional microenvironments within the organelle. Evidence for such zoning and potential dependence on IFT is presently sparse, but suggestive. For example, several signaling molecules of olfactory cilia, including the G proteins $G_{s\alpha}$ and $G_{olf\alpha}$, adenyl cyclase type III, and cyclic nucleotide-gated (CNG) channel, preferentially localize to the distal segment where singlet microtubules are present and odorant exposure is greatest [95]. As briefly discussed earlier, functional compartmentalization is also observed in *Chlamydomonas*, where IFT-dependent extension of the distal segment produces a compartment required for cell-to-cell adhesion and signal transduction during fertilization [61]. Functional demarcation of the proximal segment also occurs. In *Drosophila*, the IFT subcomplex A component IFT140 is required for the formation of, and is enriched at, the ciliary dilation, which prevents the TRPV mechanosensory channel from localizing to the distal segment [96]. In *C. elegans*, the cGMP-gated channel TAX-2 localizes to the proximal (middle) segment of AWB cilia [94]. The murine polycystic kidney disease- and *situs inversus*-associated nephrocystin-2 (*inversin*) protein is confined to a restricted region within the proximal segment but excluded from the transition zone or basal body region [97]. Such a distinctive localization is probably crucial for its function because it is evolutionarily conserved for the *C. elegans* orthologue (unpublished observation). Finally, axonemal components required for motility can be differentially localized; for example, some of the *Chlamydomonas* dynein heavy chains occur only in a restricted proximal region of the cilium [98].

Conclusions

The dawning of a ciliary organelle with motility and sensory functions conferred selective advantages to the proto-eukaryote that are still exploited today in most protists and metazoan cell types. Although much attention is now centered on elucidating the signal transduction roles of cilia in animals, evidently much remains to be understood about how cilia with different compositions, ultrastructures, morphologies and subcompartments are elaborated in the first place and can be altered dynamically. The IFT machinery, which has evolved to become what might seem excessively complex, with its different anterograde motors, multi-subunit protein assemblies and regulatory components, could well have a paramount role in these processes – much beyond simply building the microtubule-based axoneme. As discussed in Box 3, elucidating not only how the IFT machinery operates as a molecular train, but also uncovering the full complement of its passengers, should help provide crucial insight into not only the biogenesis, but also the functions of diverse cilia.

Acknowledgements

We thank Oliver Blacque for his critical appraisal of the manuscript. We apologize to our colleagues for not mentioning or citing many of their important contributions to the field of cilia research owing to space constraints. This work was supported by grants from the Canadian Institutes of Health Research (CIHR; MOP-82870) to M.R.L. and the National Science and Engineering Research Council (NSERC; 327100–06) to M.A.S. M.R.L. acknowledges scholar awards from CIHR and the Michael Smith Foundation for Health Research (MSFHR).

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