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Shared and distinct mechanisms of compartmentalized and cytosolic ciliogenesis

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Abstract

Most motile and all non-motile (primary) eukaryotic cilia possess microtubule-based axonemes that are assembled at the cell surface to form hair-like or more elaborate compartments endowed with motility and/or signaling functions. Such compartmentalized ciliogenesis depends on a core intraflagellar transport (IFT) machinery and associated Bardet-Biedl syndrome complex (BBSome) for dynamic delivery of ciliary components. The transition zone (TZ), an ultrastructurally complex barrier ('gate') at the base of cilia, also contributes to the formation of compartmentalized cilia. Yet, some ciliated protists do not encode IFT components, and together with some metazoan spermatozoa, use IFT-independent mechanisms to build axonemes exposed to the cytosol. Moreover, various ciliated protists lack TZ components, whereas *Drosophila* sperm surprisingly requires the activity of dynamically-localized TZ proteins for cytosolic ciliogenesis. Here we discuss the various ways eukaryotes use IFT and/or TZ proteins to generate the wide assortment of compartmentalized and cytosolic cilia observed in nature. Consideration of the different ciliogenic pathways allows us to propose how three types of cytosol-exposed cilia (primary, secondary, tertiary), including that found in the human sperm proximal segment, are likely evolutionary derivations of compartmentalized ciliogenesis.

Keywords

cilium; cilia; compartmentalized ciliogenesis; cytosolic ciliogenesis; cytoplasmic ciliogenesis; intraflagellar transport; IFT; BBS; BBSome; transition zone; flagella; basal body; centriole; sperm

Introduction

The cilium represents a complex organelle with essential roles in motility and/or sensory-signaling that has existed since the dawn of eukaryotes [1, 2]. But while cilia are conserved in most extant eukaryotes and all metazoans, they have been lost in several clades, such as flowering plants and most fungi [1, 3, 4]. In other eukaryotes, cilia evolved, losing some of their ancestral characteristics. Comparative genomics of organisms with prototypical cilia, modified cilia, or without cilia have predictably confirmed the purging of most known

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ciliary proteins in non-ciliated species. Such genomic arithmetic has been employed as a powerful tool for the discovery of numerous ciliary protein candidates [3, 4].

Hence, comparative genomics affords tremendous predictive power when contrasting highly divergent or closely related organisms that display cellular and morphological differences. It is within this framework that we consider how different macromolecular complexes, the transition zone (TZ) and a core intraflagellar transport (IFT) machinery with its BBSome adaptor, are differentially employed to support two distinct categories of ciliogenesis—compartmentalized and cytosolic—and the different types of cilia present across diverse eukaryotes.

The ancestral cilium: structure, function and formation

The wide phylogenetic distribution of motile cilia in extant eukaryotes provides compelling evidence that the last eukaryotic common ancestor possessed one or two cilia (flagella) capable of providing essential motility to the organism [1]. The proteins enabling this motility, including axonemal dynein molecular motors and accessory proteins present along the axoneme, are highly conserved in evolution [1]. The ancestral motile cilium is also thought to have possessed sensory and signal transduction properties [5]. Indeed, present-day motile cilia are known to engage in signaling; and, liberated from the need to move, many metazoan cell types evolved to have exclusively non-motile (primary) cilia. Together, motile and non-motile cilia play essential roles in cellular signaling that are critical for organismal physiology (*e.g.*, olfaction, mechanosensation, vision), organ homeostasis (e.g., kidney function), and development [2, 5-8].

Aside from a centriole-related basal body that is universally required for extending the microtubule-based ciliary axoneme (Fig. 1), two other large macromolecular complexes can be inferred to have been present in the last eukaryotic common ancestor [3, 9, 10]. One complex, the TZ, is present at the base of cilia and is characterized by a short repeating pattern of structures (often Y-shaped) that connect the axoneme to the overlying ciliary membrane (Fig. 1) [7, 8, 11]. Evidence points to the TZ having at least two modules (NPHP and MKS) that function as a ‘ciliary gate’ or diffusion barrier, helping compartmentalize and concentrate signaling proteins within the organelle [7, 8, 12, 13]. The second complex, the IFT machinery, is mobilized along the axoneme by kinesin and dynein molecular motors and harbors two evolutionarily conserved modules, named IFT-subcomplexes A and B [2, 9, 14]. IFT particles associate with the BBSome, a protein complex altered in the multi-organ ciliopathy Bardet-Biedl syndrome (Fig. 1). The core IFT machinery, together with BBSome, mediate the dynamic trafficking of cilia structure and signaling proteins [2].

The basic ciliary apparatus, and its dependence on IFT/BBSome and TZ for its formation and function, has been conserved for over one billion years; for example, it remains largely unchanged between Trypanosome flagella and human respiratory airway motile cilia [1]. Any significant deviation from the conserved cilium structure and mechanism of formation might be unexpected. However, some ciliated organisms or cell types (gametes) manage without IFT, BBSome or TZ proteins, whereas the BBSome and/or TZ are selectively lost in others [1, 9, 10, 15] (Fig. 2A,B).

Roles of IFT and BBSome in forming compartmentalized cilia

Most cilia are discrete compartments, or organelles, whose membranes and overall compositions are largely distinct from the cell bodies. Such ciliary proteins are not made *in situ*, given the absence of cilium-localized ribosomes. Instead, a trafficking machinery delivers structural components made in the cytoplasm to the plus-end of the growing microtubule-based axoneme—including tubulin building blocks and motility apparatus if necessary—to form the axoneme, and maintain its correct length [2]. There is now outstanding molecular evidence for the direct transport of tubulin by specific IFT subunits (IFT74 and IFT81), and physical interactions between the IFT machinery and ciliary motility apparatus have been documented [14, 16]. Furthermore, signaling proteins are also dynamically transported into (or out of) cilia; this task is principally ascribed to the IFT-associated BBSome, although there is emerging evidence that other IFT subunits play similar roles [2, 17, 18]. In vertebrates, such IFT/BBS ciliary cargo includes Hedgehog signaling components and several GPCRs, such as somatostatin Receptor 3 (SSTR3) [18-20]. Hence, core IFT machinery may be present in organisms lacking BBSome components, presumably because of reduced requirements for ciliary signaling; the reverse is not observed, since the BBSome requires IFT for its operation (Fig. 2B).

Cilia are compartmentalized, in part, *via* transition fibers at the distal end of the basal body that make contacts with the base of the ciliary membrane (Fig. 1) [8, 11]. These help form a ‘gate’ that prevents vesicles from entering the cilium and may organize together with the TZ a ‘ciliary pore’ analogous to the nuclear pore for modulating the trafficking of soluble ciliary proteins [2, 7, 8, 22, 23]. Importantly, transition fibers also serve as docking sites for the IFT machinery, positioned to accept incoming vesicle-bound signaling proteins prior to ciliary entry, or to discharge ciliary proteins (Fig. 1) [2, 8, 12].

Hypothetically, compartmentalized cilia could form in an IFT-independent, but diffusion-dependent manner [21] if the cilium were sufficiently short. This appears to be the case in fly sperm cells, with primary cilia being only 1-2 μm long and consisting mainly of a TZ (Fig. 3Av) [22, 24]. One potential reason for IFT is that virtually all compartmentalized cilia are too long to permit unaided assembly. Another possibility, not mutually exclusive, is that cilium formation and maintenance require dynamic control that can be modulated by IFT. This may be essential, for example, when cilia need to disassemble prior to cell division, which liberates the centriole to act as a centrosome [25]. Yet another possibility is that post-translational modifications of axonemal tubulins (including acetylation and polyglutamylation) may need to be specifically enriched within the ciliary compartment. Indeed, at least one IFT component, IFT70 (fleer/TTC30/DYF-1) plays a critical role in axoneme polyglutamylation [26]. Altogether, It appears that IFT provides several advantages for compartmentalized cilia, making it sufficiently long, dynamic, and specifically enriched in ciliary components ot modifications.

The transition zone forms the gate of compartmentalized cilia

Recent findings implicate a ciliary region immediately distal to the basal body, the TZ, as a *bona fide* diffusion barrier that compartmentalizes signal transduction machinery within the

organelle (Fig. 1) [7, 8, 12, 19, 22, 27, 28]. The molecular basis by which over 12 different TZ proteins creates this gate is not understood. However, it is notable that most TZ-localized proteins are membrane-associated, *via* transmembrane domains as well as lipid-binding C2 or structurally-related B9 domains [7, 8]. These proteins may help create a lipid microdomain at the ciliary base that prevents free membrane diffusion of signaling proteins into and out of the ciliary compartment. Many core IFT and BBS proteins, by virtue of their resemblance to protein coats used for vesicular trafficking, would help ferry signaling proteins across this TZ barrier [2, 3, 9]. The TZ is important for an early stage of ciliogenesis, whereby Y-link structures and associated 'ciliary necklace' appear on the emerging ciliary membrane (Fig. 3Ai-ii) [8]. Perhaps unexpectedly, TZ formation does not appear to require IFT; only the axoneme that extends past this ciliary subdomain does (Fig. 3Ai-iii) [2, 12].

Interestingly, an IFT-BBSome machinery exists in *Giardia* in the absence of detectable TZ proteins (Fig. 2). The function of IFT can thus be decoupled from the TZ, and indeed, complete removal of TZ ultrastructure in *C. elegans* has little effect on IFT motility [12]. These observations suggest that signaling (or other) proteins can be targeted to and retained within cilia lacking a TZ. Thus, in most eukaryotes, the TZ likely influences the entry or exit of only certain ciliary proteins (whether IFT-associated or not); in contrast, this function is evidently not critical in *Giardia, Toxoplasma*, or *Plasmodium*, which lack a TZ (Fig. 2). Notably, although the TZ can form in an IFT-independent manner, no organisms appear to have TZs but lack IFT machinery (Fig. 2B). This suggests that having a barrier without the ability to dynamically create/maintain the cilium using IFT may not be useful. Yet, the mechanism of ciliogenesis that occurs during *Drosophila* spermatogenesis, discussed below, argues otherwise.

Cytosolic ciliogenesis pathways are evolutionary derivations of IFT- and TZ-dependent compartmentalized ciliogenesis

While most motile cilia (flagella) are entirely compartmentalized, the basal body of some cilia is not docked to the plasma membrane and the axoneme is exposed entirely or partly to the cytoplasm (Fig. 3Aiv-vi, 3B) [3, 15, 29, 30]. This exposure, generated through the process of cytosolic ciliogenesis (Box 1), provides at least three potential advantages. First, axonemal components (microtubules and motility apparatus) may be assembled faster, as in *Plasmodium* [31]; longer cilia can be produced, as in *Drosophila* sperm [32]; and finally, ciliary motility could benefit from greater proximity to energy-generating mitochondria, as in mammalian sperm [23]. Since the exposed axoneme allows free exchange with cytosolic proteins, IFT and TZ proteins are not expected to play a direct role in cytosolic ciliogenesis. However, the situation is more complex, and considering the four best-described types of cytosolic cilia and roles of IFT and TZ in their formation allows us to propose three different categories—*primary, secondary, tertiary*—and a potential evolutionary scenario for their emergence from a compartmentalized ciliogenesis pathway.

Mammalian sperm tail

The initial steps in mammalian sperm flagellum formation are indistinguishable from that of compartmentalized ciliogenesis [33, 34]. However, following cilium formation, the basal body, together with axoneme, invaginates into the cell and attaches to the nucleus (Fig. 3Aiv). Differentiation continues, creating a complete, compartmentalized motile cilium. Finally, a structure found at the base of the compartmentalized cilium that contains membrane-barrier forming septins, termed the annulus, migrates away from the basal body along the axoneme, leaving behind what appears to be a structurally mature motile axoneme that is exposed to the cytoplasm [33, 35]. As a result, the mature axoneme consists of two portions: a short proximal cytosolic region found in the mid-piece of the sperm tail, and a longer distal compartmentalized region found in the principle piece.

Both IFT/BBSome and TZ machinery are essential for mammalian sperm formation [36-41]. One possible reason is that these components are needed for compartmentalized ciliogenesis, which precedes the formation of the cytosolic axoneme [33, 38-40]. In addition, both KIF3A and IFT88 are components of the compartmentalized portion in rodents [34, 38], suggesting that the IFT machinery participates in the maintenance of the remaining compartmentalized axoneme after the onset of cytosolic ciliogenesis.

Similar to *Drosophila*, as discussed below, TZ proteins may also play a role in separating the short proximal cytosolic region from the longer distal compartmentalized region found in the principle piece. This possibility is supported by the observation that CEP290 and MKS1 localize together with the annulus at the boundary between the middle piece (cytosolic cilium) and principle piece (compartmentalized cilium) [22, 24]. Notably, some IFT proteins likely have other functions within the spermatid cell, including at the manchette, the acrosome complex, and the head–tail coupling apparatus [34, 38, 42]. Therefore, IFT/BBSome and TZ proteins may perform non-canonical roles during compartmentalized (and potentially also cytosolic ciliogenesis) that may collectively explain their important roles in sperm cell differentiation. This potentially explains why murine BBSome and TZ mutants fail to form sperm flagella, while most other cilia type do form [36, 37, 41].

Giardia flagella

The formation of *Giardia* flagella has parallels with that of mammalian sperm (Fig. 3Aiv). The *Giardia* flagellar axoneme harbors a proximal cytosolic region, and a distal compartmentalized region like the mammalian sperm tail. Like other eukaryotes, the flagella elongate from a basal body [43]. How cytosolic cilia are formed is not clear, although the lack of TZ proteins in *Giardia* suggests a mechanism distinct from that of *Drosophila* (see below). Interestingly, GFP-tagged kinesin-2 and IFT proteins localize to both the cytosolic and membrane-bound ciliary segments [44]. However, overexpression of a dominant-negative kinesin-2 protein, the principle IFT anterograde motor, only shortens the axoneme of the compartmentalized portion, leaving the cytosolic axoneme unaffected [44]. Similar results were obtained using a kinesin-2 morpholino knockdown [45]. Together, these results suggest that IFT is only essential for compartmentalized ciliogenesis in *Giardia*.

Drosophila sperm flagella

Drosophila and other insects have compartmentalized sensory cilia and largely cytosolic sperm flagella. Like in mammalian sperm and *Giardia* flagella, *Drosophila* spermatid flagella comprise two regions. However, in *Drosophila*, the proximal cytosolic axoneme is very long (~1800μm) whereasthedistal compartmentalized cilium is very short (~2μm) (Fig. 3Av) [32, 46].

In spermatocytes, flagella formation initiates with compartmentalized ciliogenesis, whereby a basal body docks at the plasma membrane and forms a short (~1μm) primary cilium (Fig. 3Av). However, this compartmentalized ciliogenesis is independent of IFT, and the cilium is composed mainly of a TZ [22, 24]. Later during meiosis, the basal body, together with the cilium, invaginates into the cell and attaches to the nucleus. Subsequently, in spermatids, axonemal microtubules extend in the short compartmentalized region derived from the spermatocyte primary cilium. While this extention takes place, the TZ migrates distally along the axoneme and away from the basal body, leaving behind a cytosolic microtubule-based axoneme. The cytosolic portion elongates and ultimately forms the strikingly long cytosolic cilium. Cytosolic ciliogenesis continues by addition of dynein arms and other motile axoneme components directly from the cytoplasm [32, 46]. Finally, the cytosolic axoneme associates with the plasma membrane through a process called individualization (reviewed in [47]).

Several studies strongly argue that IFT is dispensable for *Drosophila* sperm cytosolic ciliogenesis. Mutations in kinesin-II or core IFT subcomplex components (*i.e.*, nompB/IFT88/Polaris/OSM-5, Oseg1/IFT122, and Oseg2/IFT172) disrupt sensory cilia, but not sperm function [3, 29, 30]. In contrast, the TZ was recently shown to play a role in separating the cytosolic sperm region from the compartmentalized flagellar tip. Specifically, several TZ proteins, namely Cep290, B9d1, B9d2, and Mks1, migrate away from the centriole to expose the sperm axoneme to the cytoplasm [22]. The TZ protein Cep290 is essential for the compartmentalization and axoneme assembly of the flagellar tip [22]. The axoneme forming at the flagellar tip later becomes the cytosolic axoneme and, therefore, disruption of Cep290 impacts the cytosolic axoneme.

Because the formation of a short compartmentalized cilium that depends on TZ proteins precedes the formation of cytosolic axoneme, the TZ machinery may be indirectly essential for cytosolic ciliogenesis in *Drosophila* sperm. Having the short distal compartment assembling axonemal microtubules might be advantageous when highly elongated cilia are formed, since this isolation may relieve the assembly machinery from the potential slowdown introduced by cytoplasmic proteins, and/or could facilitate post-translational modifications to the axoneme. Notably, both the mammalian sperm annulus and the *Drosophila* sperm compartmentalized cilium contain TZ proteins that appear to provide barrier-like functions outside of canonical Y-links. Such a divergent role for TZ proteins are likely not unique, as it has also been ascribed to a signaling compartment found proximal to the cilium of a *C. elegans* thermosensory neuron [48].

Plasmodium flagella

Plasmodium flagella (Fig. 3Avi) emerge during male gametogenesis (reviewed in [49]). In the cytoplasm, basal bodies and then axonemes form quickly (~15 minutes) but in an error-prone manner from a preexisting amorphous microtubule organization center at the vicinity of the plasma membrane. Shortly thereafter, in a process termed exflagellation, each nucleus and its attached axoneme interacts with the plasma membrane; forceful flagellar beating separates each membrane-enclosed axoneme from one another, forming individual gametes. *Plasmodium* flagella therefore appear to form completely in the cytoplasm. However, considering the rapidity of flagellar biogenesis, one cannot exclude the possibility that the distal portion of the axoneme is tethered to, or partially enveloped by, the plasma membrane during this process. Yet, the absence of membrane involvement is supported by the apparent lack of TZ and IFT proteins in this organism (Fig. 3A vi) [3, 15].

How did cytosolic cilia evolve?

Compartmentalized cilia form *via* a universal mechanism, whereby a basal body docks first to a ciliary vesicle that will fuse with the plasma membrane, or directly to the plasma membrane; this is followed by formation of the TZ, and then the membrane-ensheathed axoneme (Fig. 3A, steps **i-iii**) [2, 7, 8, 23]. On the other hand, cytosolic cilia form by at least three distinct mechanisms. We propose that these mechanisms evolved as modifications of compartmentalized ciliogenesis.

Primary cytosolic cilia form by exposing the axoneme to the cytoplasm after the initial formation of a TZ-dependent and IFT-dependent compartmentalized cilium (arrows from Fig. 3A, step **iii** directly to **iv**). This relatively simple modification of compartmentalized ciliogenesis is observed in mammalian sperm, and may also have evolved independently in *Giardia* and some chromalveolates (*Toxoplasma, Thalassiosira*); however this still awaits demonstration. We propose that this pathway represents an initial first step in the evolution of cytosolic ciliogenesis. Since *Drosophila* and mammals share a common ancestral sperm flagellum, primary cytosolic ciliogenesis may represent the ancestral mechanism of spermatogenesis in the last common ancestor of protostomes and deuterostomes that existed about 800 million years ago. Consistent with this notion, more basal metazoans, including Cnidaria (corals/jellyfish) and Porifera (sponges) have fully compartmentalized sperm cilia.

Secondary cytosolic cilia form when the centriole connects to the membrane, first forming a TZ and only then forming a cytosolic cilium (arrows from Fig.3A, step **ii** directly to **v**). In this scenario, cytosolic ciliogenesis consists of two steps: exposure of the newly-made axoneme to the cytoplasm, and addition of dyneins and other axonemal proteins. *Drosophila* sperm represents an example of this, with cytosolic ciliogenesis being independent of IFT, but dependent on membrane-associated TZ proteins. We suggest that such a pathway represents a second step in the evolution of cytosolic ciliogenesis that is conserved in insects.

Tertiary cytosolic cilia form when the centriole extends the axoneme and assembles all elements of the motility apparatus directly within the cytoplasm (arrows from Fig. 3A, steps **i** directly to **vi**). We envision that this process, observed in *Plasmodium*, represents the most

extreme modification of compartmentalized ciliogenesis and may have evolved following the two intermediate steps discussed above.

Concluding remarks

The ciliary structures and phylogenetic distribution of ciliary proteins present in extant eukaryotes indicate that the last eukaryotic common ancestor had one or two motile, compartmentalized cilia which formed in an IFT/BBSome and TZ-dependent manner [2, 9, 10]. All metazoan non-motile (primary) cilia still retain these ancient properties, likely reflecting the critical importance of being able to regulate the compartmentalization and composition of signaling proteins within cilia. We propose that different mechanisms of cytosolic cilia formation, as observed in some protists and metazoan gametes, represent evolutionary derivations of compartmentalized ciliogenesis. Moreover, cytosolic cilia, and more generally, motile cilia, make variable use of IFT, BBSome and TZ proteins. For example, Trypanosomes and *Chlamydomonas* possess a full complement of IFT, BBSome and TZ proteins, whereas *Giardia* lacks TZ proteins even though they have the IFT/BBSome transport system needed to modulate ciliary composition. *Drosophila* spermatogenesis makes use of a highly dynamic TZ, and does not require IFT to make a short primary cilium. Hence, further study of cytosolic ciliogenesis in divergent organisms, and the differences and similarities compared to compartmentalized ciliogenesis, promise to provide us with unique opportunities to better understand central aspects of cilium formation and function.

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Box 1

What is cytosolic ciliogenesis?

Classically, cilia are defined as organelles that protrude from the cell surface and contain a basal body-templated, microtubule-based axoneme that is enveloped by an extension of the plasma membrane. In most cells, cilia maintain these definitive characteristics from initiation to maturation, and their formation is referred to as *compartmentalized ciliogenesis*. In some cell types, at least temporarily, all or a portion of the ciliary axoneme is not enveloped by plasma membrane and is exposed to the cytoplasm. Maturation of such an axoneme may involve incorporation of proteins directly from the cytoplasm, and ultimately, requires association with the plasma membrane. We use the term *cytosolic ciliogenesis* to collectively describe the processes that lead to the exposure of the axoneme to the cytoplasm, maturation of the axoneme within the cytoplasm, and membrane association of the axoneme. Cytosolic ciliogenesis can occur following compartmentalized ciliogenesis.

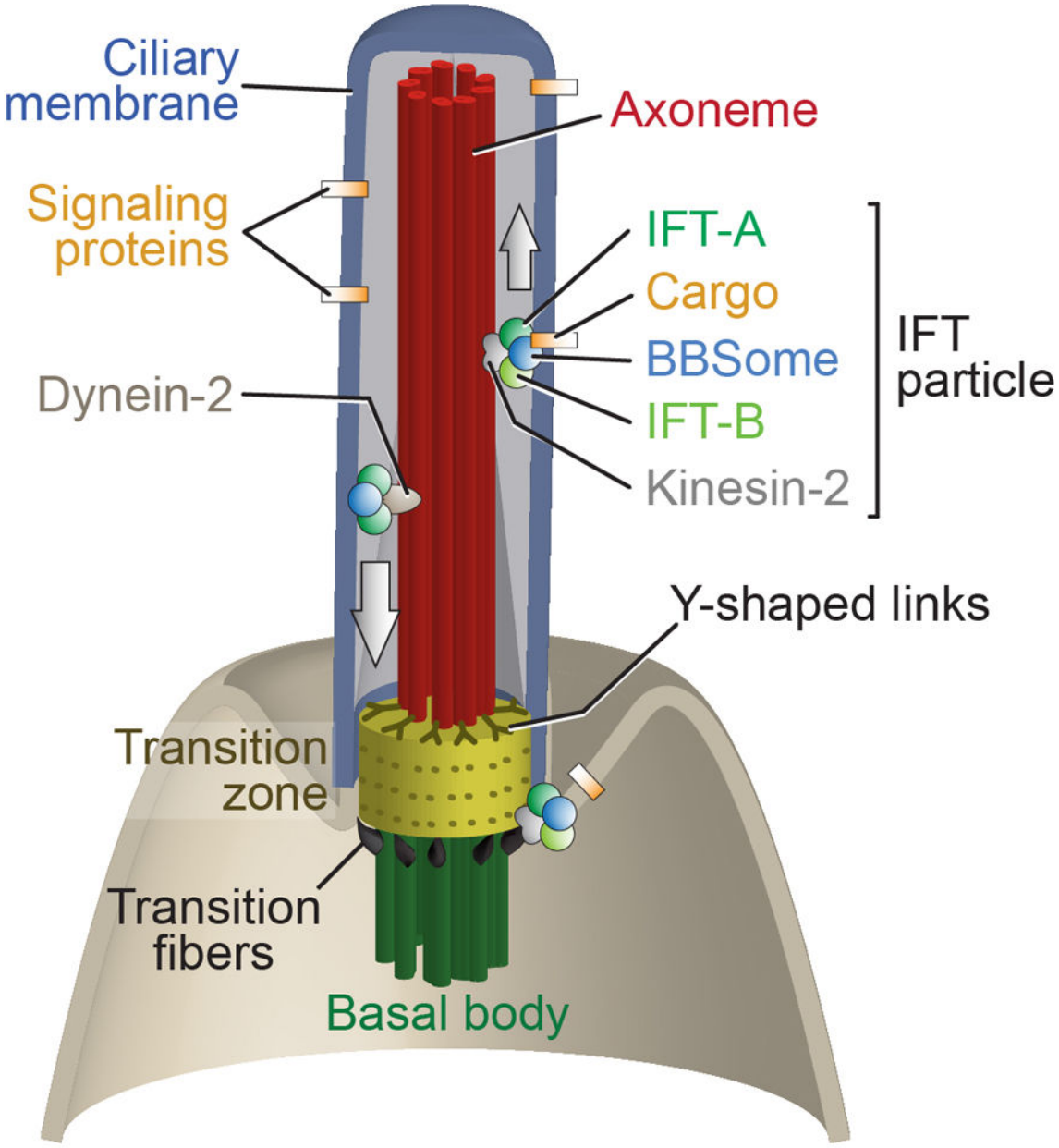

Figure 1.

Evolutionarily conserved components of a compartmentalized basal body-cilium organelle. The centriole-derived basal body connects to the plasma membrane using transition fibers. These serve as docking sites for the intraflagellar transport (IFT) machinery, which is critical for cilium formation and functional maintenance. The IFT machinery consists of one or more Kinesin-2 anterograde molecular motors which mobilize two ‘core’ IFT subcomplexes (IFT-A and IFT-B) and a BBSome adaptor, and associated ciliary cargo. A Dynein-2 molecular motor, transported to the tip by the anterograde IFT machinery (not shown), brings the IFT components back to the base. A transition zone containing axoneme-to-ciliary membrane connectors (typically Y-shaped) serves as a selective membrane diffusion barrier.

Together, the transition fibers, transition zone and IFT machinery help to compartmentalize the ciliary organelle and dynamically maintain its composition.

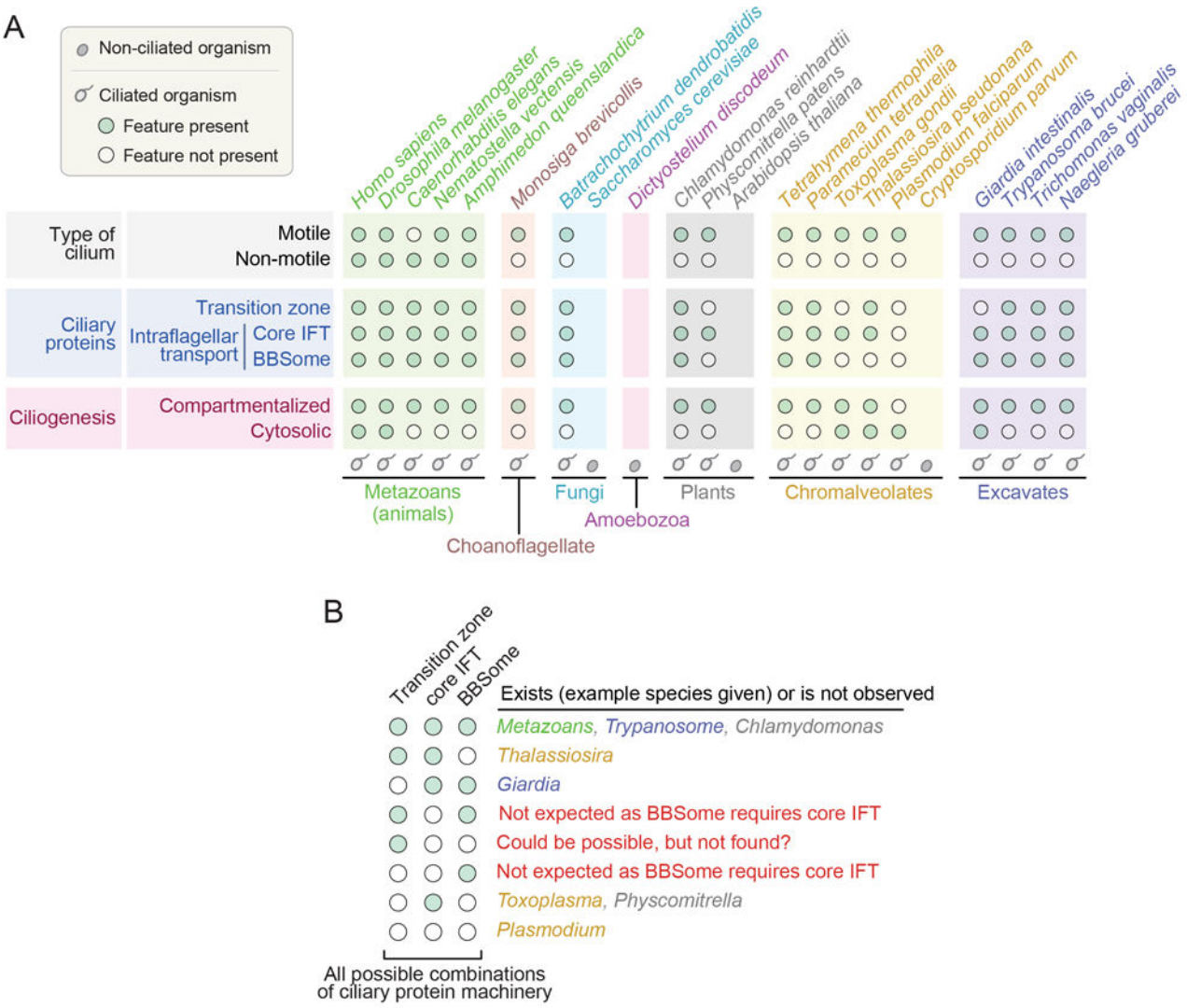

Figure 2.

Types of cilia, ciliary components, and modes of cilium formation (compartmentalized and/or cytosolic) across eukaryotes.

(**A**) Distribution of ciliary features throughout the main eukaryotic kingdoms. The major classes of cilia are motile and non-motile. Ciliary proteins used for cilium formation and functional maintenance are transition zone (TZ) proteins, as well as core intraflagellar transport (IFT) proteins and the associated BBS protein complex (BBSome). Compartmentalized ciliogenesis refers to the formation of an axoneme that protrudes from a basal body docked to the cell surface, and cytosolic ciliogenesis is the formation of an axoneme that also stems from a basal body but is at least in part exposed to the cytoplasm. (**B**) Ciliated organisms make variable use of TZ, core IFT, and BBSome machinery. Shown are all eight possible combinations of these machineries, and whether representative organisms possess such combinations or not.

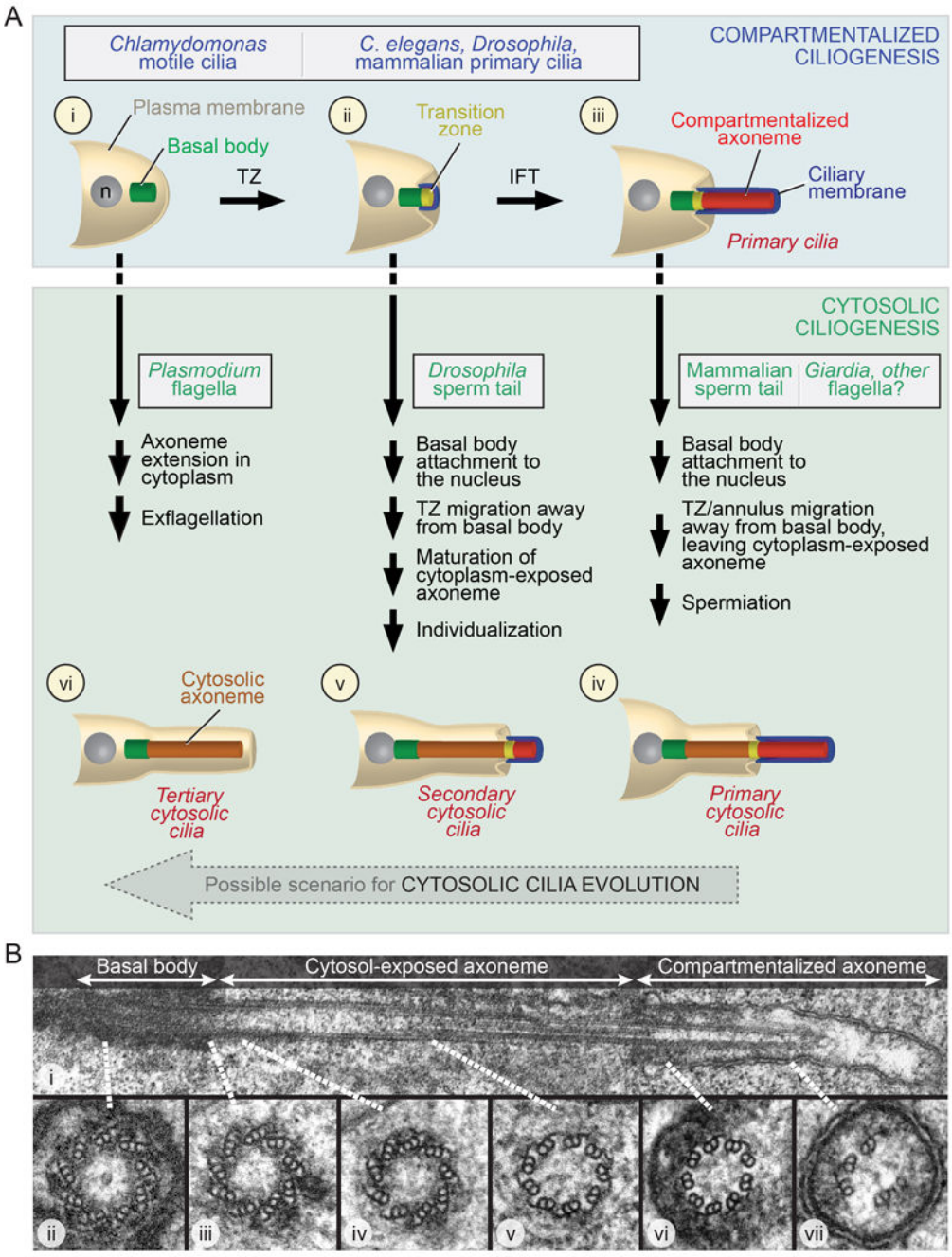

Figure 3. Compartmentalized and cytosolic ciliogenesis pathways, and model for cytosolic ciliogenesis evolution

(**A**) Non-ciliated cells (i) have centrioles (green) near the nucleus (n). Compartmentalized ciliogenesis (upper horizontal arrows) begins when a centrioles docks to the plasma membrane, or a vesicle that later fuses with the plasma membrane (not shown) and forms a transition zone (TZ; yellow) (ii). Intraflagellar transport (IFT) then mediates the formation of an elongated compartmentalized axoneme (red) ensheathed by a ciliary membrane (blue) (iii). Primary cilia are formed by this process, as are various motile cilia (e.g., in Chlamydomonas or vertebrate respiratory airway). Three types of cytosolic ciliogenesis

pathways (vertical arrows) can each be recognized to begin at distinct points after, during, or before compartmentalized ciliogenesis. We refer to these three as forming primary, secondary, and tertiary cytosolic cilia, respectively. The three different types of cytosolic ciliogenesis pathways may represent gradual/distinct steps of evolution from an ancestral compartmentalized ciliogenesis pathway. First, as exemplified by the mammalian sperm tail and possibly *Giardia* (as well as *Toxoplasma* and *Thalassiosira*) flagella (step iii directly to iv), after completion of compartmentalized ciliogenesis, the centriole attaches to the nucleus, and the TZ migrates away from the centriole along the complete axoneme to expose it to the cytoplasm. Second, as exemplified by *Drosophila* sperm (step ii directly to v), after the centriole docks to the plasma membrane and forms a TZ, it then attaches to the nucleus. Then the TZ migrates away from the centriole, while a rudimentary axoneme forms. Here, the exposed axoneme completes ciliogenesis by recruiting proteins directly from the cytoplasm (axoneme maturation). Finally, as exemplified by *Plasmodium* flagella (step I directly to vi), the centriole forms the axoneme directly in the cytoplasm. In both the mammalian and *Drosophila* sperm, the centriole migrates toward the nucleus and attaches to it. The various cytosolic cilia undergo a final process of reattachment of the cytosolic axoneme to the plasma membrane. These processes are refered to as spermiation (iii), individualization (iv), or exflagellation (vi).

(**B**) Example of a cytoplasmic cilium, from *Drosophila*. Transmission Electron Microscopy image showing the basal body, cytosol-exposed axoneme, and compartmentalized axoneme (i). Cross-sections reveal the microtubule architecture along the length: basal body with triplet microtubules (ii, iii), cytosol-exposed axoneme with doublet microtubules (iv, v), and compartmentalized (ciliary membrane-associated) axoneme with doublet microtubules (vi) or incomplete set of microtubules (vii). Image adapted from Gottardo *et al.*[50].