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The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization

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Both the basal body and the microtubule-based axoneme it nucleates have evolutionarily conserved subdomains crucial for cilium biogenesis, function and maintenance. Here, we focus on two conspicuous but underappreciated regions of these structures that make membrane connections. One is the basal body distal end, which includes transition fibres of largely undefined composition that link to the base of the ciliary membrane. Transition fibres seem to serve as docking sites for intraflagellar transport particles, which move proteins within the ciliary compartment and are required for cilium biogenesis and sustained function. The other is the proximal-most region of the axoneme, termed the transition zone, which is characterized by Y-shaped linkers that span from the axoneme to the ciliary necklace on the membrane surface. The transition zone comprises a growing number of ciliopathy proteins that function as modular components of a ciliary gate. This gate, which forms early during ciliogenesis, might function in part by regulating intraflagellar transport. Together with a recently described septin ring diffusion barrier at the ciliary base, the transition fibres and transition zone deserve attention for their varied roles in forming functional ciliary compartments.

Keywords: cilium; transition zone; transition fibre; basal body; ciliopathy

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See the Glossary for abbreviations used in this article.

Introduction

Cilia are finger-like organelles that project from the surfaces of most eukaryotic cell types. Motile cilia move fluids and cells, and immotile cilia transduce environmental stimuli and regulate signalling pathways important for development (reviewed in [1]). The biomedical importance of cilia is becoming increasingly appreciated, as defects in ciliary structure and function are found to cause human diseases or 'ciliopathies', typified by cystic kidneys, blindness, obesity, skeletal malformations and nervous system anomalies [2,3].

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All cilia arise from a basal body; a centriolar barrel anchored to the base of the ciliary membrane by transition fibres (Fig 1A,B). At or near this attachment point are docking sites for intraflagellar transport (IFT) particles (Fig 1C; [4,5]). These membrane-associated multimeric protein complexes travel along microtubules by using kinesin 2 anterograde and cytoplasmic dynein retrograde molecular motors, and are required for building and maintaining cilia [6–8]. At the distal end of the basal body, the plasma membrane gives way to a compositionally distinct membrane that envelops the entire cilium [9]. Indeed, an important feature of cilia is their compartmentalization, and this property might be bestowed in part by its singular membrane composition. Protein transport between the cytoplasm and cilium is regulated, and facilitates control over cilium-based signalling [10,11]. Regulation of ciliary protein entry and exit might depend on proteins at the ciliary base, leading to the idea of a diffusion barrier or 'gate' that prevents nonspecific movement of membrane proteins into and out of the cilium [12]. In this review, we focus on two substructures at the base of cilia, transition fibres and the transition zone, that are important for ciliogenesis and control of ciliary protein composition.

Evolutionarily conserved basal body–ciliary structures

The occurrence of cilia within all major eukaryotic clades and the evolutionary conservation of core ciliary proteins, indicate that the last eukaryotic common ancestor had a structurally sophisticated motile, and probably sensory, cilium that largely resembled that of extant eukaryotes [13,14]. The most prominent part of the cilium is the axoneme, which is comprised of nine peripheral microtubule doublets surrounding a central pair—the so-called 9+2 arrangement—in most motile cilia, with the central pair usually lacking in non-motile (primary) cilia—the 9+0 arrangement (Fig 1A; see [15] for treatment of cilium ultrastructure). Axonemes extend from the mother centriole, which is the oldest centriole, inherited during mitosis. During ciliogenesis, the typically triplet microtubule-containing mother centriole becomes a basal body, distinguished by adornment with appendages projecting from the centriolar barrel.

These appendages come in several forms (Fig 1B). Most proximal (closest to the nucleus) are the subdistal appendages and basal foot. At the basal foot of mammalian cells, ninein, a subdistal appendage component, is recruited by the Odf2 protein and promotes centriolar microtubule nucleation and anchoring [16,17]. Odf2 itself is required for basal foot formation, which in turn helps coordinate

the beat of motile cilia [18]. Beyond the subdistal appendages lie the distal appendages. During ciliogenesis, the mother centriole anchors to a membrane—the ciliary vesicle or plasma membrane—through the distal appendages, which then become the pinwheel-shaped transition fibres or alar sheets. In mammalian cells, these structures contain Cep164, and depend on distal centriolar proteins such as Ofd1 for their formation [19]. Human mutations in *OFD1* are associated with orofaciodigital syndrome 1, a ciliopathy characterized by digit malformations and kidney cysts [20]. Without Ofd1, cells cannot recruit Cep164 to the centriole, form distal appendages, dock the mother centriole to membranes or form cilia. Ofd1 localizes to the distal centriole in a region central to the appendages, a domain also occupied by other distal centriole components, including Poc5 [19,21]. Super-resolution microscopy revealed a new centriolar protein, Ccdc123/Cep123, found to co-localize with Cep164 at the distal appendages [22]. In all likelihood, additional components of transition fibres will be discovered and their specific roles in ciliogenesis will be uncovered.

Beyond the basal body lies the transition zone, an evolutionarily conserved ciliary subdomain characterized by distinctive Y-shaped fibres that connect the doublet microtubules to the overlying ciliary membrane (Fig 1A). Y-links organize, terminate in, or constitute the ciliary necklace, which are circumferential strands of intramembrane 'decorations' [23] that might in fact form a spiral (Fig 1D; [24]). The Y-links are immediately distal to the transition fibres, as both can sometimes be observed, but only in slightly oblique transmission electron microscopy cross-sections [25]. Although the composition of the Y-link and necklace structures is largely unknown, with the probable exception of CEP290/NPHP6 in *Chlamydomonas* [26], proteins with specific transition zone localization and associated functions are good candidates. These include a growing cohort of other ciliopathy proteins implicated in Meckel syndrome (MKS), Joubert syndrome (JBTS), nephronophthisis (NPHP), Senior–Loken syndrome (SLSN) and Leber congenital amaurosis (LCA). A non-exhaustive list of these proteins is presented in Table 1. Extra features observed within the transition zone of motile cilia, detailed in [15], are not discussed further here. These include, for example, the basal plate, which might anchor the central pair required for motility.

There is ambiguity concerning the boundaries of the transition zone with respect to the basal body and ciliary axoneme. For example, one review article on ciliary nomenclature [27] suggests that the transition fibres might comprise part of the transition zone. Here, we ascribe the transition fibres as components of the basal body, because they stem directly from the distal end of the centriolar barrel (see the three-dimensional reconstruction in [28]). By contrast, we define the transition zone as being part of the proximal-most region of the axoneme containing doublet microtubules and Y-links—structural features that form only in ciliated cells (Fig 1). Just beyond the transition zone is the 'inversin compartment', which in mammalian cells and *Caenorhabditis elegans* contains, among other proteins, inversin/NPHP2 [29,30].

Earliest steps of ciliogenesis

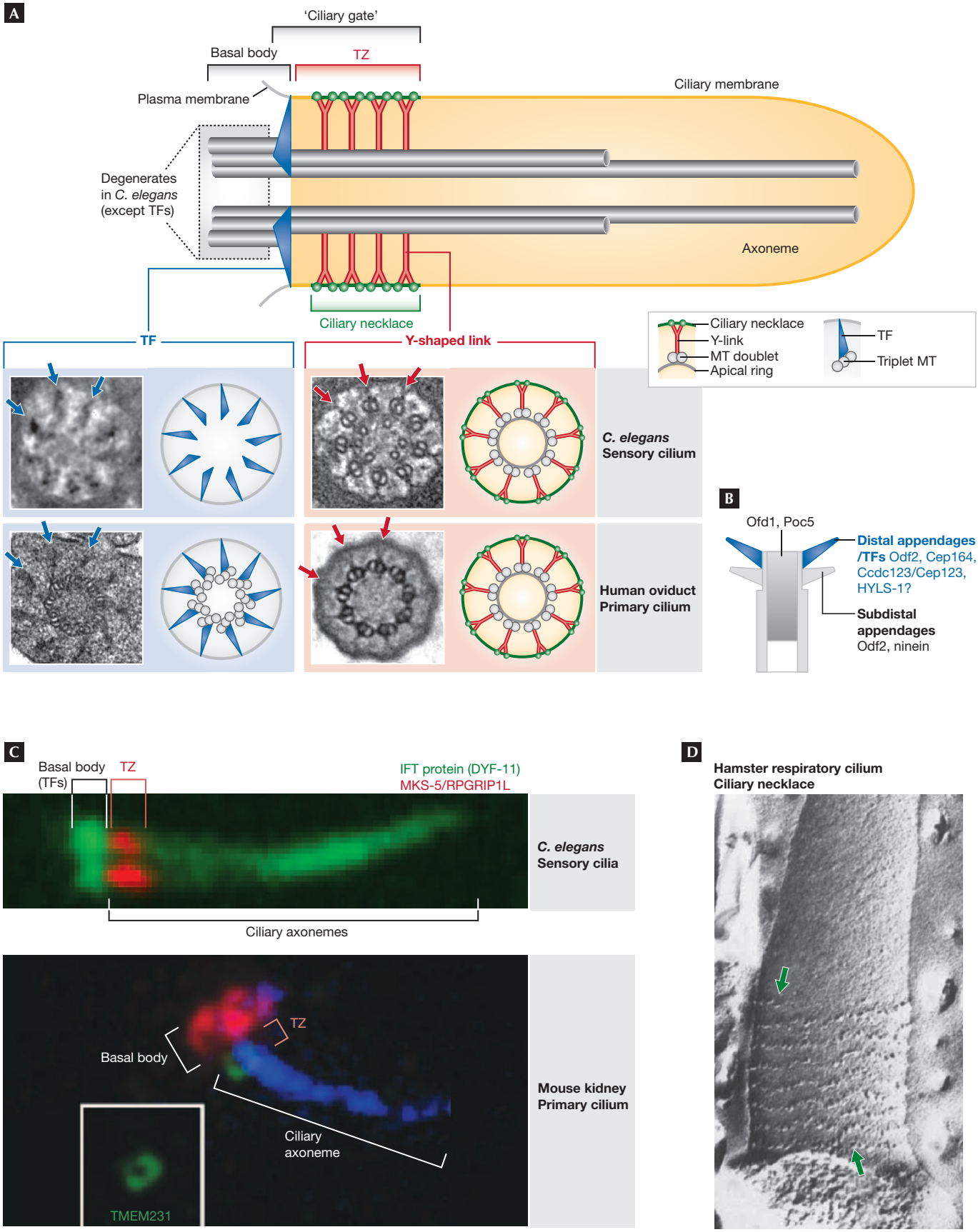
Formation of primary cilia depends on liberating the mother centriole from its centrosomal role in cell division after cytokinesis. In some mammalian cell types, the earliest detectable ciliogenic event is the docking of a ciliary vesicle to the mother centriole (Fig 2; [31,32]). Whether the centriole selects a ciliary vesicle with special ciliogenic properties for docking, or imposes ciliary characteristics on a naive vesicle, is unclear. Furthermore, whether the basal body appendages implicated in vesicle binding represent mature distal appendages and transition fibres is not certain. Subsequently, the basal body-ciliary vesicle often, but not always, migrates to the cell surface, an event which in tissue culture cells seems to be dependent on the actin cytoskeleton and, intriguingly, membrane-associated components of the transition zone (MKS1 and MKS3; [33–35]). A second intracytosolic event then initiates, whereby the axoneme elongates, invaginating one face of the ciliary vesicle to create the ciliary membrane. Alternatively, in other cell types, such as multiciliated cells, direct interaction of the basal body with the plasma membrane occurs, followed by extension of the axoneme (Fig 2; see review [33] for further details on the ciliogenic pathways observed). In both cases, the forming cilium acquires transition-zone-like features along the growing axoneme and ciliary membrane.

On the basis that disruption of one or more transition-zone-associated proteins can result in centriole migration or membrane docking and attachment defects, in mammalian cells and the nematode *C. elegans*, it has been argued that transition zone proteins participate in these early steps of ciliogenesis [5]. These transition zone proteins probably form supramolecular complexes, the nature and ultrastructural organization of which is only starting to be understood in genetic and proteomic terms [5,36–38]. Evidence that these expansive complexes might constitute features of transition zone ultrastructure comes from the observed disruption of Y-links after abrogation of several pairs of transition zone proteins in *C. elegans* or CEP290 in *Chlamydomonas* [5,26,39]. In addition to potential structural roles, the inferred transmembrane and membrane-associated properties of transition zone proteins (for example, MKS2/TMEM216, MKS3/TMEM67 and JBTS14/TMEM237; see Table 1) could confer extra functions during ciliogenesis, such as interaction with vesicular transport machinery, fusion of the ciliary vesicle with the plasma membrane and facilitating the proper anchoring of the basal body to the membrane by securing Y-link to membrane connections.

Although formation of the ciliary axoneme is widely acknowledged to depend on IFT, there is evidence from different model systems that basal body docking and anchoring, or transition zone

Glossary

AC3	adenylyl cyclase 3
DYF-1	abnormal DYe-Filling 1 gene
CEACAM1	carcinoembryonic antigen-related cell adhesion molecule 1
Cep	centrosomal protein
HYLS-1	hydrolethalus syndrome protein 1
GPI	Glycosylphosphatidylinositol
KIF17	kinesin family member 17
OFD	orofaciodigital
Odf2	outer dense fibre 2
PKD2	polycystic kidney disease 2
Poc5	proteome of centriole 5
RP2	retinitis pigmentosa 2
RPE1	retinal pigment epithelial cell line
SAS-4	spindle assembly abnormal 4
SEPT2	septin 2
siRNA	small interfering RNA
TRAM	translocating chain-associating membrane



◀ **Fig 1** | The transition fibres, and the transition zone with its associated ciliary necklace, represent evolutionarily conserved features of the basal body–ciliary organelle. (**A**) All cilia have a microtubule-based axoneme that emerges from a centriolar structure termed the 'basal body'. The TFs and TZ are depicted schematically together with representative TEMs from a *Caenorhabditis elegans* sensory cilium and human oviduct primary cilium. Y-link structures organize, or make up, the ciliary necklace present on the ciliary surface (shown as beads). In nematodes, the basal body almost completely degenerates after ciliogenesis, retaining only TFs. The nature and function of the inner singlet microtubules seen in the TEM cross-sections of *C. elegans* cilia are unknown. The ciliary compartment is highlighted in yellow. (**B**) Substructures of the centriolar barrel, with the reported localization of several components shown. (**C**) Fluorescence microscopy images of markers showing the basal bodies, TZs and axonemes of *C. elegans* and mammalian cilia. The two *C. elegans* cilia show an IFT protein (DYF-11; green) marking the basal body (TF region) and axoneme, and MKS-5 (red) marking the TZ. The mouse cilium shows the basal body (γ-tubulin; red), TZ (membrane-associated TMEM231 forming a ring; green) and the axoneme (acetylated tubulin; blue). (**D**) Freeze–fracture scanning electron micrograph of a hamster respiratory cilium, showing evidence that the ciliary necklace is in fact a spiral, with an approximate 8° angle of pitch with respect to the ciliary axis (arrows show apparent start and end points of the bead-like particles on the membrane). *C. elegans* images modified with permission from [5]; oviduct TEM from [55]; TMEM231 data from [36]; ciliary necklace from [24]. IFT, intraflagellar transport; MT, microtubule; TEM, transmission electron migrograph; TF, transition fibre; TZ, transition zone.

formation, does not require this transport system. For example, transition zone formation seems normal in the *Chlamydomonas IFT52* mutant, which cannot otherwise build the rest of the axoneme [40], as well as in IFT gene mutants in *C. elegans* [5,41]. Instead, it is probable that IFT complexes associate with the ciliary or other vesicles, and the basal body, during the early stages of ciliogenesis [42,43]—ostensibly primed for elongating the axoneme after basal body (transition fibre)–transition zone attachment, to the membrane (Fig 2). Although how these events are coordinated is not understood, at least two IFT proteins, IFT20 and Elipsa/DYF-11, Bardet–Biedl syndrome (BBS) proteins, and vesicular transport components (such as Rab11, Rab8, rabin 8 and rabaptin 5) seem to partake in ciliary cargo transport from the Golgi to the ciliary base near the basal body [44–48]. Other IFT proteins must be recruited to the ciliary base and assembled to allow IFT particle interactions with ciliary proteins, but such spatio-temporal details remain to be elucidated.

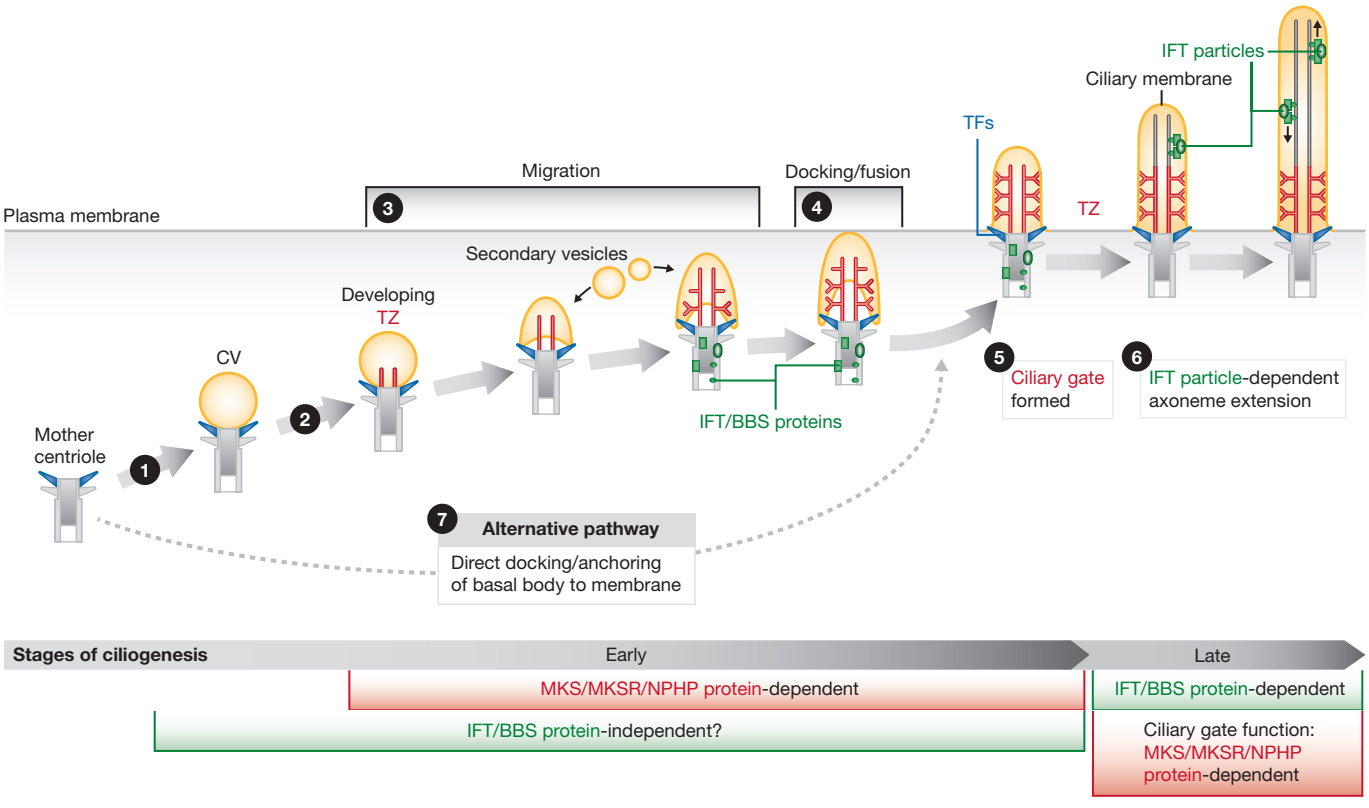


Fig 2 | Roles of the basal body distal end and transition zone region during two phases of primary cilium formation ('early' and 'late'). In many mammalian cell types, the first ciliogenic event involves the binding of a CV to the distal end of the mother centriole, probably through distal appendages (1). A (presumably immature) TZ region begins to emerge and invaginate the CV, the membrane surface of which grows through fusion of secondary vesicles (2–3). The basal body-CV can migrate to the plasma membrane (3) and then fuse with it (4), at which point the maturing TZ forms the ciliary gate (5). Complete formation of the axoneme and functional cilium is an IFT/BBS protein-dependent process (6); IFT/BBS proteins present on the undocked basal body might perform specific transport roles or might simply be trafficked for eventual assembly as functional IFT particles. A different pathway (7) followed by other cells types might not involve a CV, but rather, have the basal body docking directly with the membrane; a TZ might start to form just before, during and after this step. Also, basal body migration does not occur in all cell types. CV, ciliary vesicle; IFT, intraflagellar transport; BBS, Bardet–Biedl syndrome; TZ, transition zone.

Table 1 | Functions and associated disorders of proteins that localize to the ciliary transition zone

Mammalian protein	Other names/related disease	Function	Representative references
Mks1	Bbs13; MKS-1 (*Ce*)	Formation and function of ciliary gate; Hedgehog signalling	[5,37,38,95]
B9d1	Mksr1, Mks9; MKSR-1 (*Ce*)	Formation and function of ciliary gate; Hedgehog signalling	[5,36,38,96]
B9d2	Mksr2, Mks10, Stumpy; MKSR-2 (*Ce*)	Formation and function of ciliary gate	[5,38,96,97]
Tmem216	Mks2, Jbts2; MKS-2 (*Ce*)	Formation and function of ciliary gate	[37,39,98]
Tmem67	Mks3, Jbts6, Meckelin, Nphp11, COACH syndrome; MKS-3 (*Ce*)	Formation and function of ciliary gate	[5,37,99]
Rpgrip1L	Mks5, Nphp8, Jbts7, Ftm, COACH syndrome; MKS-5 (*Ce*)	Formation and function of ciliary gate; scaffold for many transition zone proteins; Hedgehog signalling	[5,37,38,100]
Rpgr	Rp3	NPHP4-interacting protein required for cilium maintenance	[101,102]
Rpgrip	Lca6	Rpgr- and NPHP4-interacting protein required for cilium maintenance	[101,102]
Cc2d2a	Mks6, Jbts9, COACH syndrome; MKS-6 (*Ce*)	Formation and function of ciliary gate; Hedgehog signalling	[5,36–38,103]
Cep290	Nphp6, Mks4, Jbts5, Slsn6, Lca10	Formation and function of ciliary gate; possible component of Y-links	[26]
Tmem17	ZK418.3 (*Ce*)	Function of ciliary gate	[36]
Tmem231	T26A8.2 (*Ce*)	Function of ciliary gate; Hedgehog signalling	[36]
Tmem237	Jbts14; JBTS-14 (*Ce*)	Formation and function of ciliary gate; regulation of Wnt signalling	[39]
Tctn1	Tect1, Jbts13, E04A4.6 (*Ce*)	Function of ciliary gate; regulates Hedgehog signalling	[37]
Tctn2	Tect2, Mks8	Neural tube patterning, Hedgehog signalling	[37,38]
Tctn3	Tect3	Forms complex with Tctn1,Tctn2, Mks1, B9d1, Cc2d2a, and Tmem67	[37]
Lca5	Lebercilin	Interaction with IFT proteins; ciliary photoreceptor maintenance	[67,104]
Ahi1	Jbts3, Jouberin	Cilium biogenesis; photoreceptor maintenance	[38,105–107]
Nphp1	Jbts4, Slsn1; NPHP-1 (*Ce*)	Formation and function of ciliary gate	[5, 37, 38, 106]
Nphp4	Slsn4; NPHP-4 (*Ce*)	Formation and function of ciliary gate	[5, 37, 38, 108]
nucleoporins	Many	Ciliary entry of KIF17 kinesin, perhaps other proteins	[72]

Virtually all transition-zone-localized proteins are associated with one of the following disorders, as indicated by their corresponding gene designations: Bbs, Bardet–Biedl syndrome; Mks, Meckel syndrome; Mksr, MKS1-related; Jbts, Joubert syndrome; Nphp, nephronophthisis; Lca, Leber congenital amaurosis; Rp, retinitis pigmentosa; Slsn, Senior–Loken syndrome.

Formation of ciliary compartment in early ciliogenesis

An essential attribute of the cilium is that it represents a *bona fide* organelle, with its own transport system, signal transduction molecules and, in the case of motile cilia, force-generating machinery. Yet, unlike an organelle such as a mitochondrion, which is fully sequestered from the cytosolic environment by a contiguous membrane, the cilium is built whilst ostensibly exposed to non-ciliary cell body components. If the initial establishment of a ciliary compartment requires isolation from cytosolic and plasma membrane protein pools, then ciliary gate formation might represent one of the earliest steps of ciliogenesis.

Freeze–fracture electron microscopy studies have provided insights into the timing of ciliary necklace formation, which could act as a membrane diffusion barrier. In *Tetrahymena*, a distinct arrangement of intramembrane particle arrays, termed 'fairy rings', form before substantial axonemal outgrowth and the mature ciliary necklace are apparent [49]. The ring, corresponding roughly to the diameter of basal bodies, is evident on a flat area of the plasma membrane. The emergence of a ciliary membrane protrusion is accompanied by a concentration of intramembraneous particles on the membrane slope. A similar progression of events is reported for motile and non-motile cilia of vertebrates [50], suggesting that this process is conserved.

Presumably, ciliary necklace formation coincides with the assembly of transition zone ultrastructure; however, the electron miscroscopic evidence for this is sparse. Studies of quail oviduct cilium biogenesis hint at the appearance of bead structures in the lumen of ciliary vesicles, as well as the presence of Y-links in a potentially early phase of transition zone formation [51]. Studying moth spermatogenesis, the authors of [52] visualized regular repeating patterns of membrane-associated beads in ciliary stumps protruding into the ciliary (flagellar) vesicle. More time-resolved experiments with molecular markers of the ciliary vesicle, the plasma membrane region in which the basal body docks, the transition zone and the necklace are required to better understand this early ciliogenic pathway.

Apart from the ciliary necklace, other structures probably contribute to the restriction or modulation of ciliary entry and exit. These include transition fibres, Y-links, nucleocytoplasmic transport machinery and a septin ring. Similarly to the transition zone, the spatio-temporal events

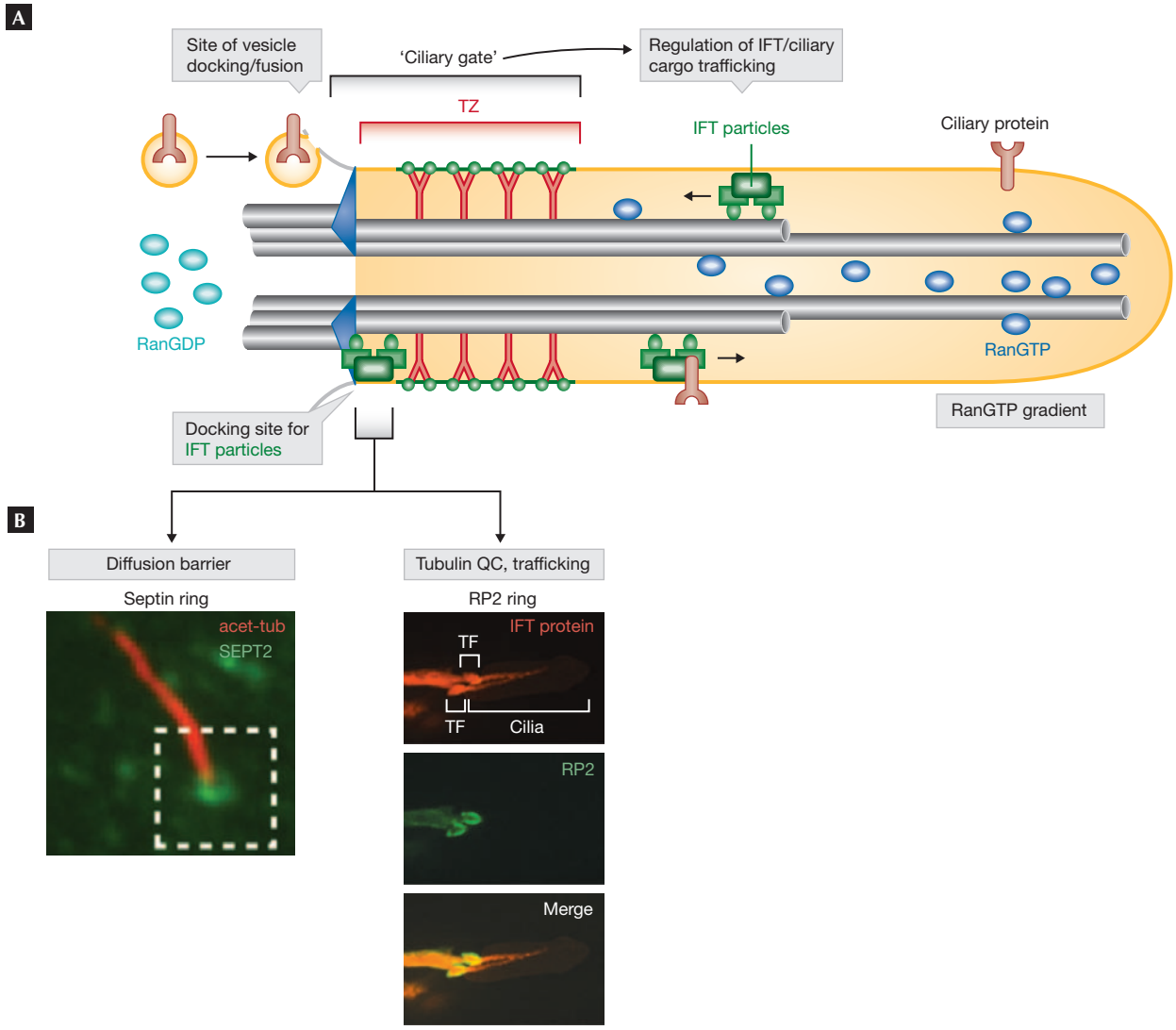


Fig 3 | Regulation of membrane diffusion and transport by ciliary gate structures present in the TF–TZ region. Vesicles bearing ciliary membrane proteins dock at the base of cilia in proximity to the TF physical barrier. Ciliary cargo might be transported into cilia by IFT particles (shown moving bidirectionally along the axoneme), which dock at the TFs and functionally interact with TZ proteins. Shown below are fluorescence images of SEPT2 (green) at the base of a mammalian cilium (marked by acetylated tubulin; red), as well as of the tubulin quality control protein RP2 (green) within the basal body (TF region; marked by IFT protein in red) of *C. elegans* cilia. A RanGDP-RanGTP gradient across the cytosol and ciliary compartment is also depicted. Refer to text for further details. SEPT2 images modified with permission from [62]; original finding of RP2 ring at base of cilia published in [61]. RP2, retinitis pigmentosa 2; SEPT2, septin 2; TF, transition fibre; TZ, transition zone.

involved in establishing these systems during ciliogenesis remain to be established. Furthermore, how these structures or functional units cooperate to ensure that the protein composition of the cilium remains optimal is not understood and requires further investigation.

Transition fibres: the 'business' end of the basal body

In many respects, transition fibres represent the functional region of the basal body. First, they serve as the main membrane attachment point for the basal body. Second, they represent a physical block to transport, as electron micrographs from multiple cell types indicate that the inter-fibre spaces are too small to allow the passage of vesicles [53–56]. Consistent with this idea, vesicles are typically not observed in cilia [57,58]. Hence, the point of transition fibre attachment to the plasma membrane defines the limit at which cilium-destined vesicles can fuse (Fig 3). Indeed, in photoreceptor cells, IFT protein- and rhodopsin-positive vesicles are found at the periciliary ridge membrane, which extends apically ~0.5 μm from the transition fibre–plasma membrane junction, and some vesicles are observed at the junction itself [42,43,59,60].

Third, various proteins are targeted to the transition fibre region. *C. elegans* and trypanosome RP2 and human SEPT2 both localize in a doughnut-like fashion (~500 nm diameter) at the ciliary base, consistent with the transition fibre ring diameter (Fig 3; [61–63]). RP2, related to the tubulin folding cofactor C, might provide quality control for tubulins before incorporation into the axoneme, and SEPT2, which we discuss below, is a component of a membrane

diffusion barrier. Immunofluorescence studies from multiple cell types and organisms show pools of IFT proteins at the ciliary base, immediately proximal to the transition zone (Figs 1B,3; [5,42,43]). Close examination using immunoelectron microscopy revealed *Chlamydomonas* IFT52 at the distal ends of transition fibres [4]. Similarly, all studied *C. elegans* IFT-associated proteins, namely core IFT and BBS proteins, concentrate within the transition fibre region (Fig 1C; [5]). In photoreceptor cells, IFT proteins are found at the centriolar barrel subdistal and distal appendages (Fig 2; [42,43]). Several IFT proteins, IFT57/88/140, are also observed at the groove in which the ciliary membrane transitions to periciliary membrane, consistent with the transition fibre attachement region [42]. Hence, transition fibres are important docking and assembly sites for IFT proteins, and are integral to cilium formation and function.

Finally, it is striking that in *C. elegans*, basal bodies degenerate almost completely post-ciliogenesis, with the exception that transition fibres persist (Fig 1A; [5,41]). At least some core centriolar proteins, for example SAS-4, disappear completely, whereas another protein implicated in cilium formation, HYLS-1, is retained [64]. It is not clear whether HYLS-1 comprises part of the transition fibre or another distal end basal body structure, but uncovering its precise localization might help elucidate its function in ciliogenesis. What is clear from the *C. elegans* findings, however, is that in the nematode at least, the distal end of the basal body, including the transition fibres, is sufficient for sustaining the function of IFT and cilia.

Function of the transition zone region as a ciliary gate

As discussed above, a transition zone forms in the early stages of ciliogenesis, probably stabilizing basal body–membrane connections and contributing to compartmentalization. There is increasing evidence that the mature transition zone functions as a ciliary gatekeeper. It has been ascribed two functions so far—a membrane diffusion barrier and a modulator of IFT.

The earliest concrete indication that the transition zone operates as a selective membrane diffusion barrier was obtained by the authors of [65], who observed the distribution of rhodopsin in mechanically dissociated rod photoreceptor cells. Vertebrate photoreceptors are comprised of a cell body, the inner segment, a transition zone often termed connecting cilium, and an expanded ciliary tip, the outer segment. Rhodopsin was found to diffuse from its normal site of action, in the outer segment, to the inner segment, but only if the two were serendipitously fused together rather than separated by the transition zone. Indeed, the diffusion rate of the membrane-associated rhodopsin to the inner segment was found to be identical to that of its usual diffusion rate within the outer segment, demonstrating that an intact transition zone limits this diffusion.

Studies in divergent species have corroborated roles for specific components of the transition zone in regulating entry or exit of membrane proteins into the cilium. In mammalian cells, several proteins display altered ciliary localization on siRNA-mediated disruption of transition zone proteins. These include membrane-associated GPI–GFP and GFP-tagged CEACAM1, which accumulate at higher levels in cilia with dysfunctional transition zones [36]. Conversely, some mammalian ciliary proteins, namely AC3 and PKD2, are lost from transition zone protein-deficient cilia [37]. In *C. elegans*, abrogation of six different transition-zone-localized proteins causes abnormal ciliary entry of the TRAM protein and membrane-associated RP2 homologues [5]. Interestingly, the cilia of one of the *C. elegans* mutants analysed, *mks-6*, shows an essentially normal cilium transition zone ultrastructure, suggesting specific disruption and malfunction, for example, of the necklace rather than complete removal of the Y-links or ciliary gate. This situation might be comparable with that found in vertebrates, in which abrogation of individual transition zone proteins, including Mks6/Cc2d2a, impairs cilium formation in some tissues but is dispensible for ciliogenesis in others [37]. Further evidence of a role for the transition zone in bidirectional gating has come from comparing the ciliary proteomes of wild-type and mutant *Chlamydomonas*. Specifically, disruption of the Y-link-localized *Chlamydomonas* CEP290/NPHP6 orthologue results in defects in the linker structures and altered composition of the motile cilia, including accumulation of the IFT-associated BBS4 protein and reduction in PKD2 [26].

Although it is striking that many transition zone proteins uncovered so far are membrane-associated (i.e. have transmembrane or lipid-interacting C2/B9 domains), consistent with a role in regulating the diffusion of membrane-associated proteins, how they do so remains unknown. One possibility, which remains to be explored, is that the transition zone proteins alter the lipid composition compared with that of the plasma membrane and perhaps the rest of the cilium, helping to restrict membrane protein diffusion. Another intriguing possibility, not mutually exclusive, is that they functionally interact with the IFT machinery, which itself is implicated in the entry and exit of proteins into cilia. In mammalian cells, the IFT protein Fleer/DYF1 interacts with a transition zone protein, B9D2/MKS10, that is necessary for the transport of opsin to the outer segment of photoreceptor cells [66]. Similarly, another transition zone localized protein, LCA5/lebercilin, associates with IFT particles and is required for opsin localization to the photoreceptor outer segment; most significantly, LCA5 mutations found in patients affect binding to IFT proteins [67]. Finally, the authors of [68] demonstrated that abrogation of the connecting cilium (transition zone)-localized NPHP1 protein impairs transport of opsins to the outer segment, coincident with the reduced presence of some, although not all, IFT proteins in this region.

In *C. elegans*, disrupting any single transition zone protein does not abrogate ciliogenesis, nor impair IFT [5,69]. However, genetics studies define two transition zone modules that are collectively required for cilium formation—an NPHP module comprised of NPHP1 and NPHP4, and an MKS module consisting of MKS1, B9D1/MKSR-1, B9D2/MKSR-2, MKS3, MKS6/CC2D2A, and probably also TMEM216/MKS2 and TMEM237/JBTS14 [5,39]. Simultaneously deleting one component from each module abrogates ciliogenesis in most cilia, because of impaired attachment of the basal body-transition zone to the membrane. In those cilia that remain, IFT velocities along axonemes are not markedly affected, suggesting that the transition zone is not absolutely necessary for IFT [5]. Hints of a functional interaction between the transition zone and IFT machinery do exist in *C. elegans*, as overexpression of OSM-6/IFT52 and loss of NPHP4 function result in a synthetic ciliogenic defect [70]. Thus, further testing for genetic and physical interactions between IFT and transition zone genes in *C. elegans* and other species, such as zebrafish, is probably a useful avenue of investigation.

Whilst evidence for regulated entry or exit of ciliary membrane proteins is mounting, the question of whether the transition zone controls the ciliary localization of all or most components deserves attention. Two recent studies addressed this question by showing that soluble proteins and dextrans above ~40–50 kDa were excluded from the *Xenopus* photoreceptor outer segment and from RPE1 primary cilia [71,72]. However, by using photoactivatable GFP, the *Xenopus*

study also showed that flux of monomeric, dimeric and trimeric GFP across the photoreceptor connecting cilium was essentially identical, thus arguing against a diffusion barrier model of exclusion for soluble proteins at the connecting cilium [71]. Consistent with these findings, a previous study showed that monomeric GFP diffusion across the connecting cilium was only moderately reduced compared with inner segment flux, and that it is much faster than outer segment flux [73]. Thus, for soluble cytoplasmic proteins, transition zone gating might not involve a diffusion barrier mechanism such as that proposed for membrane-assocated proteins.

Parallels between nucleo- and ciliocytoplasmic transport

An early suggestion that the ciliary base forms a 'flagellar and ciliary pore complex', analogous to the nuclear pore complex (NPC; [74]) was prescient, as recent studies suggest functional similarities between nucleocytoplasmic and ciliary transport machineries. Importin-β1, importin-β2/transportin, and RanBP1 are found within cilia, with a gradient of RanGTP and RanGDP across the basal body, similar to that observed across the nuclear envelope (Fig 3; [75–78]). The accumulation of RP2 and the IFT anterograde motor KIF17 in cilia requires importin-β2, and elegant experiments that use a transient fast upregulation system showed that KIF17 ciliary targeting requires a ciliary RanGTP gradient [75,78]. Moreover, RanGTP was found to promote ciliogenesis through a mechanism dependent on RanBP1 [77]. Consistent with these findings, the importin-β2 interaction with RP2 and KIF17 depends on nuclear-import-related sequences [75,78]. A recent and exciting study from the Verhey lab has now identified further NPC components—mainly nucleoporin proteins—at the base of cultured mammalian cell cilia in a region that includes the transition zone; furthermore, disruption of NPC function by using an antibody blocking approach restricts KIF17 ciliary entry [72].

Other connections between nucleocytoplasmic and ciliary transport are that numerous nuclear transport proteins are present in ciliary proteomes [79,80], and various IFT and BBS proteins share rare domain architectures—β-propeller-toroid domains—with membrane curvature-inducing and stabilizing NPC proteins [81]. As cilia are not fully bound by membrane, it remains unclear how cilium-based NPC proteins and associated nucleocytoplasmic transport machineries would regulate the ciliary entry of membrane-associated (RP2) and cytoplasmic (KIF17) proteins. One possible scenario mirrors nuclear-envelope-independent functions already known for the nucleocytoplasmic transport machinery. For example, when the nuclear envelope is broken during mitosis in *Xenopus* embryos, RanGTP concentration gradients emanating from condensed chromosomes regulate local release of spindle assembly factors from inhibitory interactions with importin-α/β complexes [82,83]. For ciliary transport, similar modes of regulation involving Ran, nucleoporins and importin proteins, could facilitate protein transport across the base of cilia near the transition fibre/transition zone gate. Further understanding requires more knowledge of the full complement of nuclear import and export components that drive ciliary transport, where exactly they localize in cilia, and how they interact with known ciliary transport, for example IFT, and with transition zone, for example MKS/NPHP, functional modules.

Septins as part of a diffusion barrier at the base of the cilium

Septins are GTPases that form large-order structures, including rings, gauzes and filaments. In budding yeast, a septin ring assembles at the bud neck during cytokinesis to prevent missegregation of organelles and proteins between mother and daughter cells [84,85]. Septins are also implicated in forming barriers in other locales, including the mammalian midbody, neural dendrite spines and sperm annulus [86,87]. To form high-order structures, septins associate with other family members. For example, in sperm, septin 7 co-localizes with septin 4 at the annulus [88,89].

The annulus forms early in sperm flagellum biogenesis at the base, where it might help anchor the overlying membrane to the flagellum [90]. Septin 4 localizes to this annulus and is essential for male mouse fertility; mice lacking this protein show retention of cytoplasm and defects in flagellar kinesin localization [88,89]. Septin 4 also controls the levels and distribution of basigin, a dynamically regulated flagellar component, consistent with an essential role for septin 4 as part of a flagellar diffusion barrier.

Septin 2, which associates with septin 7, localizes at the ciliary base (Fig 3; [62,91]). In IMCD3 mouse kidney cells, GFP fusions of several transmembrane proteins, including serotonin receptor 6, Smoothened and fibrocystin, localize to the cilia. Fluorescence recovery after photobleaching indicates that there is little exchange between the ciliary and nonciliary pools of these transmembrane proteins, despite both populations being mobile [12]. Knockdown of septin 2 causes defects in ciliogenesis, and the short cilia that do form show increased entry of ciliary transmembrane proteins [12]. Thus, septin 2 probably functions as an important part of a diffusion barrier at the base of the cilium.

In *Xenopus*, septin 2 is expressed with septin 7 during gastrulation, when it stabilizes the plasma membrane and coordinates convergent extension [92]. Similar to septin 2 in IMCD3 cells, septin 7 forms a ring at the base of motile cilia in the *Xenopus* epidermis, and knockdown results in fewer and shortened cilia. Consistent with an essential role for cilia in vertebrate Hedgehog signal transduction, knockdown of either septin 2 or septin 7 abrogates Hedgehog signalling [62,92]. Thus, septin 2 and septin 7, which can interact, could partner to participate in the diffusion barrier at the ciliary base.

Sidebar A | In need of answers

(i) What is the full complement of proteins that make up transition fibres and the different elements of the transition zone, for example Y-links and ciliary necklace?

(ii) Can the transition fibres or Y-links be isolated biochemically to ascertain composition?

(iii) What are the precise three-dimensional shapes of transition fibres and Y-links? Cryo-electron microscopy reconstruction analyses could provide sufficient structural details such that, ultimately, crystal structures of individual components could be 'fitted' or modelled into the macromolecular assemblies. Understanding the spatial relationships between the various components is necessary for understanding, at a molecular level, the potentially diverse functions of the ciliary gate structures.

(iv) What are the interaction partners of proteins present in the transition fibre–transition zone region? Some of these might bind only transiently, play roles in the formation of the ciliary gate, or could regulate docking and transport of intraflagellar transport particles.

(v) Where exactly, in the context of the transition fibres and transition zone, are the septin and RP2 rings localized, and how might they collaborate with these ciliary domains in ciliary gating or transport?

(vi) To what extent do nuclear pore-associated proteins regulate ciliary transport? Is it only to modulate ciliary entry of the kinesin motor KIF17 (OSM-3) and RP2, or other proteins as well?

The findings raise interesting questions. How might septins promote ciliogenesis? Septin 2 and septin 7 can associate with and arrange the distribution of microtubules to control vesicular transport [93,94]. Do septins coordinate the distribution of microtubules required to deliver ciliary components to the basal body, or stabilize the microtubules of the cilium itself? If so, this might explain how septins promote ciliogenesis. Given that the authors of [36] demonstrated mislocalization of transition zone proteins on siRNA knockdown of septin 2, it will be interesting to further probe the functional relationship between the ciliary septin ring and the transition zone. For example, are they part of the same or are they distinct mechanisms for regulating ciliary composition?

Conclusions

It has been known for over 30 years that the transition fibres, together with the transition zone Y-links, represent evolutionarily conserved subdomains of the basal body–ciliary organelle that connect microtubules to the ciliary membrane [15]. We have gained valuable insights into their roles in cilium biogenesis, maintenance and functions. Yet, a deeper understanding of the molecular mechanisms of transition fibre–transition zone function is only possible once we enumerate their components and define their macromolecular organization (Sidebar A). Furthermore, continuing to uncover the specific functional associations between transition fibre and transition zone components, and other ciliary components as well as transport machinery, for example IFT, will help to elucidate their crucial roles in not only the formation, but also the function of the cilium.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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