HHS Public Access

Author manuscript

*Curr Opin Cell Biol*. Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

*Curr Opin Cell Biol*. 2015 August ; 35: 98–105. doi:10.1016/j.ceb.2015.04.015.

# The essential roles of transition fibers in the context of cilia

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## Abstract

Once thought of as a vestigial organelle, the primary cilium is now recognized as a signaling hub for key cellular pathways in vertebrate development. The recent renaissance in cilia studies significantly improved our understanding of how cilia form and function, but little is known about how ciliogenesis is initiated and how ciliary proteins enter cilia. These important ciliary events require transition fibers (TFs) that are positioned at the ciliary base as symmetric nine-bladed propeller fibrous structures. Up until recently, TFs have been the most underappreciated ciliary structures due to limited knowledge about their molecular composition and function. Here, we highlight recent advances in our understanding of TF composition and the indispensable roles of TFs in regulating the initiation of ciliogenesis and the selective import of ciliary proteins.

## Introduction

Microtubule-based cilia fulfill important sensory functions in most eukaryotic cells and are critical in vertebrate embryonic development and tissue homeostasis [1, 2]. Cilia dysfunction is correlated with an expanding spectrum of human genetic diseases (collectively termed ciliopathies) [3, 4]. Since cilia are ubiquitous on cell surfaces, most ciliopathies occur as syndromic disorders that affect many vital organs during development, including the central nervous system (CNS), eyes, cardiovascular system, kidney, liver, limbs, bones, and fat storage tissue. Cilia dysfunction might affect as many as 100 human disorders [4, 5]. Ciliopathies are probably the fastest growing category within human disease family: ~60 new causal loci were identified in last decade, and more are suspected [6].

Despite the physiological and clinical relevance of cilia, our understanding of how cilia form remains poor, and several key questions remain to be answered. For example, how is ciliogenesis initiated? At a morphological level, the mother centriole must dock to the membrane to initiate ciliogenesis. In different cell types, the mother centriole first attaches to vesicles, presumably Golgi-derived, in smooth muscle and endothelial cells [7] or the cell

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membrane in some epithelial cells [8, 9]. Distal appendages (DAs), the fibrous structure at the distal end of the mother centriole, might mediate the centriole-membrane docking [10]. During the docking, the mother centriole transforms into the basal body, and its DAs mature into TFs. After that, the ciliary axoneme begins to elongate with the assistance of intraflagellar transport (IFT) machinery, which moves bidirectionally inside cilia to transport ciliary proteins essential for cilia formation, maintenance, and signaling [11–13].

As another question, how are cilia functionally separated from the cytosol? Unlike other membrane-enclosed cellular organelles, the cilium is open to the cytoplasm at its base. The ciliary base needs to control the selective entry of ciliary proteins and, thus, functionally separates the cilium from the cell body and makes it a discrete sensing organelle. The morphology of the ciliary base is highly conserved [14]: a basal body with fibrous apparatuses TFs and basal feet, and the transition zone (TZ, the proximal part of the axoneme that contains Y-links) (Fig. 1). TFs form a 9-bladed propeller-like structure, and their anchoring points with the membrane define the border between the plasma membrane and the ciliary membrane [10]. Above the TFs, the Y-links of the TZ connect axonemal microtubules to the ciliary membrane [15]. The distinct locations of TFs and the TZ make them good candidates for the enigmatic ciliary gate or “ciliary pore complex” that regulates the selective import of the ciliary proteins [13, 14].

For many years, many roles were proposed for TFs in ciliogenesis initiation [10] and cilia import [13, 16, 17]. However, only recently, characterization of the molecular identities of DAs/TFs made it possible to elucidate and confirm the roles of TFs at a molecular level. Here, we review the insights that establish and expand our views of TFs as the indispensable structures in the context of cilia.

## TF composition and assembly

So far, five proteins have been identified as genuine DA/TF components. These include CEP164, CEP83 (CCDC41), CEP89 (CCDC123), SCLT1 (sodium channel and clathrin linker 1) and FBF1 (Fas (TNFRSF6) binding factor 1) [18–23]. CEP83 was reported to regulate the TF targeting of the other four proteins, and SCLT1 specifically affects the localization of CEP164 and FBF1 (Fig. 2) [20]. However, the strict role of CEP83 in regulating the TF localization of CEP164 has been questioned [23]. Also Cby (Chibby) and TTBK2 (Tau tubulin kinase 2), two newly identified CEP164 interactors, localize to TFs and regulate ciliogenesis in different types of mammalian cells [24, 25]. In super-resolution studies, the CEP164 ring is actually larger and more proximal than the Cby ring at the distal end of the mother centriole, suggesting that Cby attaches apically on TFs [24].

The key structural TF components have not been determined due to the fact that the electron density of TFs in mammalian cells is too low for conclusive EM studies. TTBK2 and Cby are likely effectors of CEP164 that are recruited to TFs during ciliogenesis [24, 25]. Since TFs exist in all ciliated organisms, the core structural component(s) should be evolutionarily conserved. However, of the other five components, only the homolog of FBF1 can be found in the genome of non-vertebrate ciliated organism (Table 1), and depletion of DYF-19, the worm homolog of FBF1, does not affect TF biogenesis [21]. These observations suggest that

either some of the identified TF components are only mammalian-specific TF structural component(s), or more likely, the seven identified candidates are just the functional components of TFs, and the key structural components of TFs remain to be identified.

Several proteins have been implicated in the proper formation of DAs on mother centrioles. These include OFD1 (oral-facial-digital syndrome 1), C2CD3 (C2 calcium-dependent domain containing 3), ODF2 (outer dense fiber 2), and DZIP1 (DAZ-interacting zinc finger protein 1). Yet none of them appears to be the TF component [26–31] (Fig. 2). OFD1 localizes to both centrioles and centriolar satellites and seems to have different roles in different locations. In murine OFD1-lacking ES cells, centriole distal ends elongate abnormally and show severe defects in distal appendage formation and ciliogenesis [27]. Conversely, autophagy-dependent depletion of OFD1 from centriolar satellites can actually promote ciliogenesis independent of the role of centriolar OFD1 [32]. C2CD3, another OFD syndrome protein, might physically interact with OFD1, regulate DA formation, but functionally antagonize OFD1 in centriolar elongation [28, 29]. ODF2 and DZIP1 are required for the assembly of both sub-distal appendages (sub-DAs) and DAs [26, 30, 31]. Therefore, although the mechanisms underlying TF malformation are not clear when these proteins are depleted, they are probably secondary to other centriole anomalies, such as a malformed distal end or altered outer wall structure of the centriole. Notably, ODF2's regulation of DA formation was challenged: when ODF2 was knocked down, none of DA components was missing from the ciliary base [20]. Whether the discrepancy is due to the efficiency of RNAi knockdown or differences in cell types is unknown.

## TFs and ciliogenesis initiation

As seen in transmission EM studies, the earliest event in ciliogenesis is the docking of the mother centriole to the primary cilia vesicle (PCV) or the apical membrane [7–9]. Most recent studies have focused on the roles of TF components in centriole-vesicle-docking. Depleting CEP164, CEP83, CEP89, or Cby leads to defects in either centriole-vesicle-docking or PCV formation [20, 23, 24, 33, 34]. Intriguingly, a group of small vesicles called the distal appendages vesicles (DAVs) was recently suggested to first anchor to the mother centriole. Then the membrane shaping proteins EHD1 and a SNARE membrane fusion regulator SNAP29 regulate DAV-mediated PCV formation (Fig. 3) [35]. How the DAVs and the PCV dock on DAs/TFs is unknown. After PCV formation, the physical association between the CEP164-Cby complex and the Rabin8/Rab8 trafficking machinery likely recruits more Rab8-positive vesicles to TFs to ensure PCV extension and support axoneme elongation [24, 33, 35, 36] (Fig. 3).

In addition to their indispensable role in membrane docking, TFs are required to initiate axoneme elongation. When cells exit the cell cycle to form cilia, CEP164 recruits TTBK2 to TFs [25]. Once associated with TFs, TTBK2 regulates the removal of microtubule cap protein CP110 and the recruitment of IFT components [37]. In TF-defective cells, CP110 is not removed from the distal end of the mother centriole, and ciliogenesis is blocked [20, 25, 29]. Although both are regulated by CEP164, TTBK2 recruitment and CP110 removal appear to occur before Rab8-dependent membrane extension in the early steps of ciliogenesis [35] (Fig. 3).

## TFs, diffusion barrier, and the ciliary gate

Mounting evidence suggests that diffusion barriers exist for either membrane [38] or soluble proteins [39–42] at the ciliary base. For soluble proteins, different approaches gave different estimations for the physical size or the diffusion rate of the barrier. Proteins larger than 100 kDa are commonly assumed not to efficiently pass through the ciliary base without the assistance of active importing mechanisms. A group of proteins mutated in different ciliopathies, including NPHP, MKS, and JBTS syndromes, form multimeric complexes to regulate the integrity of the TZ and the function of the diffusion barrier [43–48]. In *C. elegans*, the TRAM protein abnormally enters cilia when the integrity of the TZ is compromised [46]. In mammalian cells, the plasma membrane proteins GFP-CEACAM1 and GFP-GPI accumulate abnormally inside cilia when the TZ was disrupted [44]. However, the role of TFs as part of the diffusion barrier for the ciliary proteins has not been directly confirmed. Unlike with TZ, disruption of TFs completely abolishes ciliogenesis initiation and would thus make it impossible to study whether TFs act as part of diffusion barrier or not. To this end, a hypomorphic mutant that only partially disrupts TF integrity or the identification of structural component(s) of the barrier on or near TFs would answer the question.

The existence of a diffusion barrier at the ciliary base indicates that active transport is required for the import of large proteins. Remarkably, the IFT complex is a large multimeric complex comprising >20 proteins [11–13], with many components larger than 100 kDa. Some IFT cargoes, such as radial spokes and dynein arms, are also large protein complexes assembled before they enter cilia [49, 50]. Thus, certain mechanism must facilitate the ciliary import of the IFT machinery. The discovery of the pore-like structures formed by the TFs and the TZ (Fig. 1) led to the hypothesis that they are analogous to the nuclear pore complex (NPC) and may act as a passive diffusion barrier and an active cilia gate to actively transport soluble proteins into cilia [13, 17]. Immuno-EM studies revealed that the IFT-B component IFT52 clusters along the TFs and especially where the TFs connect to the flagellar membrane in *Chlamydomonas* [16]. Recruitment of IFT proteins is abnormal in CEP164- and CEP83-deficient cells [20, 23, 25, 33]. All these data lead to the assertion that TFs might be the site for actively regulating the ciliary entry of IFT particles.

Recently, a study in *C. elegans* revealed how TFs facilitate the ciliary entry of assembled IFT particles. DYF-19 (the homologue of human FBF1) facilitates the ciliary import of assembled IFT particles through direct interaction with IFT component DYF-11 (the homologue of human IFT54) (Fig. 3) [21]. FBF1 (DYF-19) might provide a docking site on TFs for the assembled IFT particles. After docking, the proposed ciliary pore complex (CPC), possibly positioned at inter-fiber space of TFs, facilitates the import of assembled IFT particles. The molecular identities of the CPC remain mysterious, and molecular evidence supports [40, 51, 52] or challenges [42] the similarity of the CPC and NPC. It is worth determining if this active transport mechanism applies to other non-IFT ciliary proteins, how the CPC recognizes the cilia-targeting signal of ciliary proteins, and what is the functional and structural relationship among the CPC, TFs, and the TZ.

## TFs and cilia-targeted vesicular trafficking

Apart from the roles in guiding ciliogenesis initiation and membrane extension, cilia-related vesicles presumptively regulate the transport of ciliary proteins from post-Golgi network or recycling endosomes to the periciliary membrane [13, 39, 53]. Small GTPases along with their regulators have been implicated in regulating the sorting, docking, or fusion of vesicles at the ciliary base [54, 55]. In particular, Rab8 has been implicated in facilitating cilia trafficking by several studies [36, 56–61]. Notably, ultrastructure studies also suggest that polarized exocytosis takes place at the base of either *Ochromonas* flagella or photoreceptor cell connecting cilia [62, 63]. Polarized exocytosis adjacent to the basal body has been widely accepted as an early step for the ciliary import of membrane proteins [13, 39, 53]. Multiple components of the exocyst, a multiprotein complex that mediates the polarized exocytosis, localize at the ciliary base or on the ciliary membrane, interact with either Rab11 or Rab10, and are required for ciliogenesis [64–67]. Since the Rab11-Rab8 small GTPase cascade and the TFs are involved in ciliogenesis initiation, it would be interesting to determine if Rabs and the exocyst also regulate the docking of ciliary vesicles on TFs and the polarized exocytosis at the base of mature cilia.

## Tight connection between TFs and ciliopathies

Consistent with the important roles of TFs in the context of cilia discussed here, many identified TF components were recently identified as causal loci for specific human ciliopathies (Table 1). Mutations in CEP164 (also called NPHP15) and CEP83 (also called NPHP18) cause nephronophthisis-related ciliopathies (NPHP-RC) [68, 69]. SCLT1 is mutated in patients with orofaciodigital syndrome (OFD) type IX [70], and mutations in TTBK2 cause neurodegenerative disease spinocerebellar ataxia type 11 [37]. In addition, mutations in OFD1 and C2CD3, two centriole proteins required for TF formation, are associated with OFD syndrome [28]. It will not be surprising to see that other identified TF components are identified as causal loci for certain ciliopathies in the near future. Exploring how TF components and their modifiers/effectors function *in vivo*, as well as their functional crosstalk with other characterized ciliary proteins, especially ciliopathy proteins, will greatly enhance our understanding of the role of TFs in the pathogenesis of various ciliopathies.

## Perspectives

In recent years, our understanding of TFs in the context of cilia has deepened significantly. However, many questions remain unanswered and will likely be the focus of future research. First, what are the structural components of DAs/TFs? Given the highly conserved structure of cilia and flagella during evolution, TFs are not likely built from different structural parts in different ciliated species. Thus, dissecting the interaction network of highly conserved TF components, such as FBF1 and TTBK2, will likely have a greater chance of identifying the *bona fide* structural components of TFs. Second, what signals trigger the initial docking of DAVs at the tip of the mother centriole? When cells exit the cell cycle and form cilia, a checkpoint must initiate recruitment of DAVs to the DAs of the mother centriole. Only after this checkpoint can the mother centriole mature into the basal body and the DAs transform into TFs, which ultimately support the formation of the sensory antenna for the cells. Third,

while we have amassed a reasonable candidate list for the roles of TFs in basal body docking, ciliary gate formation, vesicle trafficking, and IFT docking and sorting, how are these players are integrated to execute their function? Different protein modules are likely responsible for different aspects of TF function. For example, CEP164 and its interacting partners regulate basal body docking and the initiation of ciliogenesis; while the FBF1 protein network regulates active import through the ciliary gate. Finally, how do TFs molecularly regulate the direct docking of the mother centriole to the cell membrane in those cell types that do not use DAV-PCV vesicle route in ciliogenesis initiation? To this end, identification of structural and other functional components of TFs and a better understanding of how TF dysfunctions lead to human ciliopathies will provide seminal insights into our understanding of cilia development and function in normal and pathological states, and represent an interesting challenge for cell biology.

## Acknowledgments

We thank our many colleagues who have contributed to the work described herein, and apologize to those whose work we could not cite due to the space issue. We are grateful for funding from the National Institutes of Health research grant 1R01DK090038, 1R01DK099160-01A1, and P30 center grant P30DK90728. K.L. was supported by the National Cancer Institute (NCI; 1R01CA149039-01A1), Susan G. Komen for the Cure (KG100902).

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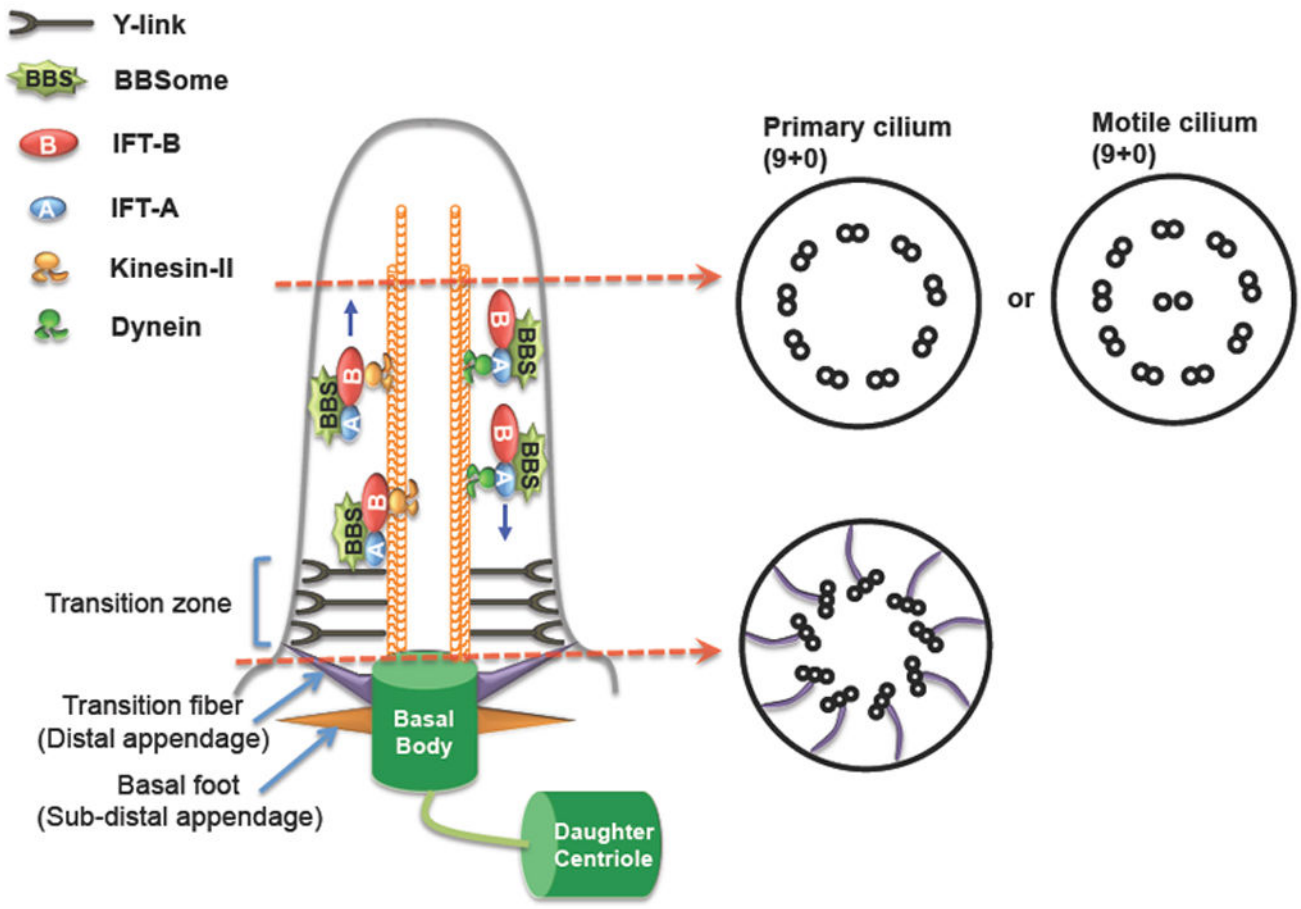


**Figure 1. Schematic diagram of a cilium**

All cilia have a microtubule-based core structure, called the axoneme, which projects from the basal body and is tightly surrounded by the ciliary membrane. Based on the motility, cilia can be divided into primary cilia (non-motile cilia) and motile cilia. The axoneme of primary cilia typically consists of nine microtubule doublets (9+0), typical motile cilia have an extra pair of microtubule singlet in the center of the ring of nine outer doublets (9+2). The basal body is transformed from the mother centriole during ciliogenesis. At the ciliary base, there are two structurally distinct sub-regions: TFs and the TZ. TFs are analogous to DAs of the mother centriole and form a 9-bladed propeller-like structure linking the basal body to the ciliary membrane. Basal feet (analogous to sub-DAs) locate below TFs on the basal body. Above TFs is the TZ that is characterized by the Y-links connecting axoneme microtubules to the ciliary membrane. Extension, maintenance and function of cilia require intraciliary transport machinery IFT, which is composed of IFT-A, IFT-B, BBSome and motors. IFT moves bidirectionally along the ciliary axoneme to transport cargos into or out of cilia. TFs, transition fibers; TZ, transition zone; DAs, distal appendages; IFT, Intraflagellar transport; Sub-DAs, sub-distal appendages.

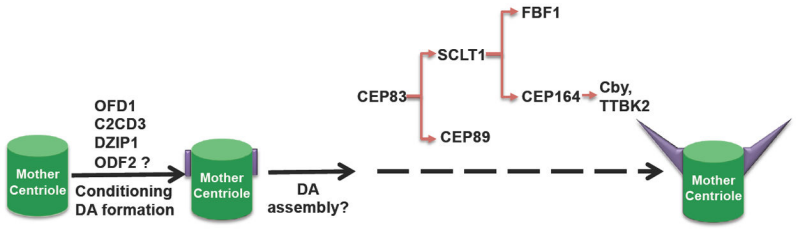


**Figure 2. The assembly of distal appendages/transition fibers**

OFD1, C2CD3, DZIP1, and ODF2 condition the distal end of the mother centriole for DA formation. Then different structural/functional components of DAs/TFs are recruited in a sequential manner. DAs, distal appendages; TFs, transition fibers.

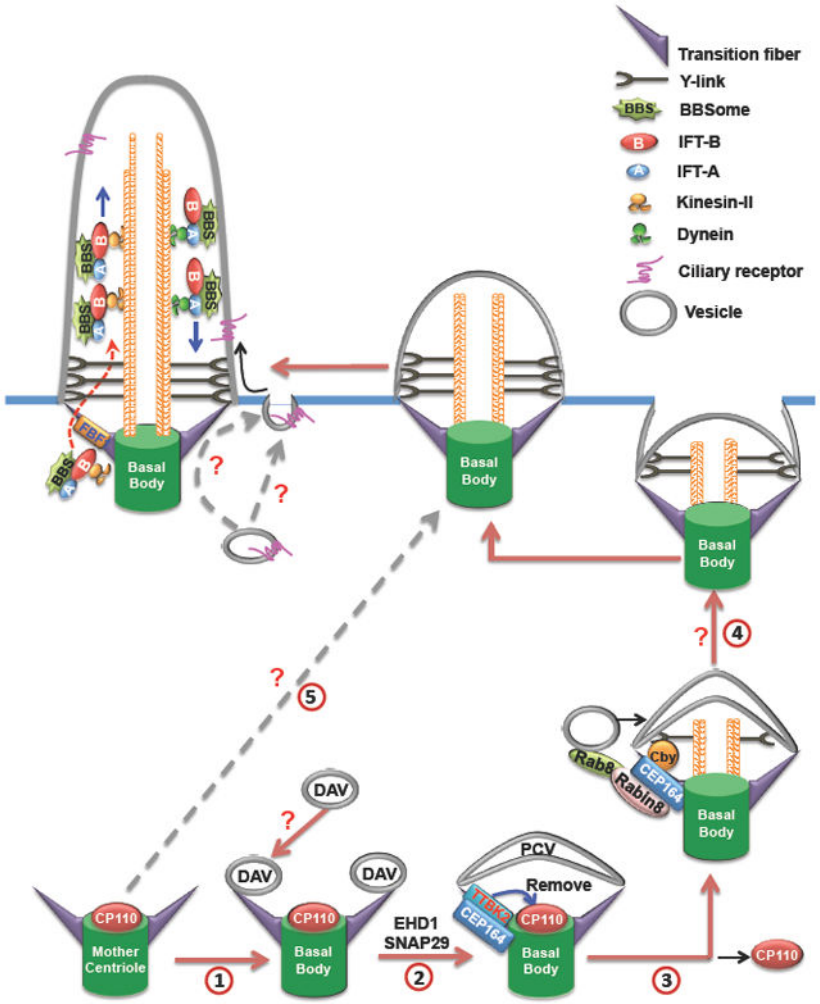


**Figure 3. The roles of transition fibers in the context of cilia**

In some mammalian cell types, during the very early stage of ciliogenesis, small DAVs first dock to the DAs of the mother centriole through an unknown mechanism (1). EHD1 and SNAP29 then regulate the fusion of DAVs into the large PCV (2). During the formation of PCV, TTBK2 is recruited to TFs by CEP164 to remove microtubule cap protein CP110 to initiate axoneme elongation. Then the interaction between Rabin8/Rab8 complex and CEP164/Cby complex mediates the recruitment of more Rab8 positive vesicles to support membrane extension of the PCV (3). Meantime, the TZ starts to form, the basal body-PCV migrates to the plasma membrane, and then the PCV fuses with the cell membrane through an unknown mechanism (4). Lastly, IFT regulates the extension of the axoneme. FBF1 acts as the functional component on TFs to facilitate the ciliary import of assembled IFT complex. Polarized vesicle trafficking and exocytosis have been implicated in mediating ciliary cargos targeting to periciliary membrane, and then these cargos enter into cilia through lateral diffusion. But, whether TFs play a role in this process is not clear. In other cell types, an alternative ciliogenesis pathway (5) may be employed: the basal body directly docks to the plasma membrane independent of DAV/PCV route. TFs, transition fibers; TZ,

transition zone; DAs, distal appendages; DAVs, distal appendage vesicles; PCV, primary ciliary vesicle.

**Table 1**

Function and associated diseases of reported transition fiber related proteins

| | Protein name | Conservation in ciliated model organisms* | Mutation-related diseases | TF formation | Function | References |
|---|---|---|---|---|---|---|
| **TFs proteins** | CEP164 | *Hs, Mm, Dr, Dm* | Nephronophthisis | No | Basal body docking | [18, 20, 24, 25,32, 67] |
| | CEP83 | *Hs, Mm, Dr,* | Nephronophthisis | No | Basal body docking | [20, 23, 68] |
| | SCLT1 | *Hs, Mm, Dr,* | Orofaciodigital syndrome | No | | [20, 69] |
| | CEP89 | *Hs, Mm, Dr, Dm* | | | Vesicle formation | [20, 22, 33] |
| | FBF1 | *Hs, Mm, Dr, Dm, Ce* | | No | IFT entry | [20, 21] |
| | TTBK2 | *Hs, Mm, Dr, Dm,Ce* | Spinocerebellar ataxia | No | Ciliogenesis initiation | [25, 36] |
| | Chibby | *Hs, Mm, Dr, Dm* | | No | Vesicle formation, basal body docking | [24] |
| **TF-related proteins** | OFD1 | *Hs, Mm, Dr, Dm?* | Oral-facial-digital syndrome | Yes | Inhibit centriole elongation | [27] |
| | C2CD3 | *Hs, Mm, Dr, Dm, Ce?* | Oral-facial-digital syndrome | Yes | Promote centriole elongation | [28, 29] |
| | ODF2 | *Hs, Mm, Dr, Dm,* | | Yes | Formation of both distal appendages and sub-distal appendages | [26, 31] |
| | DZIP1 | *Hs, Mm, Dr, Dm,* | | Yes | Formation of both distal appendages and sub-distal appendages | [30] |

Note: *Hs, Homo sapiens; Mm, Mus musculus; Dr, Danio rerio; Dm, Drosophila melanogaster; Ce, Caenorhabditis elegans.*

*, Conservation data are based on literatures and NCBI BLASTP.

?, Potential homolog with low sequence similarity