Roles of kinesin-2 motor proteins involved in intraciliary protein trafficking

2017

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GENERAL INTRODUCTION

Cilia are microtubule-based organelles protruding from the surface of most eukaryotic cells and serve as cellular antennae by mechanosensation of extracellular stimuli, such as fluid flow, and chemosensation of developmental signals, such as the Hedgehog (Hh) signal (Mukhopadhyay and Rohatgi, 2014; Bangs and Anderson, 2017). To perform these functions, cilia contain a large number of unique proteins, including a variety of G protein–coupled receptors (GPCRs) and ion channels. The crucial roles of cilia as cellular sensory antennae have been highlighted by findings that defects in ciliary assembly and functions result in a variety of hereditary disorders, collectively called the ciliopathies, with a broad spectrum of symptoms, including retinal degeneration, polycystic kidney, morbid obesity, and polydactyly (Schwartz *et al.*, 2011; Madhivanan and Aguilar, 2014; Braun and Hildebrandt, 2017). Although the ciliary membrane is continuous with the plasma membrane, protein and lipid compositions of cilia differ greatly from those of the cell body, because the ciliary transition zone (TZ), which is located at the ciliary base, serves as a diffusion/permeability barrier (Sung and Leroux, 2013; Wei *et al.*, 2015). Proteins required for the assembly and functions of cilia are transported from the cell body. The transport process includes entry of the proteins into cilia across the TZ and trafficking of proteins along axonemal microtubules within cilia.

The ciliary TZ is structurally characterized by the presence of transition fibers and Y-linked structures, which have been proposed to constitute a barrier similar to the nuclear pore complex (NPC; Takao and Verhey, 2016). Therefore, some ciliary proteins, such as KIF17 and RP2, contain a nuclear localization signal (NLS) and enter the cilium by a mechanism dependent on the Ran GTPase and importin β2 (also known as transportin 1 [TNPO1]; Dishinger *et al.*, 2010; Hurd *et al.*, 2011).

On the other hand, bidirectional trafficking of ciliary proteins along the axoneme is mediated by intraflagellar transport (IFT) particles containing two large multisubunit complexes, IFT-A and IFT-B, powered by the kinesin-2 and dynein-2 motor proteins. At the ciliary base, the IFT-A and IFT-B complexes assemble to form IFT particles, to which kinesin-2 and dynein-2 bind via the IFT-B and IFT-A complexes, respectively (Fig. 1) (Taschner *et al.*, 2012; Lechtreck, 2015; Katoh *et al*., 2016; Hirano *et al.*, 2017; Nakayama and Katoh, 2018). Subsequently, ciliary soluble cargo proteins,

including α/β-tubulin dimers, are loaded onto the assembled particles, and membrane cargo proteins, such as GPCRs, are connected to the IFT-A complex through TULP3, which interacts with the IFT-A complex (also see Fig. 2A). The IFT particles then enter the cilium across the TZ and undergo anterograde trafficking along the axoneme powered by the kinesin-2 motor. At the ciliary tip, IFT particles are thought to disassemble to release cargo proteins, and kinesin-2 is inactivated. IFT particles are then reassembled and cargoes are loaded, and the assembled particles undergo retrograde trafficking powered by the dynein-2 motor. The particles exit the cilium across the TZ and are disassembled.

Our laboratory recently demonstrated the overall architecture of the IFT-A and IFT-B complexes. The IFT-A complex can be divided into the core subcomplex composed three subunits (IFT122/140/144), which interacts with TULP3, and the peripheral subcomplex also composed of three subunits (IFT43/121/139) (Fig. 2A) (Hirano *et al.*, 2017; Nakayama and Katoh, 2018). The IFT-B complex can also be divided into the core and peripheral subcomplexes, which are composed of 10 subunits (IFT22/25/27/46/52/56/70/74/81/88) and 6 subunits (IFT20/38/54/57/80/172), respectively; these subcomplexes are linked by the connecting tetramer involving composite interactions of IFT38/52/57/88 (Fig. 2B) (Katoh *et al.*, 2016; Nakayama and Katoh, 2018). Deficiency in any of the IFT-B subunits often leads to extremely short or no cilia, indicating a crucial role of the trafficking of ciliary proteins in assembly of cilia.

Two different types of kinesin-2 motors have been implicated in ciliary anterograde protein trafficking; heterotrimeric kinesin-II composed of motor subunits, KIF3A and KIF3B, and an accessory protein KAP3 (KLP-20, KLP-11, and KAP-1 in *Caenorhabditis elegans*), and homodimeric KIF17 (OSM-3 in *C. elegans*) (Scholey, 2013). In *C. elegans*, heterotrimeric kinesin-II and the OSM-3 homodimer have been shown to participate in IFT within the axoneme proximal/middle segment and the distal segment, respectively (Pan *et al.*, 2006; Scholey, 2013; Prevo *et al.*, 2015). However, in vertebrates, differential roles of the two types of the kinesin-2 motors in ciliary anterograde protein trafficking are unclear. Furthermore, although previous studies suggested that these kinesin-2 motors interact with the IFT-B complex (Baker *et al.*, 2003; Snow *et al.*, 2004; Insinna *et al.*, 2008; Howard *et al.*, 2013; Liang *et al.*, 2014), it

remains uncertain which IFT-B subunit(s) directly participate in the interaction with these kinesin-2 motor proteins.

In the present study, I set out to determine the subunit(s) of the IFT-B complex involved in its interaction with kinesin-2 motor proteins and elucidate the differential roles of these kinesin-2 motors in ciliary protein trafficking. I revealed that KIF17 interacts with the IFT-B complex through the IFT46–IFT56 dimer and is a cargo of, rather than serving as an anterograde motor for, the IFT-B complex (Chapter 1). On the other hand, I found that heterotrimeric kinesin-II interacts with the IFT-B complex through the connecting tetramer and showed that the interaction between heterotrimeric kinesin-II and the IFT-B-connecting tetramer is crucial for ciliogenesis (Chapter2).

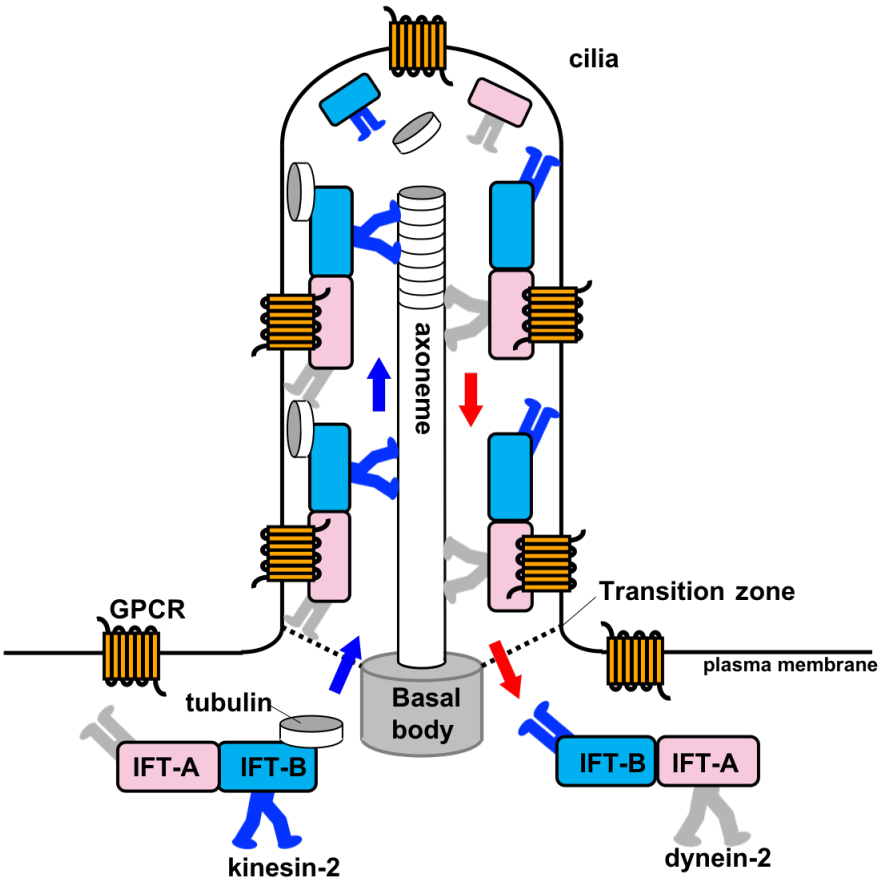

Fig. 1. Schematic illustration of a cilium and IFT.

Cilia are the microtubule-based structures projecting from the surface of most eukaryotic cells. Bidirectional trafficking of ciliary proteins along the axoneme is mediated by IFT particles, which contain the IFT-A and IFT-B complexes. At the base of cilia, the IFT-A and IFT-B complexes assemble to form IFT particles. Kinesin-2 and dynein-2 bind to IFT-B and IFT-A, respectively. Subsequently, ciliary soluble cargo proteins, such as α/β-tubulin dimers, are loaded onto the assembled particles, and membrane cargo proteins, such as GPCRs, are connected to the IFT-A complex via TULP3, which interacts with the IFT-A core subcomplex (also see Fig. 2A). The particles then enter the cilium across the TZ and undergo processive anterograde trafficking along the axoneme powered by heterotrimeric kinesin-2. At the ciliary tip, IFT particles are thought to disassemble to release cargo proteins, and kinesin-2 undergoes inactivation. IFT particles are then reassembled and cargos are loaded, and the assembled particles undergo retrograde trafficking powered by dynein-2. The particles exit the cilium across the TZ.

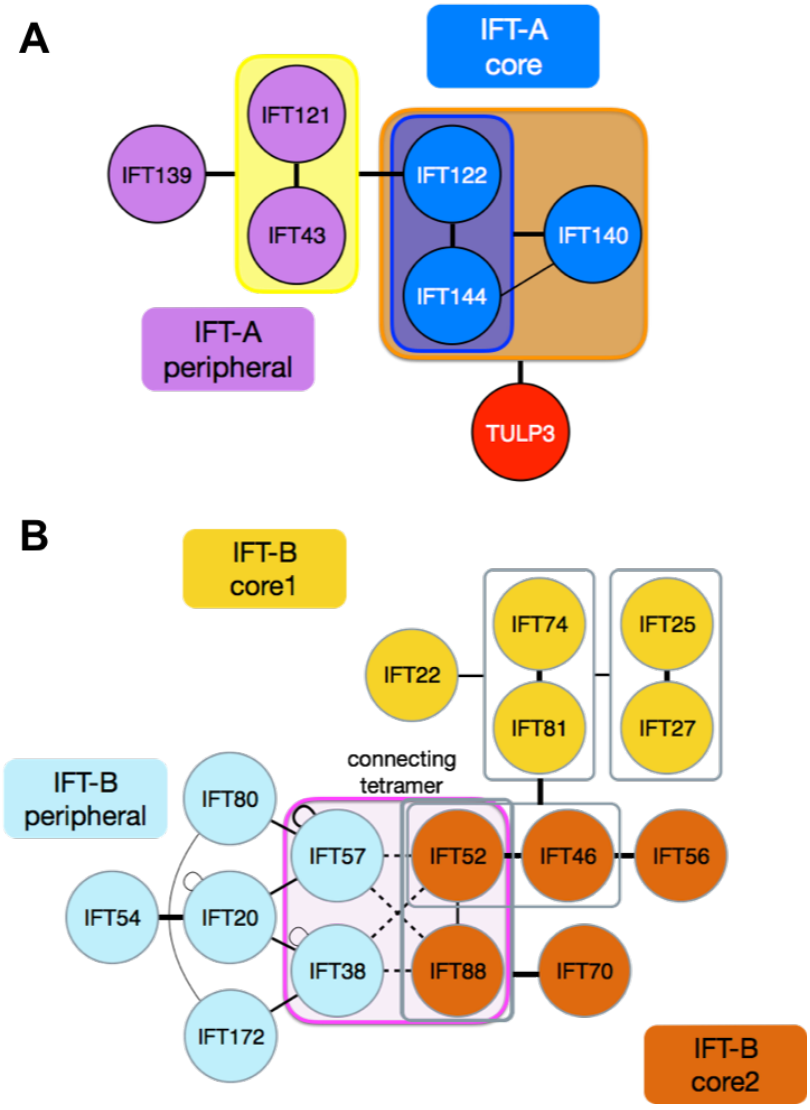

Fig. 2. Architectures of the IFT-A and IFT-B complexes

Architectures of the IFT-A and IFT-B complexes predicted from our previous studies (Katoh *et al.*, 2016; Hirano *et al.*, 2017; Nakayama and Katoh, 2018). (A) The IFT-A complex can be divided into the core subcomplex composed of IFT122/140/144, which interacts with TULP3, and the peripheral subcomplex composed of IFT43/121/139. (B) The IFT-B complex are also composed of the core and peripheral subcomplexes. The former is composed of 10 subunits (IFT22/25/27/46/52/56/70/74/81/88) and the latter is composed by six subunits (IFT20/38/54/57/80/172); these subcomplexes are linked by composite interactions involving the connecting tetramer composed of IFT38/52/57/88.

Chapter 1: KIF17 is a cargo of the IFT-B complex rather than being a motor for the IFT machinery

ABSTRACT

Cilia function as cellular antennae to sense and transduce extracellular signals. A number of proteins are specifically localized in cilia. Anterograde and retrograde ciliary protein trafficking are mediated by the IFT-B and IFT-A complexes in concert with kinesin-2 and dynein-2 motors, respectively. However, the role of KIF17, a homodimeric kinesin-2 protein, in protein trafficking has not been fully understood in vertebrate cilia.

In this chapter, I first demonstrated by taking advantage of the visible immunoprecipitation (VIP) assay, that KIF17 interacts with the IFT46–IFT56 dimer in the IFT-B complex through its C-terminal sequence located immediately upstream of the NLS. I then showed that KIF17 requires IFT-B binding for its entry into cilia, rather than for its intraciliary trafficking. I further showed that KIF17 ciliary entry is dependent not only on its binding to IFT-B but also on its NLS, to which importin α proteins bind. Taken together, I conclude that in mammalian cells, KIF17 is dispensable for ciliogenesis and IFT-B trafficking, but requires IFT-B as well as its NLS for its ciliary entry across the TZ located at the ciliary base.

Chapter 2: Heterotrimeric kinesin-II is essential for ciliogenesis serving as an anterograde motor for the IFT-B complex

ABSTRACT

In the previous chapter, I demonstrated that KIF17 interacts with the IFT-B complex through the IFT46–IFT56 dimer and is trafficked toward the ciliary tip along the axoneme as a cargo of the IFT-B complex, rather than serving as an anterograde motor for IFT-B. I therefore predicted that heterotrimeric kinesin-II serves as an anterograde motor for the IFT-B complex, although there has been no direct evidence showing that heterotrimeric kinesin-II interacts with the IFT-B complex.

In this chapter, using the VIP assay, I identified a three-to-four protein interaction involving the kinesin-II trimer KIF3A/KIF3B/KAP3 and the IFT-B-connecting tetramer IFT38/IFT52/IFT57/IFT88; among the kinesin-II subunits, KIF3B contributed mainly to IFT-B binding. Furthermore, I showed that the ciliogenesis defect of *KIF3B*-KO cells can be rescued by the exogenous expression of wild-type KIF3B, but not by that of its mutant compromised with respect to IFT-B binding. Thus, interaction between heterotrimeric kinesin-II and the IFT-B-connecting tetramer is crucial for ciliogenesis, via the powering of IFT particles to move in the anterograde direction.

CONCLUSIONS

The summary of the results presented in this study is as follows:

Chapter 1

1. KIF17 accumulates at the ciliary tip regardless of presence or absence of its motor domain.
2. KIF17 interacts with the IFT46–IFT56 dimer of the IFT-B complex via its conserved sequence located immediately upstream of the NLS in its C-terminal region.
3. In addition to its NLS, KIF17 requires its binding to the IFT-B complex to enter the cilium across the TZ.
4. *KIF17*-KO cells are normal with respect to ciliogenesis, and anterograde trafficking of the IFT-B complex and ciliary proteins, such as Arl13b, Smo, and DRD1.

These results indicate that KIF17 is a cargo of the IFT-B complex and transported into the cilium across the TZ to the ciliary tip, rather than serving as an anterograde motor for the IFT-B complex to transport the proteins required for the ciliogenesis or ciliary functions.

Chapter 2

1. Heterotrimeric kinesin-II, composed of KIF3A, KIF3B, and KAP3, interacts with the IFT-B complex through the IFT-B connecting tetramer composed of IFT38, IFT52, IFT57, and IFT88; among the kinesin-II subunits, KIF3B contributed mainly to the IFT-B binding.
2. *KIF3B*-KO cells exhibit the severe defects in ciliogenesis, although the IFT-B complex localizes at the basal body.
3. The ciliogenesis defect observed in *KIF3B*-KO cells can be rescued by the exogenous expression of wild type KIF3B but not its C-terminally truncated mutant that retains the ability to form heterotrimer with KIF3A and KAP3, but is compromised with respect to IFT-B binding.

These results indicate that the interaction between heterotrimeric kinesin-II and the IFT-B complex through the IFT-B connecting tetramer is essential for the anterograde trafficking of the IFT-B complex, by which proteins essential for the ciliogenesis, such as α/β-tubulin dimers, are transported from the basal body toward the ciliary tip.

Together, the results presented in this study unequivocally showed that, among two types of kinesin-2 motors, heterotrimeric kinesin-II serves as an anterograde motor for the IFT-B complex, and thereby plays essential roles in ciliary anterograde trafficking of proteins required for ciliogenesis. On the other hand, KIF17 is transported into the cilium across the TZ to the ciliary tip as a cargo of the IFT-B complex along the axoneme, rather than as an anterograde motor for the IFT-B complex, although the roles of KIF17 at the ciliary tip remain unclear (Fig. 3). The present study will provide a new insight into understanding the molecular basis of ciliogenesis regulated by the IFT-B complex and the pathogenic mechanisms of the ciliopathies.

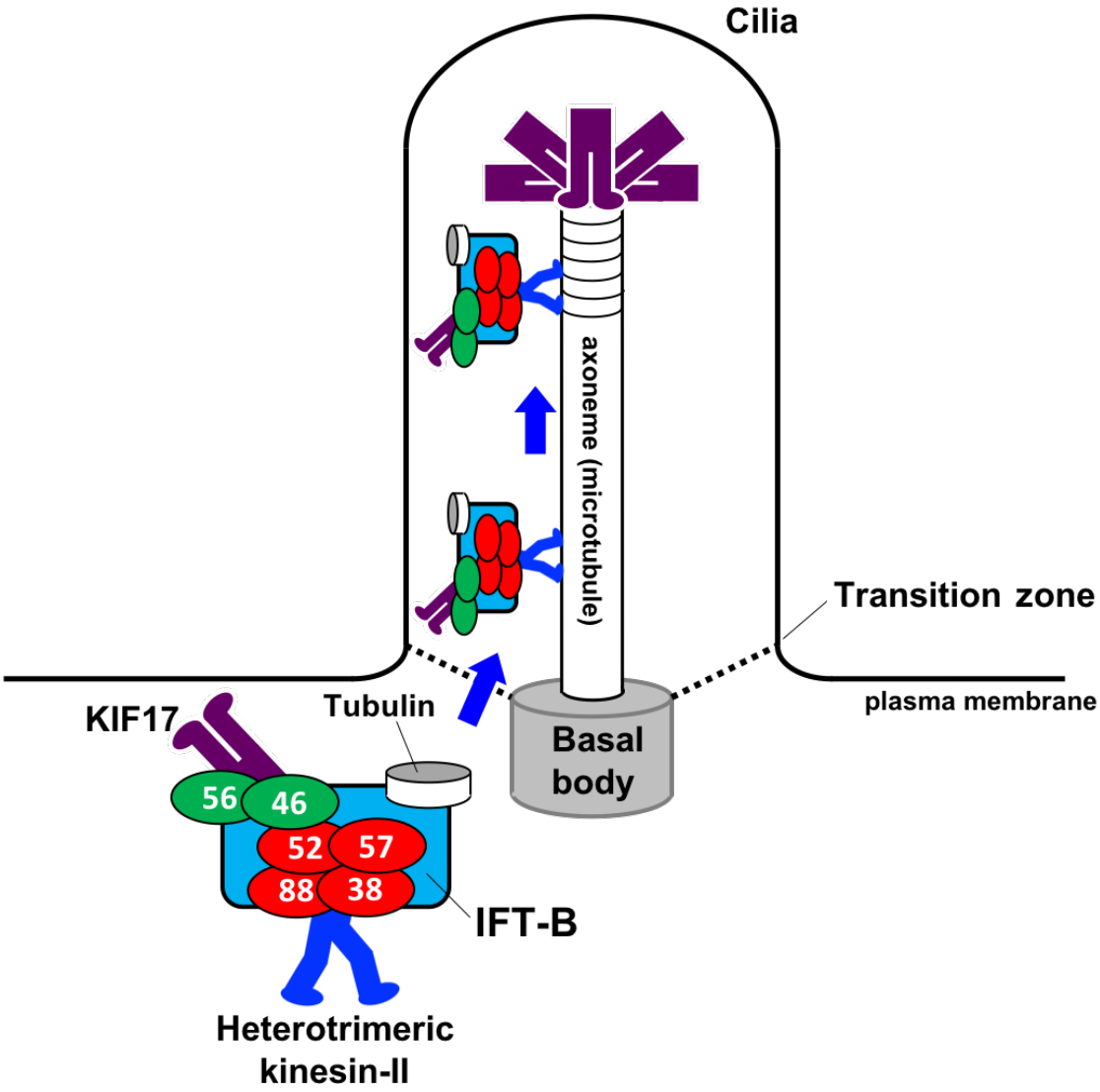

Fig. 3. Schematic illustration of the conclusions of this study.

Heterotrimeric kinesin-II, composed of KIF3A, KIF3B, and KAP3, interacts with the IFT-B complex through IFT-B–connecting tetramer composed of IFT38, IFT52, IFT57, and IFT88. Furthermore, heterotrimeric kinesin-II is serving as an anterograde motor for the IFT-B complex, and thereby plays essential roles in ciliary anterograde trafficking of proteins required for ciliogenesis, such as α/β-tubulin dimers, from the basal body toward the ciliary tip. On the other hand, KIF17 interacts with the IFT-B complex through the IFT46–IFT56 dimer and is transported as a cargo of the IFT-B complex into the cilium across the TZ to the ciliary tip, where KIF17 accumulates, although the roles of KIF17 at the ciliary tip remain unclear.

ACKNOWLEDGEMENTS

I would like to express the deepest appreciation to Professor Kazuhisa Nakayama, Graduate School of Pharmaceutical Sciences, Kyoto University, and Assistant Professor Yohei Katoh, Graduate School of Pharmaceutical Sciences, Kyoto University, for their superior guidance and suggestions through the course of this work.

I am deeply grateful to Mr. Masaya Terada, Ms. Saki Michisaka, Ms. Maho Sugawa, and Ms. Misato Okazaki for technical supports and helpful discussions.

I would like to express my gratitude to Dr. Hye-Won Shin, Dr. Hiroyuki Takatsu, Dr. Senye Takahashi, Dr. Kouhei Takashima, Dr. Yuki Imoto, Dr. Tomoki Naito, Ms. Ayako Hanai, Mr. David Hartanto, Mr. Yohei Hagiya, Ms. Minako Kobayashi, Ms. Rie Miyano, Ms. Chikako Yagi, Mr. Takashi Matsumoto, Mr. Shohei Nozaki, Mr. Tomoaki Hirano, Mr. Yuya Nishijima, Ms. Mariko Takahara, Mr. Yuki Hamada, Mr. Ryota Takei, Ms. Luxiaoxue Liang, Mr. Takuya Tone, Mr. Yuta Tsurumi, Mr. Takuya Kobayashi, and Mr. Kentaro Nakamura for their technical advice and helpful discussions.

My heartfelt appreciation also goes to the former and present members of the Department of Physiological Chemistry, Graduate School of Pharmaceutical Sciences, Kyoto University, for their continuous encouragements, helpful discussions and technical supports.

Finally, I would like to thank my family, Takashi, Toshiko, Yuko, and Eri, for persistent understanding and support for many things.

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