JCB Journal of Cell Biology

SPOTLIGHT

IFT trains overcome an NPHP module barrier at the transition zone

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Cilia harbor diffusion barriers for soluble and membrane proteins within their proximal-most transition zone (TZ) region and employ an intraflagellar transport (IFT) system to form dynamic motile and signaling compartments. In this issue, De-Castro and colleagues (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202010178) uncover a long-suspected role for the TZ in gating IFT particles.

The cilium is a complex and functionally versatile cellular extension that emerged, some two billion years ago, in the lineage leading to the last eukaryotic common ancestor. To this day, motile cilia continue to enable locomotion in most unicellular eukaryotes and power sperm movement or fluid flow in metazoans. The sensory functions of motile cilia have also been adopted by and expanded in different metazoan cell types, to create specialized nonmotile cellular antennae (1).

The formation and functions of cilia depend on a basal body from which stems the microtubule-based axoneme, as well as two additional, evolutionarily conserved macromolecular complexes: a transition zone (TZ) "ciliary gate" and an intraflagellar transport (IFT) machinery (Fig. 1). Understanding the functions and potential interactions between these ancient complexes is important, as they are involved in multiple human disorders—ciliopathies—that affect virtually all organ systems (1).

The TZ, comprising over one dozen components, is characterized by Y-link structures that connect the axoneme to the membrane at the ciliary base. Studies in model systems, including *Chlamydomonas*, *Caenorhabditis elegans*, and mammals, establish the TZ as a diffusion barrier for membrane-associated proteins (2, 3). Mechanistically, how the TZ achieves this is unclear. One possibility is that membrane-associated TZ proteins create a lipid microdomain that limits the diffusion of membrane proteins (2, 4). The TZ also creates a separate barrier for soluble proteins. This gate, or ciliary pore complex, has the properties of a size-selective matrix that may share components and functional similarities with the nuclear pore complex (4, 5).

How the different TZ proteins assemble in the context of Y-links to create these two distinct diffusion barriers remains unclear. Protein–protein interaction studies and genetic analyses (6) point to the existence of two multi-protein modules, termed MKS (Meckel–Gruber syndrome) and NPHP (nephronophthisis; Fig. 1). The modules are anchored at the TZ by at least two scaffolding proteins needed for the formation of Y-links. CEP290 tethers the MKS module, and MKS5 (RPGRIP1L) plays a more central role, assembling CEP290, the MKS module, and the NPHP module at the TZ (7). The MKS and NPHP modules are similarly required to establish the membrane and soluble protein gates; hence, their individual roles have been difficult to ascertain.

The IFT machinery harbors ∼50 proteins and forms "trains" with relatively well-understood roles in shuttling ciliary cargo (8). IFT particles dock at basal body-associated transition fibers, travel to the tip using IFT-kinesin anterograde motors, and after remodeling, return to the base via an IFT–dynein (dynein-2) retrograde motor complex (Fig. 1). IFT trains continuously transit the TZ, motoring along doublet microtubules, contacting the overlying membrane, and passing in between Y-links (4). Evidence for physical and genetic interactions between IFT and TZ components suggests functional connections between them (6), but the obvious question of whether the TZ acts as a gate for IFT particles/trains remained largely unexplored. Until now.

To understand the role of the *C. elegans* dynein-2 subunit WDR-60 (DYNC2I1/WDR60) in retrograde IFT, De-Castro and colleagues (9) analyzed two *wdr-60* mutants, one null, and another lacking a β-propeller domain known to bind the IFT-dynein heavy chain (CHE-3; human DYNC2H1 orthologue). They found that loss of WDR-60 appears well-tolerated compared with that of CHE-3 or the light intermediate chain XBX-1 (human DYNC2LI1 orthologue). Ciliary structures in WDR-60–deficient animals appear normal; in contrast, like in other organisms, CHE-3 and XBX-1 are essential for retrograde IFT, and their disruption leads to short, bulbous structures.

Yet, closer inspection of fluorescently labeled IFT reporters in *wdr-60* mutants by

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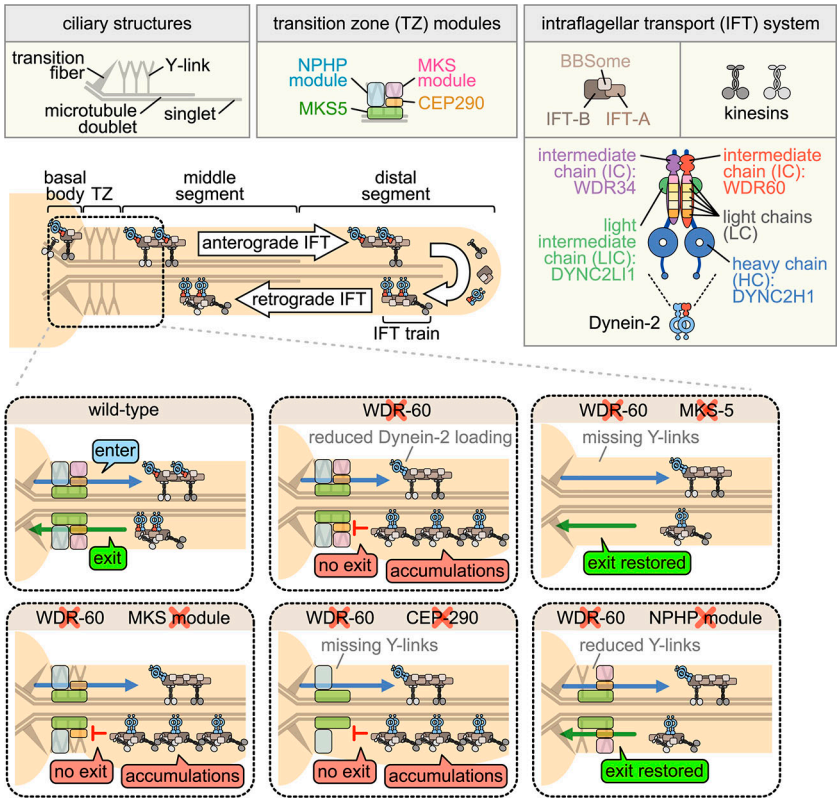

Figure 1. **The ciliary TZ acts as a barrier that must be overcome by the IFT system.** TZ modules are known to assemble into diffusion barriers for soluble and membrane proteins at the base of cilia. De-Castro et al. (9) uncover a TZ barrier for the ciliary cargo-trafficking IFT system, which consists of different modules (BBSome, IFT-A, IFT-B) moved bidirectionally by kinesin anterograde and dynein-2 retrograde motors. When the dynein-2 subunit WDR-60 is disrupted, fewer dynein-2 retrograde motors associate with IFT particles upon entering cilia, and the under-powered retrograde IFT trains fail to break through the TZ—that is, unless the entire TZ is disrupted (MKS-5 mutant) or a specific TZ module (NPHP) is removed.

live imaging revealed a significant defect: fewer IFT–dynein motors were incorporated onto anterograde IFT rafts and entered cilia (Fig. 1; 9). This in itself was not unexpected, as the Stephens and Nakayama laboratories had observed less DYNC2LI1 localized to cilia upon disrupting mammalian WDR-60 (10, 11). However, whereas mammalian cilia displayed strong IFT protein accumulations, particularly at the ciliary tip, *C. elegans* IFT particles lacking WDR-60 accumulated substantially less at the tip and were better able to traffic toward the base. Remarkably, though, the WDR-60–deficient IFT trains, with fewer IFT–dynein motors, amassed at the distal end of the TZ, apparently unable to cross the barrier (Fig. 1; 9). This finding presented an opportunity to further investigate how the TZ establishes a barrier for the IFT system.

Confirming that the TZ acts like a gate was straightforward: The IFT roadblock was cleared in the *mks-5* mutant, which completely lacks Y-links and the MKS and NPHP modules (Fig. 1). To narrow down which TZ module(s) provide the IFT-gating function, the researchers disrupted the MKS module, which contains many membrane-associated proteins and thus likely contributes to forming a membrane diffusion barrier. This did not restore the ability of WDR-60–deficient retrograde IFT trains to cross the TZ barrier. Similarly, the barrier remained intact upon mutation of CEP290, the anchor for the MKS module (Fig. 1). This left open the possibility that the NPHP module harbors the gating functionality.

That is exactly what De-Castro et al. observed. Even though MKS-5, CEP-290, the MKS module, and Y-links are still present upon disrupting the NPHP module (*nphp-4* mutant), IFT trains devoid of WDR-60 were now able to cross the TZ barrier (Fig. 1). Additionally, the *nphp-4* mutant displayed faster anterograde and retrograde IFT speeds within the TZ compared with wild-type, further implying a role for the NPHP module in restricting IFT particle movement.

Altogether, the findings by De-Castro et al. reveal that IFT trains driven by retrograde dynein motors must overcome, or "power through," a TZ barrier specifically established by the NPHP module. But what exactly is the nature of this barrier? Is there some sort of molecular switch, the equivalent of a cell-cycle checkpoint where permission for entry and/or exit is regulated? Is it the purported gel-like matrix formed by nucleoporins (5) that simply decelerates the IFT trains? This latter hypothesis was not investigated or suggested in the current study. However, using mammalian cells, the Verhey laboratory (12) showed a physical interaction between a nucleoporin (NUP62) and the same TZ protein (NPHP4) found to confer the IFT-barrier functionality in *C. elegans*. This could in principle explain the findings of De-Castro et al.: removal of NPHP4 could prevent the proper localization of a related nucleoporin and disrupt the size-selective matrix at or near the TZ. Interestingly, the Verhey laboratory also revealed that NUP62 is required for ciliary entry of KIF17 (13), an IFT-associated kinesin motor (8).

Connecting the proverbial dots from different model systems might be premature. But if correct, this hypothesis suggests that during the evolution of the ancestral eukaryote, the use of a nuclear pore complex–like system by the TZ to form a soluble protein diffusion barrier may have required specific adaptations for the ciliary entry/exit of the IFT system. Notably, that a ciliary pore complex could potentially influence ciliary entry and exit of IFT particles was suggested by Rosenbaum and Witman 20 yr ago (14). Continuing to shed light on the functional interactions between the TZ and IFT machinery will undoubtedly lead to a better understanding of how cilia create dynamic signaling compartments.

Acknowledgments

We apologize to our colleagues whose work could not be discussed and cited due to space limitations.

Our work on the transition zone and intraflagellar transport is supported by grants from the Canadian Institutes of Health Research (grants MOP-142243 and PJT-156042, respectively). M.R. Leroux is

the recipient of a Senior Scholar award from Michael Smith Foundation for Health Research.

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