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SPOTLIGHT

Can microtubule motors use every available track?

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Flagellar assembly and function depend on cargo traveling via motors on microtubule doublets. Bertiaux, Mallet et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201805030) find that only a subset of available doublets are used for this transport in trypanosomes, leading to questions about how and why this is achieved.

Within eukaryotic cilia and flagella, microtubule motors carry adapters and cargo for delivery. New proteins travel from where they are synthesized in the cell body to their final destination within the ciliary compartment. Some of these cargoes are also recycled back out of cilia by motors returning on microtubule tracks. How is this bidirectional traffic regulated? The answers dictate many properties of ciliary structure, motility, and signaling and can have important implications for cellular function and fitness.

Regardless of organism, cylindrically arranged microtubule doublets form the ciliary superstructure, called the axoneme. Usage of these doublets can tell us how traffic jams are avoided or which factors constrain cargo transport capacity. In this issue, Bertiaux et al. use both electron microscopy and structured illumination live cell microscopy to show that transport within the flagella of the protist *Trypanosoma brucei* occurs on only a subset of microtubule doublets.

Since the discovery of bidirectional transport within flagella, termed intraflagellar transport, or IFT (1), researchers have tried to analyze and quantify this behavior. How fast and frequently do motors travel? What triggers entry of motors and cargo, and is there preassembly of complexes before this entry? How long are the chains of motors or "trains," and how much cargo are they carrying? To what degree are proteins recycled from flagella versus recruited fresh from the cell body? How are collisions avoided during bidirectional transport? And finally, which of these properties dictate length or go awry when cilia and flagella are defective?

Whether IFT motors can use all of the nine doublets available will dictate the maximum cargo capacity and timing of motor entry into flagella. In other words, trafficking capacity will differ based on whether a train has to clear the docking station before another can enter or multiple trains can simultaneously travel on all doublets. If tip- and base-directed motors are able to simultaneously traffic without crashing into one another, they might travel on different doublets or individual tubules.

Bertiaux et al. have tackled the question of microtubule selection of IFT trains in trypanosomes (2). This organism contains nine microtubule doublets, with doublets 4–7 linked to a structure called the paraflagellar rod (PFR; Fig. 1 A), which is involved in flagellar motility (3). Using focused ion beam scanning electron microscopy (FIB-SEM), electron-dense particles corresponding to IFT trains can be seen mostly on two sides of the flagellar axoneme, surprisingly restricted to doublets 3–4 and 7–8 (Fig. 1 A). Both tip-directed (anterograde) and base-directed (retrograde) traffic were found on both sides of the axoneme. The authors were unable to detect any exchange of trains between sides of the flagella, suggesting trains may remodel and return on the same side (Fig. 1 B). In growing trypanosome flagella, there is some disorder at flagellar tips, likely because structural components between microtubules are temporarily absent during growth (4). In these disordered regions, the axoneme comes into contact with the membrane and may slow free exchange of IFT trains to the other side of the axoneme before retrograde trains can reengage on the same side. Mature flagella also contain structures extending from one tubule in each doublet and from the central pair of microtubule singlets to the membrane, which could also constrain diffusion of IFT trains between the two sides of the axoneme.

Fixed cell staining of the retrograde IFT protein IFT172 identified two resolvable tracks, and live-cell imaging of two anterograde IFT proteins, IFT81 and IFT52, recapitulates the two-track finding. While we can learn much about transport behavior by visualizing IFT proteins, IFT proteins can function as cargoes themselves or as adapters for other cargoes. Occupancy of motors with cargoes can also vary and can influence flagellar assembly and function. In the flagella of the green alga *Chlamydomonas reinhardtii*, for example, the size and frequency of IFT trains can vary as judged by labeled motor proteins, but the occupancy of these trains with the microtubule building block, tubulin, can also vary (5). Tubulin occupancy on IFT trains is increased in growing *Chlamydomonas* flagella compared with those maintaining their final length. Further, while most cargoes are thought to bind motors via IFT proteins, some may also interact with motors directly (6). Given the variability of motor occupancy, visualizing multiple modules of trafficking machinery is needed to give a complete picture of how transport is regulated and how this will impact function (i.e., quantifying labeled motors relative to IFT proteins and IFT cargo). Here, we have a strong indication that IFT trains (as identified by those labeled IFT components) are limited in which microtubule doublets they can use. Anterograde transport of the dynein motor also appears to be limited

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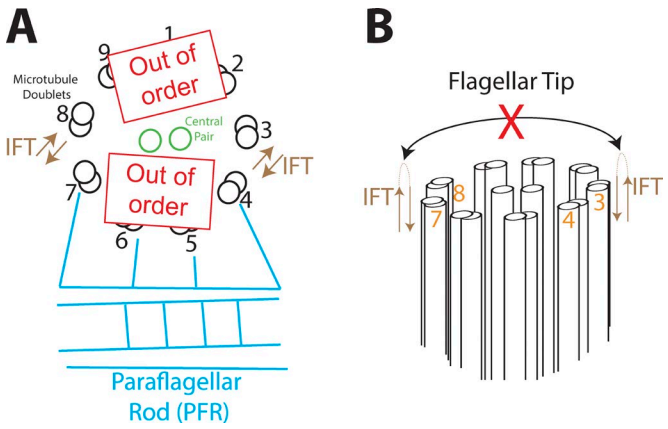

Figure 1. **Model of trypanosome IFT microtubule doublet utilization. (A)** Doublets 3–4 and 7–8 show the presence of IFT trains by electron microscopy and structured illumination microscopy. **(B)** No exchange of IFT trains is detected between sides of flagella at the tip, suggesting remodeled retrograde trains return to the base on the same side they arrived.

in track usage. In this case, dynein acts as a cargo rather than as an active motor. It remains to be seen whether motors themselves can actively transport on a small number or all of the microtubule doublets.

What might give rise to doublet selectivity? We know that both the ciliary microtubule doublets and the basal bodies/centrioles from which they extend can be radially asymmetric (7, 8). They may also differ with respect to tubulin posttranslational modification, which can affect motor behavior (9). If tagged motors demonstrate that only a subset of doublets are used for IFT, it is likely that some chemical features of the doublets themselves may restrict where motors can bind microtubule tracks. Alternatively, if anterograde motors can travel on all doublets but IFT proteins or certain cargoes can only load onto motors on certain doublets, it could be that physical constraints (PFR or potential differences in membrane proximity to the axoneme) may dictate the extent to which cargoes can be loaded. If there are physical barriers preventing the formation of large complexes on the other doublets, perhaps empty motors or those carrying cargoes via direct interaction can still traffic there.

Why would doublet specificity be needed for IFT in trypanosomes while an expanded number are used in other organisms? Since the bihelical waveform of trypanosome flagella involves bending in the plane that divides the central microtubule pair (10), could doublets 3–4 and 7–8 orthogonal to the bending plane and adjacent to the movement-constraining PFR better maintain traffic during motility or when flagellum direction reverses? Does the remaining space free of IFT trains allow for diffusion of components needed for flagellar functions in host tissue invasion or attachment? In trypanosomes, IFT seems to be responsible for flagellar growth, flagellar motility, and transport of signaling components but not structural maintenance of mature flagella (11). Perhaps restricting IFT transport on a subset of doublets allows other motors that are not involved in IFT such as KIF9B, which transports PFR components (distinct from the IFT kinesin-2 motors), to traffic on additional doublets (12). Visualization of additional trafficking components, particularly active motors and trypanosome-specific cargoes, will provide essential clues. Bertiaux et al. have taken the critical step of showing that we should not assume that traffic on all doublets is the same and that there is yet another level of regulation dictating flagellar protein transport (2).

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