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SPOTLIGHT

How do trypanosome IFT trains choose special tracks?

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Intraflagellar transport trains in *Trypanosoma brucei* are known to selectively associate with a subset of axonemal doublet microtubules. In this issue, Araujo Alves et al. (https://doi.org/10.1083/jcb.202501088) aim to elucidate the mechanisms underlying this selective association using high-resolution microscopy approaches.

Cilia and flagella are conserved eukaryotic cell extensions playing important functions in motility and signaling. Their structure and function depend on intraflagellar transport (IFT), which moves protein complexes, including the IFT trains and their cargos, bidirectionally along axonemal doublet microtubules (DMTs). In *Chlamydomonas reinhardtii*, IFT trains are associated with all nine DMTs, not only at the ciliary base where they are assembled (1) but also along the mature flagellum (2).

In stark contrast, IFT trains of *Trypanosoma brucei* primarily travel along DMTs 3–4 and 7–8 in the flagellum (3). To determine how IFT trains selectively associate with particular DMTs in *T. brucei*, in this issue, Araujo Alves et al. (4) mapped the assembly and distribution of IFT at the base, through the proximal region and along the flagellum, using a combination of advanced imaging methods, including super-resolution microscopy, ultra-expansion microscopy and focused ion beam scanning electron microscopy. All microscopy methods preserve the spatial organization of the axoneme and allow the visualization of DMTs in association with IFT trains, IFT-like densities, or IFT components. The authors found that IFT subunit IFT172 surrounded all DMTs in the transition zone at the flagellar base. From the transition zone toward the flagellum, IFT-like densities and IFT trains were progressively restricted to DMTs 3–4 and 7–8. These striking observations supported a model where selective IFT association with DMTs occurs at the proximal portion of the flagellum as the IFT trains assemble and/or disassemble (4).

What determines IFT selectivity? And what is so special about DMTs 3–4 and 7–8 in *T. brucei*? In future work addressing the molecular mechanisms for IFT selectivity, differential posttranslational modification of tubulin is likely a top priority. Tubulin isoforms and modifications have been shown to affect microtubule dynamics and mechanical properties, as well as the activities of motor proteins such as kinesins and dyneins (5, 6). In a recent study, tubulin tyrosination/detyrosination is found to regulate IFT affinity to microtubules, which may explain the differential preference of anterograde and retrograde IFT trains for different microtubules in the same DMT, thus avoiding clashes (7). *T. brucei* genome encodes a single isotype each of α-tubulin and β-tubulin, but extensive tubulin modifications such as glutamylation, tyrosination and detyrosination, and acetylation have all been shown to occur on axonemal microtubules in *T. brucei* (8).

Apart from tubulin code, other factors may also contribute to IFT selectivity. In trypanosomes, the paraflagellar rod is a para-axonemal structure occupying along DMTs 4–7 in the flagellum, physically blocking IFT access to these DMTs (4). Further, IFT trains are associated with both flagellar axoneme and flagellar membrane. Recent examination of IFT in primary cilia using serial section electron tomography notes an absence of IFT on DMTs that are further away from the ciliary membrane (9), suggesting a role of ciliary membrane in IFT-DMT association. As *T. brucei* flagellum beats and twists in three-dimensional space, whether the distance between the DMTs and the flagellar membrane change during the beating and whether the changes have any effect on IFT-DMT association are yet to be examined. The findings in *T. brucei* provide a unique opportunity to study the mechanisms of IFT-DMT selectivity in motile cilia, which can offer insights into the regulation of IFT movement and activity.

Not so long ago, the axoneme was thought to be a symmetrical structure, with the same protein components forming the same repeating pattern along each DMT. Recent advances in understanding the biochemical composition of axonemes and imaging of their structural organizations have revealed proximal-distal and radial asymmetries in axonemal structures in diverse motile cilia types (10, 11, 12). The selective association of IFT with DMTs further emphasizes the asymmetry not only in structure but also in function. Whether the selectivity is mediated by differential tubulin code, protein compositions, or association with other structures, intrinsic differences among DMTs are likely to be widely present in different cilia types. Multi-scale, in situ imaging methods that preserve the spatial arrangement of the DMTs will be crucial to understand the

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mechanisms and functions of axonemal asymmetries.

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