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SPOTLIGHT

Discriminating motilities: Coordinating IFT with flagellar beating patterns

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Intraflagellar transport has traditionally been studied in immobilized flagella. In this issue, Gray et al. (https://doi.org/10.1083/jcb.202401154) introduced a novel methodology for fast imaging in free-swimming *Leishmania*, revealing the impacts of flagellum immobilization on intraflagellar transport and its inverse correlation with cell swimming speed.

Cilia and flagella are complex structures composed of numerous proteins, most of which are found in their core, known as the axoneme, primarily composed of microtubules. It contains dynein complexes essential for flagellum beating (1). All flagellar proteins are produced in the cytoplasm, and since the axoneme grows from its tip, a specialized transport system is required to deliver them. This system is termed intraflagellar transport (IFT) and is conserved in nearly all ciliated organisms. IFT uses molecular motors to move large protein complexes, called IFT trains, along microtubules of the axoneme. Transport occurs in two directions: from the base of the flagellum to the tip (anterograde transport), which is powered by the molecular motor kinesin-II, and from the tip back to the base (retrograde transport), driven by a specific dynein motor (2).

IFT was discovered in 1993 in the laboratory of Joel Rosenbaum when they detected the movement of particles in paralyzed flagella of the green alga *Chlamydomonas reinhardtii* (3). Since then, IFT has been primarily studied by tracking the movement of fluorescently labeled IFT proteins in various organisms. Studies have typically involved either immotile cilia or immobilized motile cilia (flagella), showing that IFT moves faster in motile cilia (between 1.5 and 5.6 µm/s in *Chlamydomonas* and *Trypanosoma brucei*) compared to immotile cilia (between 0.3 and 1.3 µm/s in primary and sensory cilia) (4, 5). The speed differences could be explained by the longer length of motile cilia, possibly requiring faster IFT to grow the cilia at a reasonable rate.

Whether the IFT speed differences were an inherent property of cilia, or an artifact caused by the methods used to immobilize them for imaging was an interrogation in the field. To address this issue, Gray et al. (6) developed a technique to image IFT in free-swimming cells. The protist *Leishmania* serves as an excellent model for studying IFT as it is easy to immobilize its flagellum, both mechanically and genetically (7). Richard Wheeler's group was the first to visualize IFT in *Leishmania*, where cells were mechanically immobilized by washing them in PBS, causing them to adhere to glass coverslips and allowing imaging of IFT trains with a fluorescent protein reporter (8). Gray et al. (6) found that under these conditions, the speed of IFT trains decreased over time, suggesting that mechanical immobilization has an impact on IFT speed.

To analyze the effects of flagellum immobilization on IFT, Gray et al. (6) developed a method to image IFT in freely beating flagella. The flagellum of *Leishmania* beats at two different frequencies: a high-frequency beat (20–25 Hz) for swimming, characterized by a symmetric tip-to-base movement, and a low-frequency beat (around 5 Hz) for reorienting the flagellum, characterized by an asymmetrical base-to-tip pattern. Some other beating patterns, like symmetrical low-frequency tip-to-base and occasional uncoordinated movements, are also observed. To overcome the challenge of live imaging flagella during the high-frequency beat, Wheeler's group used high-frame-rate acquisitions (100–400 Hz) of 4.75 s. This required a strong and stable fluorescence signal from the IFT particles, achieved by expressing an IFT protein fused to three copies of mNeonGreen. Another difficulty of imaging a beating flagellum is compensating for its curvature. To address this, the authors expressed a flagellar membrane protein tagged with mCherry and used this signal to digitally straighten the flagellum during its beat, allowing analysis of IFT movement.

Using this new approach, they compared IFT in free-beating and immobilized flagella and observed that IFT trains moved slightly faster in both directions when the flagella were free to beat. This suggests that mechanical immobilization introduced constraints that affected IFT observations. This finding is consistent with studies in *Chlamydomonas*, where mechanically immobilized flagella showed static IFT trains (9). IFT trains are responsible for transporting transmembrane proteins along the flagella (10), and their processivity might be affected when the flagellar membrane

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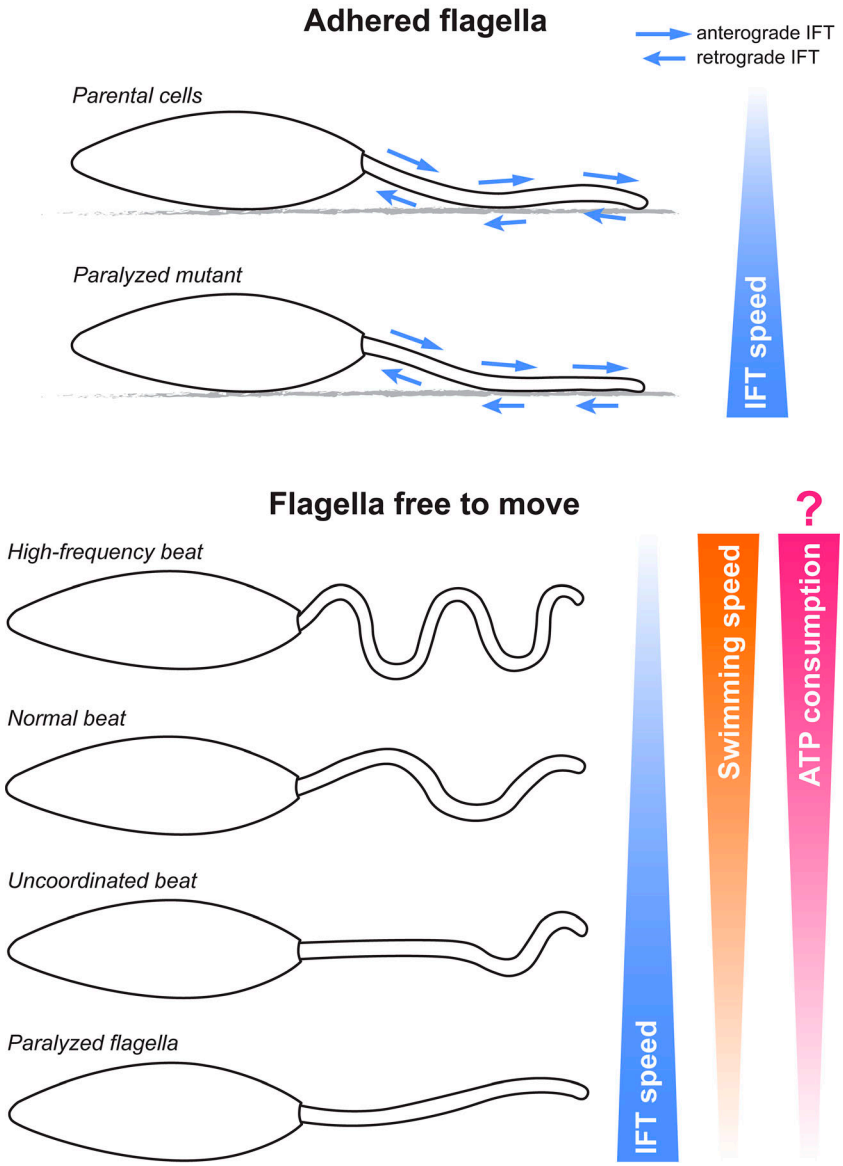

Figure 1. **Illustration of flagellar beating patterns in *Leishmania* and their inverse correlation with IFT speed.** Adhered parental cells showed slower IFT trains compared to genetically paralyzed cells. The advanced method developed by Gray and colleagues (6) enabled the observation of IFT in flagella free to move. Under these conditions, genetic modifications that altered beating patterns and swimming speeds inversely affected IFT speed: higher swimming speeds (High-frequency beat) resulted in slower IFT trains, while slower swimming speeds (Uncoordinated beat, Paralyzed flagella) led to faster IFT. The model suggests ATP competition between axonemal dyneins and IFT molecular motors.

interacts with the glass slide surface during immobilization.

Gray et al. (6) then examined the effects of genetic flagellum paralysis on IFT speed in adhered cells. They measured IFT speed after a gene deletion that causes paralyzed flagella, revealing slightly faster IFT trains when compared to normal cells in adhered (Fig. 1, Adhered flagella) but also in free-swimming conditions. Additionally, the reduction in IFT speed over time, observed in physically immobilized cells, was less pronounced in this mutant. The fact that genetic immobilization had an opposite impact on IFT speed than physical immobilization suggests the presence of physiological factors, rather than only mechanical aspects, could influence IFT displacement.

Using their novel imaging approach, the authors investigated whether changes in the flagellar beat pattern affect IFT speed. They tracked IFT particles during different beat types in free-swimming cells and found that the flagellum's beating pattern had little impact on IFT speed (Fig. 1, Flagella free to move). To clarify the correlation between flagellar beat and IFT, they examined mutants with different flagellar beating patterns and compared them to parental cells in free-swimming conditions. In mutants with reduced high-frequency symmetric beating combined with increased asymmetric and uncoordinated beats, IFT trains were faster in both directions. IFT was also faster in mutants with predominantly low-frequency symmetric beats. In mutants with mostly uncoordinated beats, only anterograde IFT trains were faster (Fig. 1, Uncoordinated beat). The paralyzed mutant, which had faster IFT in immobilized conditions, showed even higher IFT speeds in non-adhered cells (Fig. 1, Paralyzed flagella). All these mutants showed slower swimming speeds, suggesting cell swimming speed is inversely related to IFT speed (Fig. 1, blue and orange). If this hypothesis is true, cells with higher swimming speeds should show slower IFT speeds. To validate this, Gray and colleagues quantified IFT in a mutant with higher flagellar beat frequency and swimming speed, which indeed showed slower IFT in both directions (Fig. 1, High-frequency beat). Altogether, the data support an inverse correlation between swimming speed and IFT speed.

Based on these observations, Gray et al. (6) hypothesize that higher flagellum beat frequency leads to lower IFT speed due to competition for ATP between the molecular motors on the axoneme and those carrying the IFT trains (Fig. 1, pink). As the axonemal dyneins responsible for flagellar beating consume significantly more ATP than the IFT molecular motors (one to two orders of magnitude), any reduction in ATP consumption for flagellar beating would notably increase the ATP available for the IFT motors.

Overall, the findings by Gray et al. (6) solve a puzzle that has been open for 30 years. Although flagellum immobilization significantly impacted IFT speed, their reports align with the current understanding of IFT. Despite some limitations due to short time acquisitions (frequency of injection or possible speed changes along the flagellum length), this approach paves the

way for applying similar techniques to other well-known IFT models, such as *Chlamydomonas* and *T. brucei*. The discoveries reported by Gray and colleagues underscore the importance of observing biological processes under conditions that closely mimic natural situations and open new opportunities for understanding further mechanisms regulating IFT.

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