FIB-SEM and Live Cell imaging to study the localisation and composition of IFT trains in Trypanosoma

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Intraflagellar transport or IFT is a bidirectional motility along axonemal microtubules that is essential for the formation (ciliogenesis) and maintenance of most eukaryotic cilia and flagella. The process of IFT involves movement of large protein complexes called IFT trains from the base to the ciliary tip and followed by their return. 1/Where are localised IFT trains? 2/ how do they travel in the flagellum? 3/what is the composition of IFT trains? The FIB-SEM was used to reveal that IFT trains are localised specifically on 2 tracks in the flagellum of *Trypanosoma brucei*. IFT trains are heterogeneous in length: 255 ± 360 nm (n=88) for those present on doublets 3-4 and 387 ± 678 nm (n=73) for those encountered on doublets 7-8, raising the question of their identity. Since cells were fixed, it was not possible to determine in which direction trains were travelling (anterograde or retrograde). Using a strain expressing an mNeonGreen::IFT81 fusion, we shows that IFT is bidirectional on each track by high resolution live cell imaging (Bertiaux, Mallet et al, JCB 2018). Using same technical approaches we have study the composition of IFT trains. The IFT particles are composed of 20 proteins organized in two sub-complexes called complex A and B. We used 2-colour live cell imaging and electron microscopy to monitor IFT trains in *Trypanosoma brucei*. We show that IFT-A, IFT-B and dynein motor travel together and two populations of IFT trains are visible: long trains ~1µm and short trains ~300nm.