

Investigating the role of unique kinesin-2 motors in intraflagellar transport in trypanosomes

Alves, Aline A¹; Bastin Philippe¹

¹ Trypanosome Cell Biology Unit, INSERM U1201, Université de Paris and Institut Pasteur, Paris, France

Trypanosomes have a single flagellum essential for survival, motility, life cycle and host-parasite interaction. These parasites are an excellent model to study flagella assembly, as they build a new flagellum while conserving the old one. Flagella assembly requires a specific transport system called intraflagellar transport (IFT). IFT is the bidirectional movement of multiprotein complexes, or IFT trains trafficking along flagellar microtubules. The transport towards the ciliary tip is called anterograde, driven by kinesin-2. These motors comprise two subfamilies: the heterotrimeric kinesin-2, with two different motor subunits and a non-motor subunit, and the homodimeric kinesin-2, with two identical motor subunits. *Trypanosoma brucei* is unique since it lacks the heterotrimeric version but contains two kinesin-2 genes – KIN2A and KIN2B. KIN2A and KIN2B involvement on anterograde IFT transport was shown by RNAi knockdown of the two motors together. Still, how each motor contributes individually to the anterograde IFT remains unclear. To investigate KIN2A and KIN2B roles, we generated cell lines expressing the kinesins fused to the fluorescent protein mNeonGreen (mNG) and followed IFT by confocal spinning-disk microscopy. Compared to the IFT protein IFT81, KIN2A-containing particles move at a similar speed but have a lower frequency, suggesting KIN2A is not present in every anterograde train. KIN2B-containing particles present a unique pattern: only a minor part of them can reach the flagellar tip. Most KIN2B particles are restricted to the proximal region of the flagellum and are slower than IFT anterograde trains. Double-tagged cells expressing KIN2B and the IFT protein IFT140 confirmed that KIN2B proximal particles do not colocalise with the IFT140, showing they are not associated with IFT trains. In contrast, the distal KIN2B-containing particles have the expected IFT speed and colocalise with IFT140 but show a low frequency. The frequencies of KIN2A and KIN2B distal particles together reach the expected frequency of anterograde IFT trains. Together, our data suggest KIN2A and KIN2B carry different trains and function as homodimers.