Towards a mechanism of waveform-switching in the motile flagella of trypanosomatids, from cyclic-AMP signal to dynein motor

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Motile eukaryotic flagella transduce ATP chemical energy via dynein motors to produce the microtubule bending required for beat generation. Modulatory signals can give rise to varied waveforms in different species and cell types. Trypanosomatids can switch the wave propagation direction and modulate wave symmetry, shape and frequency. How conserved upstream signals like cyclic adenine monophosphate (cAMP) influence dynein motor dynamics and how they are transduced in trypanosomatids remains unclear. Here we use CRISPR, RNAi, high-speed video and expansion microscopy in *Leishmania* and *Trypanosoma* to study how cAMP influences cell swimming and waveform-types. We show that in *Leishmania*, cAMP acts within nanodomains at the flagellar tip to regulate swimming speed. Furthermore, elevated levels of cAMP promote waveform-switching. We localised Cyclic AMP Response Protein 1 (CARP1) to a radially asymmetric domain along the axoneme. Our studies of CARP1 mutants suggests that it controls waveform-switching: mutagenesis of the CARP1 cAMP binding site renders cells blind to elevated cAMP levels and the symmetric wave becomes stabilised. Upon deletion of CARP1, the cells remain blind to elevated cAMP levels and lose their preference for symmetric waveforms. It has long been known that knockdown of CARP1 protects *Trypanosoma brucei* bloodstream forms (TbBSF) from the lethal effects of elevated cAMP levels. We found that in *Leishmania,* elevated cAMP levels resulted in cell rotation through waveform-switching, with no detrimental effect on cell growth. By contrast, in TbBSF treated with a phosphodiesterase (PDE) inhibitor or dual-knockdown of PDE B1 and B2 caused persistent reverse swimming prior to cytokinesis failure and cell death. In TbBSF devoid of CARP1, no reverse swimming was observed, and elevated cAMP levels were no longer lethal. These new data directly link waveform dynamics to cytokinesis fate and demonstrates how one signal can affect the same process in two related species with very different fitness costs. Targeted mutagenesis and knockout of dynein motors revealed that inner dynein arms IDA*f*, IDAa and IDAd are required downstream of a cAMP signal and CARP1, highlighting a potential mechanistic hub within the conserved axonemal 96 nm repeat unit where the waveform-switch is executed. Together, this study brings us closer to unpicking the signal transduction pathways through which second messengers influence dynein motor activities in a beating eukaryotic flagellum.