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The role of cyclic nucleotide signalling in Leishmania mexicana flagellar motility.

Sophia Fochler, Richard Wheeler, Eva Gluenz

The second messenger cyclic AMP (cAMP) has been linked to modulation of flagellar movement in diverse eukaryotic species including *Trypanosoma* and *Leishmania* but the specific transduction pathways from second messenger to dynein driven force remains in most cases unclear. Trypanosomatids are recognised to have an unconventional cAMP signalling pathway, lacking canonical downstream effectors of cAMP and harbouring protein kinase A (PKA) complexes that are insensitive to cAMP. We aimed here to elucidate how the cAMP signalling pathway impacts on flagellar motility in *L. mexicana*. We compiled all proteins predicted to act in cAMP signalling pathways from analysis of the *L. mexicana* genome sequence, including known cAMP producers (adenylyl cyclases, ACs) and degraders (phosphodiesterases, PDEs) and proteins with predicted cyclic nucleotide binding domains. We then tagged these with mNeonGreen (mNG) to determine their subcellular localisation and generated knockout mutants to study the effect on motility. These screens identified over two thirds (24) of our proteins localised to flagellar domains. KO of tip-localised AC (RAC-a) swam slower, KO of PDEs in the distal flagellum swam faster, suggesting cAMP in the distal flagellum modulates swim speed. Our population motility screen found that the deletion mutant of the cyclic AMP response protein 1 (*dCARP1*) had the slowest swim speed. High speed video microscopy and Fourier analysis of the tip-to-base flagellar beat revealed that *dCARP1* flagella were unable to reach frequencies over 30 Hz and the *dCARP1* population showed an increased prevalence of cell turning via the asymmetric base-to-tip ciliary beat. A higher propensity for ciliary beats was also observed upon deletion of PDE B1 or treatment with high concentrations of the PDE inhibitor CpdB. mNG::LmxCARP1 localised to the detergent insoluble fraction of the flagellar axoneme and conservation of key residues in its CNBD suggests it binds cAMP. Our data identifies CARP1 as a cAMP-binding protein that may directly or indirectly modulate the activity of axonemal dyneins, which ultimately dictate swimming behaviour. Together, these data indicate that cAMP signalling modulates beat frequency and cell swim speed and contributes to the balance between flagellar beating, and ciliary beating.