CEP43 – a protein with unexpected and divergent functions in the assembly and stability of the trypanosome flagellum

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The *T. brucei* flagellum contains both a canonical 9+2 eukaryotic axoneme and extra-axonemal paraflagellar rod (PFR). In most flagellated eukaryotes, flagellum assembly depends upon intraflagellar transport (IFT), a bidirectional transport system that transports cargo along axonemal microtubules. A conserved protein complex consisting of CEP43, CEP19 and RABL2B localised at the basal body facilitates anterograde (i.e. base to tip) IFT. However, we have previously demonstrated the primary impact of *Tb*CEP43^{RNAi} depletion is on PFR assembly, with minimal impact on axoneme formation. To determine whether the entire CEP43/CEP19/RABL2B complex is involved in PFR rather than axonemal assembly in *T. brucei*, we assessed the roles of *Tb*RABL2B and *Tb*CEP19. Our data demonstrates that depletion of *Tb*RABL2B and *Tb*CEP19results in flagellum phenotypes indicative of a generalised IFT defect; mirroring published short-flagellum phenotypes of IFT-mutants, rather than a specific failure in PFR assembly. This develops our understanding of the unexpected divergence of CEP43 function in trypanosomes within a protein complex that is otherwise functionally conserved in facilitating IFT.

To further investigate *Tb*CEP43 function we studied the temporal appearance of PFR abnormalities following induction of *Tb*CEP43^{RNAi} using immunofluorescence, transmission and scanning electron microscopy. Our investigations indicate that in addition to failing to assemble a coherent PFR structure in newly forming flagella, we observe disruptions in PFR uniformity in flagella that were assembled prior to induction of *Tb*CEP43^{RNAi}, as well as an unusual rudimentary polymerisation of PFR material. These results are, as far as we are aware, the first evidence for the integrity of the highly ordered PFR structure being affected after its construction has been completed. In addition, live cell imaging of *Tb*CEP43^{RNAi} induced cells expressing a fluorescently tagged IFT protein, revealed an unexpected IFT phenotype; characterized by a reduction of IFT processivity and aberrant switching between anterograde and retrograde transport along the length of the flagellum. Our studies raise intriguing questions on how *Tb*CEP43 influences PFR assembly

and stability in the *T. brucei* flagellum, and the relationship between IFT-dysregulation and PFR maintenance.