Characterizing the phenotypes of *Leishmania* dynein assembly factor mutants in two life cycle stages

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Throughout the *Leishmania* life cycle, flagella serve as motile and sensory organelles that are critical for the parasite's progression though the life cycle. As promastigote forms within the sandfly vector, these parasites use a motile flagellum with a 9+2 microtubule axoneme and a canonical set of dynein motor proteins, which they remodel into a short non-motile 9+0 cilium upon differentiation to the amastigote form in mammalian hosts. Previous research in model organisms and human ciliopathies has established the importance of axonemal dynein assembly factors (DNAAFs) for assembly of dynein motor proteins and axonemal integrity in motile cilia. While the lack of DNAAFs in other cell types usually results in axonemes that assemble correctly but lack dynein arms, preliminary data suggested that in *Leishmania* the absence of DNAAFs led to a complete failure to assemble an axoneme. We aim to characterize the contribution of all identified *Leishmania* DNAAFs to the assembly and function of motile and non-motile flagella. To investigate whether the loss of specific assembly factors consistently results in flagellar assembly failure, we used CRISPR-Cas9 to generate seven DNAAF knockout (KO) mutants in a reporter cell line expressing a fluorescent flagellar membrane marker. Flagellar lengths were analyzed using semi-automated ImageJ-based quantification. Here, we present the results for the KOs of DNAAF3, DNAAF4, DNAAF6, DNAAF7, DNAAF13, ARL3 and CMF49. In the promastigote stage, all mutants displayed significantly reduced average flagellar lengths compared to the 13.99 µm measured for the parental cell line. DNAAF4 KO exhibited the shortest average length (2.17 µm), while ARL3 KO (12.27 µm) and DNAAF13 KO (8.82 µm) remained the longest. Distinct morphotypes were identified: DNAAF3 KO predominantly showed "stubs" (fluorescent signals that do not exit the flagellar pocket), DNAAF7 KO displayed "ball-like " structures (signal is at least as wide as thick). In the amastigote stage, all mutants remained viable, but flagellar remodeling was affected. The KOs of DNAAF4, DNAAF7, CMF49, and ARL3 had significantly shorter amastigote flagella (<1 µm), whereas DNAAF13 KO stood out as it displayed a range of flagellar lengths. These results suggests that DNAAFs in *Leishmania* are critical for axonemal assembly and/or structural stability rather than just dynein arm incorporation. In the future, we will expand this characterization to the remaining DNAAFs and evaluate the impact of these assembly defects on the ultrastructure of the promastigote and amastigote flagella and on host-parasite interactions through macrophage infection studies.