Structural and functional insights into ESAG3 and GRESAG3 proteins in African trypanosomes

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Abstract:

African trypanosomes are highly effective extracellular parasites that evade host immune responses through antigenic variation of variant surface glycoproteins (VSGs). VSGs are co-transcribed in blood-stage parasites alongside expression site-associated genes (ESAGs) that play key roles in host adaptation, including nutrient acquisition and resistance to human serum. Many ESAGs have homologues outside telomeric VSG expression sites, termed Genes Related to ESAGs (GRESAGs), which are expressed across both mammalian and insect lifecycle stages and are thought to have redundant functions with ESAGs. However, the extent to which GRESAGs can compensate for ESAG functions within their gene family is not well understood, and the specific functions of many ESAGs remain unknown.

Previous studies indicate that (GR)ESAG3 belong to the Fam53 family, is exclusively expressed in blood-stage trypanosomes, and is upregulated during chronic human infections, but the exact function of (GR)ESAG3 is unknown. Here, we show that although ESAG3 and GRESAG3 are phylogenetically distantly related gene families, both exhibits conserved key residues and a tertiary structure characteristic of class-A glycosyltransferases (GT). RNAi-mediated depletion of ESAG3 inhibits parasite growth *in vitro* without altering GRESAG3 expression levels. Biochemical fractionation and immunofluorescence analyses reveal that ESAG3 is localised to the ER, whereas GRESAG3 is localised to the Golgi apparatus, suggesting distinct functions despite their shared structural features.

Moreover, we demonstrate that purified recombinant ESAG3 exhibits GT activity. Size exclusion chromatography and native gel analysis of trypanosome extracts reveal that ESAG3 forms higher-order oligomers of ~700 kDa. Single-particle cryo-electron microscopy analysis at a resolution of 3.2 Angstroms reveals the ESAG3 complex as a ring-shaped structure formed by eighteen monomers organised into three hexamers. The structure reveals that the fundamental building block is a dimer with the GT active sites facing each other. These findings provide the first structural insights into the domain organisation of a GT from Kinetoplastids, generating testable hypotheses to uncover the precise role of potentially novel GT functionalities in Kinetoplastids in general or *T. brucei* in particular. It is tempting to speculate that (GR)ESAG3 is the GT responsible for *O*-glycosylating trimeric VSGs, which also assemble into an 18-mer structure, for which the GT remains elusive.