African trypanosomes secrete a cocktail of VSG family antigens

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Variant surface glycoproteins (VSGs) coat bloodstream-form African trypanosomes and underpin antigenic variation and immune evasion. For this, trypanosomes have a vast repertoire of sub-telomeric *VSG* genes, and they periodically switch the expressed, and RNA polymerase I transcribed, telomeric *VSG*. However, there is also a subset of genes, belonging to the wider *VSG* family, with distinct roles, in transferrin uptake and innate immunity, for example. A bioinformatics assessment revealed >50 chromosome-internally located *VSG* family genes in *Trypanosoma brucei,* typically located at the sites where polycistronic transcription converges. We also observed a similar distribution of VSG family genes in human-infective *T. b. gambiense.* Using transcriptomics and polymerase-selective RNA-seq, we confirmed that *VSG* family genes are transcribed by RNA polymerase II (pol-II). Quantitative proteomics revealed that VSG family proteins are typically expressed in the bloodstream stage, but not in the insect mid-gut stage. Although predicted to be structurally similar to VSGs, typically incorporating signal peptides, many VSG family proteins appear to lack GPI-anchor signals, suggesting that they may be secreted. Secretome data derived using quantitative (direct data-independent acquisition) proteomics revealed that many VSG family proteins are indeed secreted, along with many digestive enzymes. We suggest that bloodstream-stage specific secretion of a cocktail of VSG family antigens plays a role in modulating the host immune response. We are now incorporating signal-peptide compatible epitope-tags to further monitor the fate of a VSG and several VSG family proteins following secretion.