Control of variant surface glycoprotein expression by CFB2 in African trypanosomes and quantitative proteomic connections to translation and cytokinesis

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Abstract

Variant Surface Glycoproteins (VSG) coat parasitic African trypanosomes and underpin antigenic variation and immune evasion. These VSGs are super-abundant virulence factors that are subject to post-transcriptional gene expression controls mediated via the *VSG* 3'-untranslated region (3'-UTR). To identify positive VSG regulators in bloodstream form cells, we used genome-scale screening data to prioritise mRNA binding protein (mRBPs) knockdowns that phenocopy VSG mRNA knockdown, displaying loss-of-fitness and pre-cytokinesis accumulation. The top three candidates were CFB2 (cyclin F-box protein 2), MKT1 and PBP1 (polyadenylate binding-protein binding-protein). Notably, CFB2 was recently found to regulate VSG transcript stability, and all three proteins were found to associate. We used data-independent acquisition for accurate label-free quantification and deep proteome coverage to quantify expression profiles following depletion of each mRBP. Only CFB2 knockdown significantly reduced VSG expression and the expression of a reporter under the control of a *VSG* 3'-untranslated region (3'-UTR). CFB2 knockdown also triggered depletion of cytoplasmic ribosomal proteins, consistent with translation arrest observed when VSG synthesis is blocked. In contrast, PBP1 knockdown triggered depletion of CFB2, MKT1, and other components of the PBP1-complex. Finally, all three knockdowns triggered depletion of cytokinesis initiation factors, consistent with a cytokinesis defect, confirmed here for all three knockdowns. Thus, genome-scale knockdown datasets facilitate the triage and prioritisation of candidate regulators. Quantitative proteomic analysis confirms 3'-UTR dependent positive control of VSG expression by CFB2 and interactions with additional mRBPs. Our results also reveal connections between VSG expression control by CFB2, ribosomal protein expression, and cytokinesis.