VEX2: A Rogue Helicase Rewriting Eukaryotic Rules for Immune Evasion

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African trypanosomes demonstrate extreme molecular strategies for survival within the host. They periodically switch their homogeneous coat of a single variant-surface glycoprotein (VSG), which amounts to ~10% of their total transcriptome and proteome. Their extensive *VSG* repertoire of >2,000 *VSG* genes and pseudogenes can be further expanded through recombination, providing a virtually unlimited antigenic diversity for immune evasion. *VSG* genes are expressed solely from a limited subset of transcription units - expression sites (ES) - with only one active at a time and transcribed within the expression-site-body (ESB), a specialised sub-nuclear compartment.

VSG-Exclusion-Protein-2 (VEX2), a 224 kDa large member of the Superfamily 1 (SF1) helicases, specifically localises to the ESB, associates with the chromatin of the active-*VSG*-ES and is a master regulator of monogenic *VSG* expression, as its depletion leads to simultaneous expression of multiple VSGs within individual cells. However, the mechanistic details underpinning its function and regulation remain mysterious. Here, we present the first molecular and structural characterisation of VEX2.

Firstly, to determine if VEX2's helicase activity is the "engine" behind *VSG* exclusion, we developed a CRISPR/Cas9-mediated saturation mutagenesis screen paired with FACS sorting and amplicon-Seq. We found that replacing a single critical amino acid for any other residue disrupted allelic exclusion. Consistently, overexpressing a catalytically "dead" mutant recapitulated the transcriptional profile seen in VEX2-depleted cells. Together, these results confirm that VEX2's helicase activity is essential for maintaining monogenic *VSG* expression.

Secondly, to biochemically characterise the helicase activity, full-length VEX2 was recombinantly expressed and purified using a *Leishmania tarentolae*-based expression system (LEXSY). The protein shows RNA/DNA unwinding activity *in vitro*, confirming its helicase function and substrate preference. Using single particle cryo-electron microscopy, we determined the VEX2's apo structure at 3.5 Å resolution. Notably, the prong subdomain—required for autoinhibitory N-terminal interactions in the yeast orthologue Sen1—is absent, consistent with substantial deletions within the primary sequence compared to Sen1. Furthermore, unlike Sen1,

our cryo-EM analysis does not show evidence for a stable VEX2 N-terminal–helicase domain interaction, implying a divergent regulatory mechanism.

Thirdly, DRIP-Seq showed that VEX2 depletion leads to increased R-loop levels at *VSG*-ES promoter-proximal regions. These data suggest that VEX2 is required to mitigate the excessive R-loop formation driven by intense transcription at the active site, thereby preventing transcriptional stalling and genome instability.

Finally, we propose that VEX2 is a constitutively active helicase optimised for high-flux RNA:DNA unwinding at the active-*VSG*-ES. We suggest *T. brucei* has evolved a spatial rather than auto-inhibitory regulatory strategy, typical of the SF1 superfamily. This specialisation likely enables maximal catalytic power while precluding off-target genomic instability—a distinct evolutionary adaptation for parasitic immune evasion.