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Abstract text (4000 characters)

"Multi-allelic exclusion by an allele-selective RNA-DNA helicase in African trypanosomes"

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Allelic-exclusion mechanisms, including those that underpin antigenic variation in parasites and olfaction in mammals, are not fully understood, remaining one of the greatest outstanding mysteries in eukaryotic biology. In African trypanosomes, immune evasion involves expression of a single Variant-Surface-Glycoprotein (VSG) gene in a dedicated sub-nuclear expression factory. VSG-exclusion-1 (VEX1) and VEX2 are concentrated at the splicing and transcription compartments, respectively (PMID: 33432154).

Here, using ChIP-Seq, we show that VEX2 is associated with the active-*VSG* expression-site, forming an allele-specific bridge, via VEX1, to a *trans*-splicing locus on another chromosome. Further, single cell RNA-Seq analysis following VEX2 depletion revealed the simultaneous expression of several VSGs and showed: 1) the number of simultaneously active VSGs that can be tolerated; 2) a hierarchy of *VSG* transcriptional derepression.

Moreover, VEX2 is a large protein that forms a native multimeric complex of approximately 1 MDa. *In vitro* data using a recombinant helicase core showed that it is an RNA-DNA helicase. To assess whether this helicase activity was required for *VSG*-monogenic-expression, we established a FACS-based CRISPR-Cas9-mediated saturation mutagenesis assay combined with amplicon-Seq profiling. Replacement of a critical amino acid in the helicase core by any other amino acid disrupted allelic exclusion. This phenotype was only rescued by synonymous mutations.

Finally, we found that VEX1 and VEX2 interact via their *N*- and *C*-termini, respectively and reciprocal turnover control limits the abundance and maintains sequestration of the complex.

This work begins to reveal the mechanism by which the VEX complex sustains *VSG*-monogenic-expression in African trypanosomes.