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

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Large gene-families often exhibit monogenic expression, with contingent processes including antigenic variation for immune evasion in parasites, and olfaction and B-cell development in mammals. Despite intense study, the mechanisms that underpin these paradigmatic examples of stochastic gene choice and exclusion remain somewhat mysterious.

Substantial recent progress yielded Variant-Surface-Glycoprotein (VSG) exclusion factors in African trypanosomes and a new appreciation of the context in which they operate. Specifically, VSG-exclusion-2 (VEX2) accumulates at the active-*VSG* expression-site and binds VEX1 at a *trans*-splicing locus on another chromosome; other *VSGs* are excluded from this sub-nuclear expression-factory (PMIDs: 31289266; 33432154).

We performed single cell RNA-Seq following VEX1 or VEX2 depletion, revealing a surprisingly complex mixture of simultaneously active *VSGs* in single cells, and a striking difference between both factors. Further, this analysis showed: 1) the number of simultaneously active VSGs that can be tolerated; 2) a hierarchy of *VSG* transcriptional derepression.

ChIP-Seq indicated strong enrichment of VEX2, which forms a native complex of ~1 MDa, at the active-*VSG* expression-site, particularly accumulating at the expression-site associated genes (ESAGs) coding regions. Using super-resolution microscopy, VEX2 N- and C-termini were distinctively visualised extending towards the active-*VSG* and the splicing locus, respectively, revealing an allele-selective inter-chromosomal bridge, via VEX1, to a *trans*-splicing locus on another chromosome.

Through a combination of super resolution microscopy and native gels, we found that most of VEX1 and VEX2 sub-nuclear pools are not in complex with one another, and their interaction is dynamic and cell-cycle regulated.

To further dissect VEX interactions, we generated several VEX1 truncated forms, and found that the VEX1 N-terminal fragment (1-289 aa) interacts with VEX2, stabilising it and sustaining exclusion. Additionally, we found that the VEX1 C-terminal fragment, which includes nucleic acid binding domains, also contains regions involved in protein stability and turnover.

Finally, VEX2 is a putative DNA:RNA helicase, thus to assess whether this activity was required for *VSG*-exclusion, we established a CRISPR/Cas9-mediated saturation-mutagenesis assay using FACS followed by amplicon-Seq profiling. Replacement of a critical amino acid with any other amino acid disrupted allelic exclusion.

This work begins to reveal the mechanisms by which the VEX complex promotes stochastic *VSG* gene choice and allelic exclusion in African trypanosomes.