How is VEX2 recruited to the expression-site body in *Trypanosoma brucei*?

Beth Spink[1,2], Kiera Hayton[1,2], Sarah Stevens[1,2,3], Joana R. C. Faria[1,2]

[1] Biology Department, University of York, UK

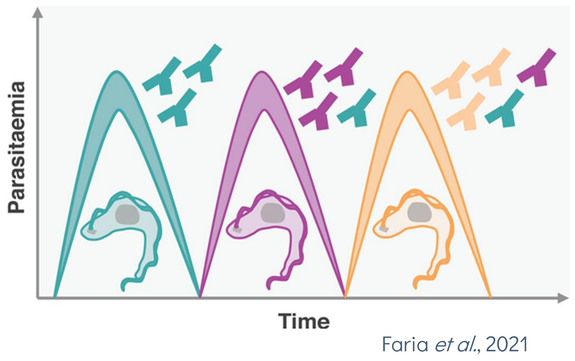
[2] York Biomedical Research Institute, University of York, UK

[3] Current address: School of Life Sciences, University of Dundee, UK

Antigenic variation is essential for parasite survival in the mammalian host

Stochastic switching of variant surface glycoprotein (VSG) allows parasites to evade the immune response and maintain persistent infections.

Antigenic variation

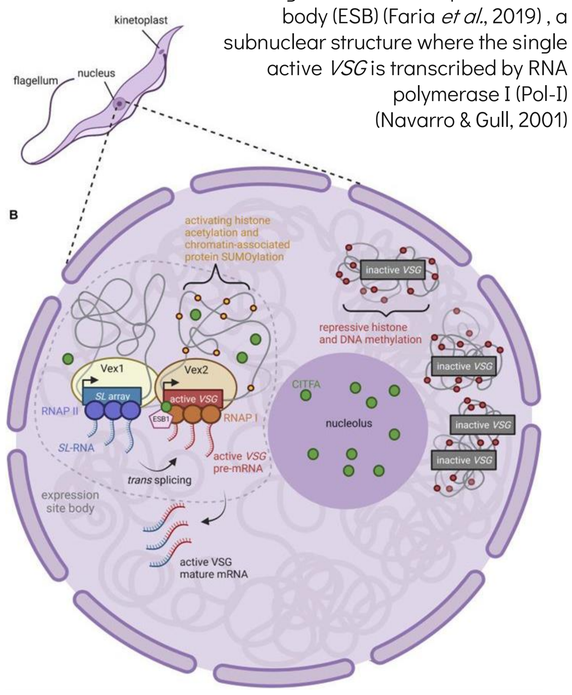


Faria *et al.*, 2021

Monoallelic expression of VSGs is essential for the survival of the parasite, as cells expressing multiple VSGs at once are easily cleared by the immune system (Aresta-Branco, 2019).

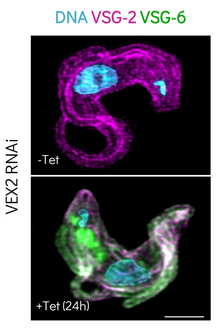
The active *VSG* is expressed in a specialised subnuclear body

VSG-Exclusion protein 2 (VEX2) forms a single focus at the expression site body (ESB) (Faria *et al.*, 2019) , a subnuclear structure where the single active *VSG* is transcribed by RNA polymerase I (Pol-I) (Navarro & Gull, 2001)



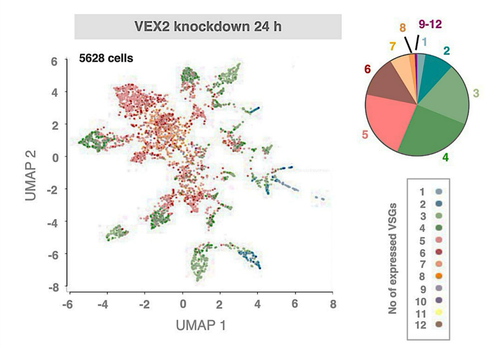
Faria *et al*, 2019
Faria *et al*, 2021
Willliams *et al.*, 2022

Monoallelic VSG expression is controlled by VEX2



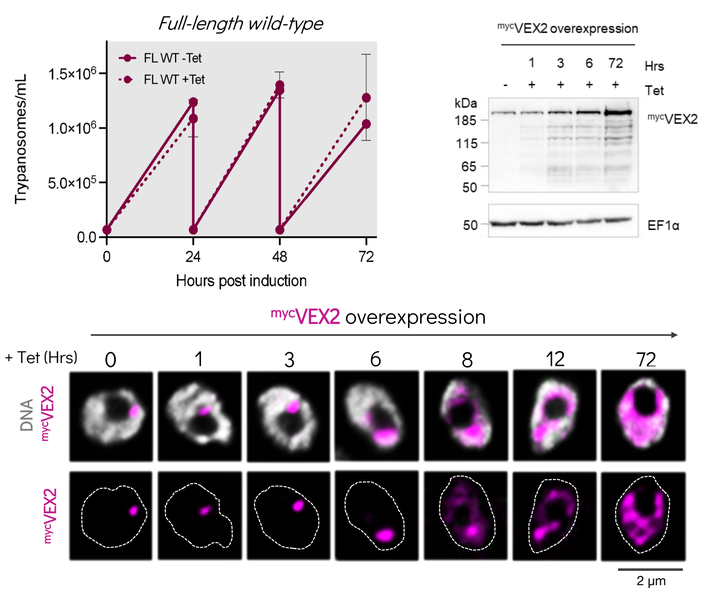
VEX2 knockdown leads to derepression of all Pol-I transcribed protein-coding genes, including all silent *VSGs* (Faria *et al.*, 2019 & 2023)

scRNA-Seq:

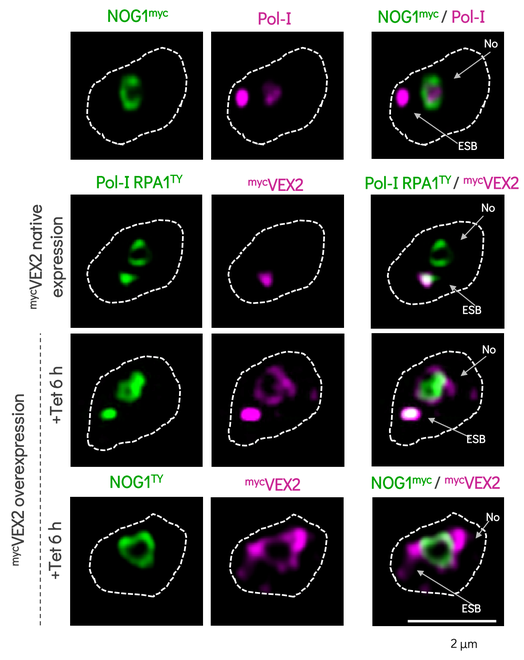


- VEX2 is an orthologue of the RNA:DNA helicase senataxin.
- **However, the specific role of VEX2 in maintaining monoallelic *VSG* expression remains unclear.**

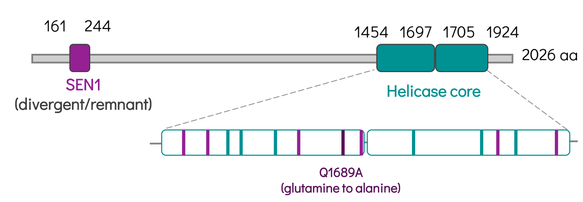
VEX2 has affinity for sites of Pol-I transcription but preferentially localises to the ESB



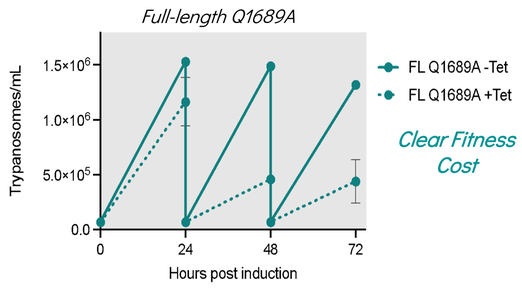
When overexpressed, VEX2 first accumulates at the ESB and then forms a ring around the nucleolus. The cells retain a single ESB and preserve *VSG* monogenic expression.



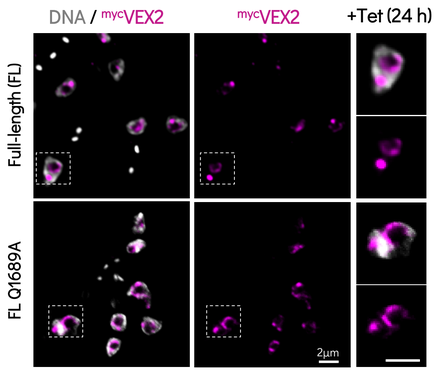
VEX2's helicase activity is required for VSG expression control

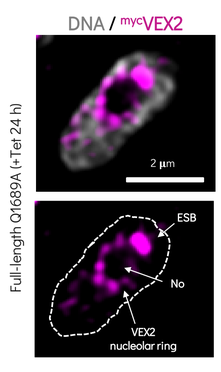


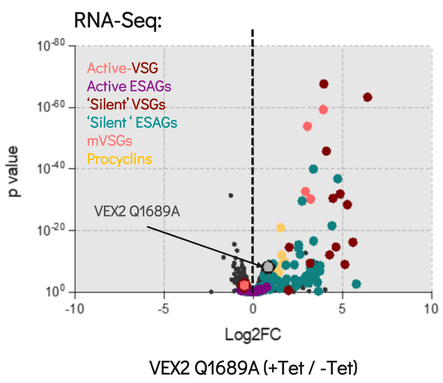
CRISPR/Cas9-mediated precision editing has shown that replacement of Q1689 by alanine is lethal (not shown).

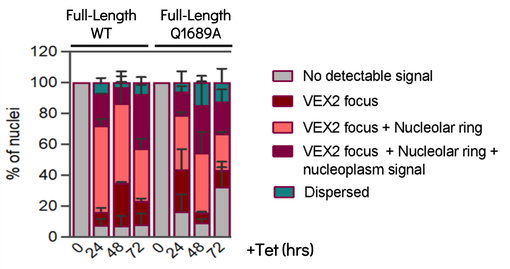


Clear Fitness Cost





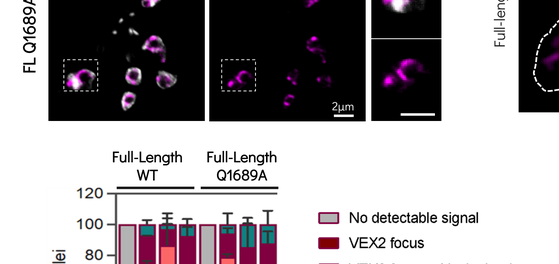


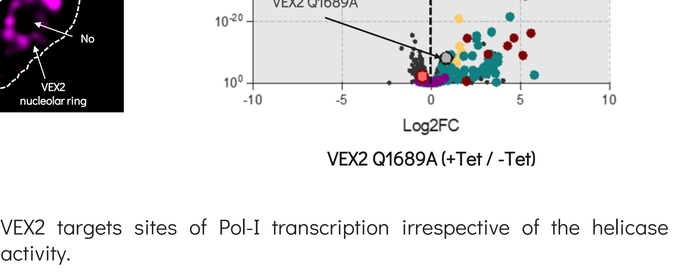


VEX2 targets sites of Pol-I transcription irrespective of the helicase activity.

However, abrogating the helicase activity produces a dominant negative phenotype similar to VEX2 knockdown, indicating that it is required for *VSG* expression control.

VEX2 compartmentalisation is dependent on active Pol-I transcription.





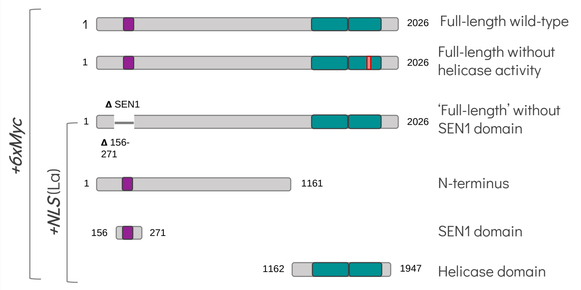
VEX2, unlike ESB1, is recruited to the ESB in a transcription-dependent manner. tSNAP remains focal, showing that the results are VEX2-specific and not a consequence of deleterious effects on general chromatin organisation.

Transcriptomics shows that we successfully blocked transcription at the active-site, and activation of silent ESs is consistent with VEX2 dispersion.

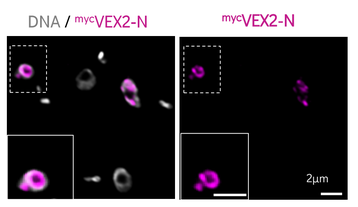
These results suggest that:

1) VEX2 recruitment occurs after transcriptional activation (likely mediated by ESB1).
2) RNA is required for VEX2 foci formation.

Which domains of VEX2 are required for its recruitment to the ESB and/or function?



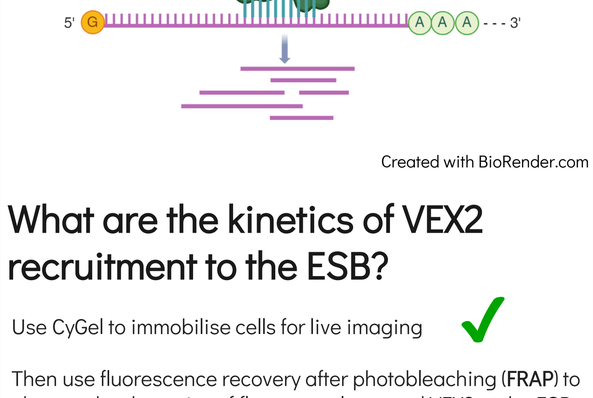
A sneak-peek...



VEX2 N-terminus overexpression (+Tet 24 h)

The N-terminus of VEX2 seems to be sufficient for recruitment to the ESB.

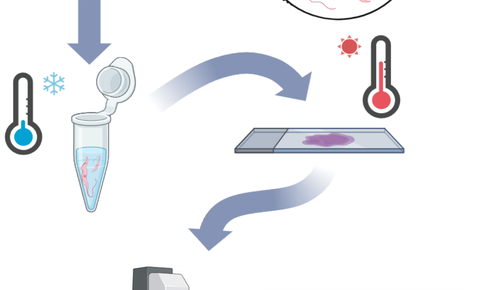
Can any of VEX2's domains rescue the RNAi knockdown phenotype?



Created with BioRender.com

Is the assembly of VEX2 foci RNA-dependent?

Use Cross linking Immunoprecipitation sequencing (**CLIP-Seq**) to identify the specific RNA sequences that interact with VEX2



Created with BioRender.com

What are the kinetics of VEX2 recruitment to the ESB?

Use CyGel to immobilise cells for live imaging ✓

Then use fluorescence recovery after photobleaching (**FRAP**) to observe the dynamics of fluorescently tagged VEX2 at the ESB.