Defining the spatial interactome of VSG-Exclusion Proteins 1 and 2 using TurboID in African Trypanosomes.

Lianne Lansink[1], Adam Dowle[1], Jose Baptista[2], Richard McCulloch[2], Joana R. C. Faria[1]

[1]York Biomedical Research Institute, University of York

[2]Wellcome Centre for Integrative Parasitology, university of Glasgow

African trypanosomes are 'masters of disguise'. They rely on a vast genetic repertoire (>2,600 genes and pseudogenes) encoding from their variant surface glycoprotein (VSG) to undergo antigenic variation and successfully evade their host immune response. Their ability to express a single *VSG* at any given time, *monogenic expression*, is imperative for successful antigenic variation; yet mechanisms governing this complex process are not fully understood in any eukaryote.

In *Trypanosoma brucei* bloodstream-form, the single active-*VSG* is transcribed by RNA-Polymerase I within the expression-site body (ESB). VSG-exclusion-2 (VEX2) accumulates at the ESB and binds VEX1 at the *Spliced-Leader* (SL) locus on another chromosome; other *VSGs* are excluded from this sub-nuclear 'expression-factory' (PMIDs: 31289266; 33432154). The VEX proteins, particularly VEX2, are critical to sustain *VSG* monogenic expression, however the mechanism remains mysterious.

To dissect VEX1/VEX2 function(s) and the sub-nuclear context in which these proteins operate, we sought to define their spatial interactome using proximity labelling combined with LC-MS/MS analysis. We generated cell lines where VEX1 or VEX2 were fused with TurboID. In the case of VEX2, because it is an unusually large protein (>200 kDa; 1 megadalton in native conditions), TurboID was placed at either the N or C- terminus to increase spatial resolution. Super resolution microscopy revealed that all the TurboID-protein fusions localised to the expected sub-nuclear compartments; upon biotin addition, highly compartmentalised biotinylation could be achieved.

Further, following LC-MS/MS analysis, the identification of known VEX-interactors (e.g. CAF-1 subunits) as well as a collection of proteins known to reside within the ESB or in close spatial proximity, validated the approach. Interestingly, we found several additional proteins of known and unknown function enriched in either or both VEX2-TurboID datasets. We are currently validating whether these proteins directly interact with VEX2.

Moreover, phylogenetic analysis suggests that VEX2 is a rather divergent form of Senataxin (SETX), an RNA:DNA helicase involved in transcription and splicing regulation in mammals, as well as resolving R-Loops at transcription termination sites. We are currently exploring whether VEX2 displays similar substrate preference and functions in trypanosomes. Interestingly, we found some potential interactors in our proximity labelling data that are consistent with SETX-related functions.

Overall, we hope that this study will improve our understanding of the molecular mechanisms underpinning allelic exclusion.