Heterogeneous elongation of RNA polymerase I transcription at the active VSG expression site in *Trypanosoma brucei*

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A Variant Surface Glycoprotein (VSG) coat protects bloodstream form *Trypanosoma brucei* within the mammalian host. VSG is the most abundant mRNA in the cell, with the active VSG gene expressed from one of ~15 VSG bloodstream-form expression sites (BES). The active BES is exclusively transcribed by RNA Polymerase I which is thought to provide continuously high levels of VSG mRNA production. However, using different microscopy approaches we have consistently observed that transcription of the active BES may be heterogeneous. To investigate this further, we used an eGFP reporter assay and directly measured nascent RNA levels across the active BES using single molecule RNA-FISH and 4sUTP nascent RNA labelling.

Interestingly, we find a decrease in pre-mRNA production along the active BES with the highest pre-mRNA levels at active BES promoter region, and the lowest pre-mRNA levels near the telomeric VSG. We find a similar effect at both long and short BESs indicating that reduced nascent RNA production at the active BES telomere is independent of BES length. In addition, previously published RNA Pol I ChIP-qPCR experiments show that RNA Pol I occupancy is higher at the active BES promoter region than at the active telomeric VSG. These data point to a model whereby RNA Pol I elongation is impaired as proximity to the telomere increases, resulting in generation of lower levels of nascent transcripts from the active BES telomere compared to the promoter. These unexpected observations challenge the paradigm that RNA Pol I provides continuously high levels of transcription throughout the active BES.