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Characterisation of the *ESAG6* and *ESAG7* 3'UTRs involved in the iron starvation response in *Trypanosoma brucei*

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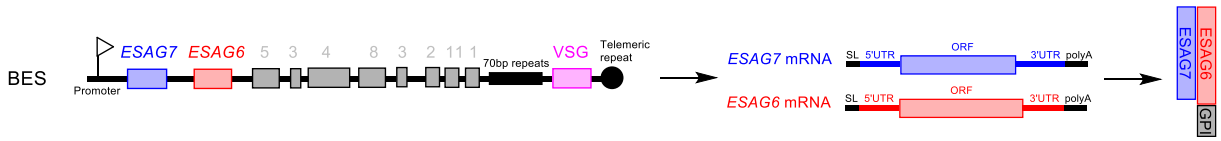
Introduction

Trypanosoma brucei obtains iron by uptake of host transferrin (Tf) through its own transferrin receptor (*Tb*TfR).

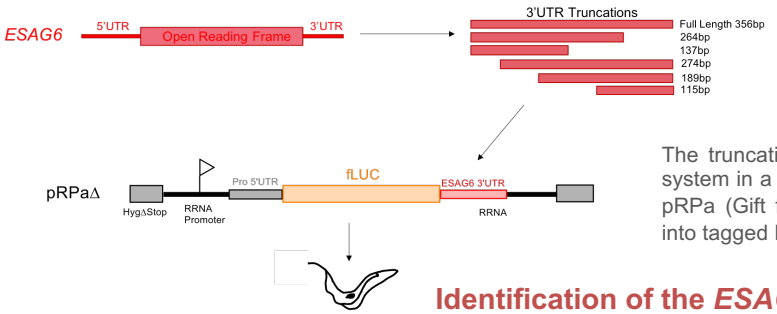
- *Tb*TfR is a heterodimer composed of the glycoproteins ESAG6 & ESAG7[1]
- ESAGs are proximal to the VSG promotor and transcribed only from the active BES by RNA Pol I
- Iron starvation rapidly increases ESAG6 & 7 equally at the mRNA and protein level (~5-fold in 6h)[2,3]
- TfR upregulation proceed VSG switching events
- Dynamic regulation of the *Tb*TfR is mediated via the *ESAG6* 3' untranslated region (UTR)[4]

Here, we aim to identify important motifs in the *ESAG6* 3'UTR and characterise the *ESAG7* 3'UTR .

Model of *Tb*TfR heterodimer. Tf recognition occurs at hyper-variable regions at top of structure. (Mehlert et al. PLoS Pathogens 2012)



Generating truncations of the *ESAG6* 3'UTR

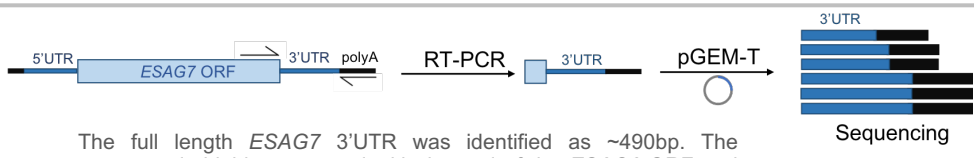


Primers were designed to truncate the *ESAG6* 3'UTR at regions of hypervariability between the BES. The truncated sections were generated by PCR using the full length as a template.

The truncations were ligated into a Firefly luciferase (fLUC) reporter system in a constitutively-expressing version of the landing pad vector pRPa (Gift from Sam Alsford, LSTHM), facilitating targeted insertion into tagged RRNA locus of the 2T1 bloodstream form cell line.

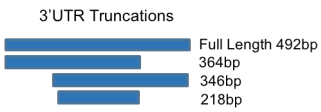
Identification of the *ESAG7* 3'UTR

The *ESAG7* 3'UTR was identified by Reverse Transcriptase – PCR from bloodstream form cells expressing VSG221 from BES1.[5]



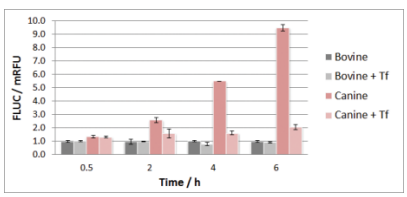
The full length *ESAG7* 3'UTR was identified as ~490bp. The sequence is highly conserved with the end of the *ESAG6* ORF and 3'UTR and between the different BES.

N and C terminal truncations of the *ESAG7* 3'UTR were generated by PCR. Primers were designed to give sequences similar in length to the *ESAG6* 3'UTR, they were ligated into the *fLUC* reporter system and transfected into 2T1 BSF cells.

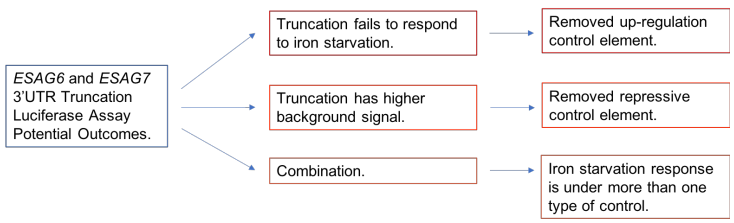


Future Work

Luciferase activity assays will be performed on the *ESAG6* and *ESAG7* 3'UTR full length and truncated sequences to identify changes in the upregulation of the TfR under iron starvation conditions. To induce the iron starvation response in the cells lines, cultures will be switched from growth in 10% bovine serum to 10% canine serum (5h). The luciferase assay will be performed on cells in log phase ($1x10^6$ cells/mL) with a stabilised firefly luciferase substrate (OneGlo, Promega), in triplicate compared to control cell lines under normal and iron starvation conditions.



Graph shows how iron starvation by serum switch results in rapid upregulation of the transferrin receptor over 6 hours. Fluorescence is proportional to expression of the TfR.



If an important motif is identified, it could be used as a selection marker to aid in identification of the signalling pathway involved in the upregulation of the transferrin receptor under iron starvation conditions.

References: 1. Steverding et al. (1994) Eur. J. Cell Biol. 64, 78-87. 2. Fast et al. (1999) Biochem J. 342, 691-696. 3. Mussmann et al (2004) J. Biol. Chem. 279, 40690-40698. 4. Benz et al. (2018) PLoS One 13, e020633. 5. Alsford et al (2005) Mol.Biochem.Parsit. 114, 142-148.