Characterising the Heat Shock Response in *Trypanosoma congolense*

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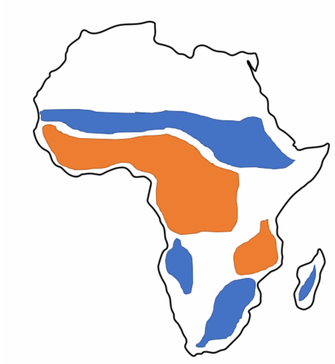
What is the impact of *T. congolense?*

T. congolense is a zoonotic parasite, transmitted by the tsetse fly. It causes a significant economic burden across the tsetse belt, estimated at $2.5 billion over 20 years[1]. It causes a wasting disease, Nagana, in cattle makes raising them a difficulty.

Currently only poor pharmaceutical treatment is available and resistance is emerging against them.



- Tsetse belt
- Cattle raising areas

Figure 1. Locations of the tsetse belt where *T. congolense* is present and cattle raising areas, there is no overlap between the two.

Why study the heat shock response?

The heat shock response is a key virulence factor helping trypanosomes survive in the mammalian host.

The host immune system elicits a fever of up to 41° C which the parasite survives by halting global translation of mRNAs[4] and upregulating heat shock proteins (HSPs).

Understanding this virulence factor may lead to the discovery of novel drug targets.

We can use information known about the well studied *T. brucei*, as it has been exposed to the same evolutionary pressures as *T. congolense*, so they are expected to have similar mechanism's to respond to the host.

Project aims

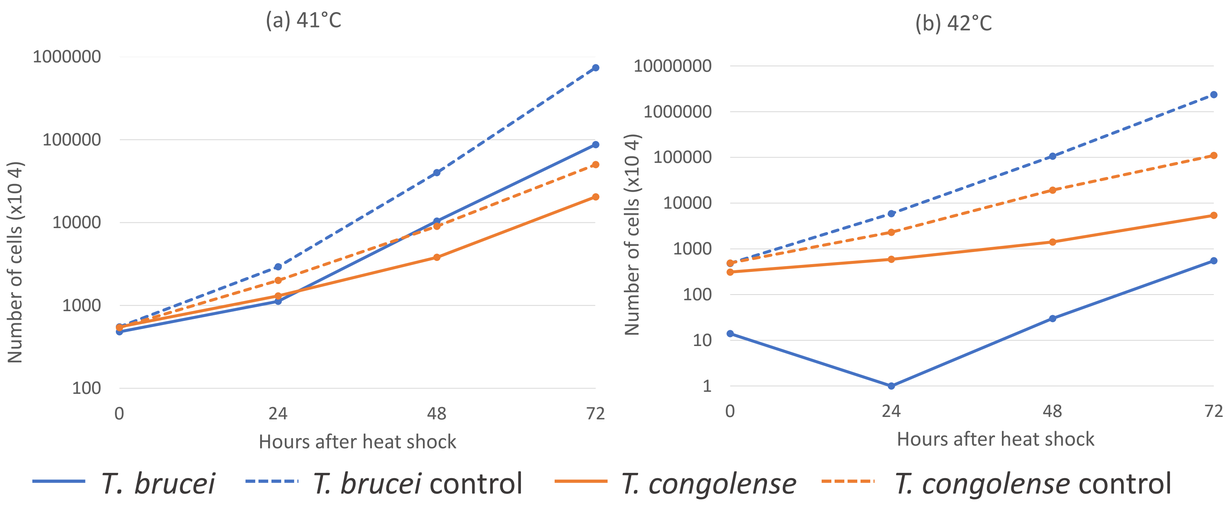
- Optimise temperatures and timepoints of *T. congolense* heat shock
- Investigate the arrest in heat shock with slow cytometry.
- Use genetic manipulation to tag heat shock related proteins to see if they localise in the same way during and after heat shock in both *T. brucei* and *T. congolense*
- Perform RNAi knockdown of ZC3H11, a protein integral to regulating the heat shock response, in *T. congolense* and observe effects.

Conservation of heat shock proteins and phosphorylation sites

Bioinformatic analysis to compare conservation of proteins and phosphorylation sites involved in heat shock was performed. Of those looked at 98% of proteins were conserved and 85% of p-sites, some key proteins shown below. These proteins will be tagged[2] to see if same localisation/change in abundance is seen in both *T. brucei* and *T. congolense* during heat shock.

Protein	Role in *T. brucei* Heat Shock Response	Conserved protein?	Conserved p-sites?
ZC3H11	Regulation of heat shock related proteins by mRNA stabilisation[3]	Yes	4 of 5 p-sites conserved
DHH1	Localises into p-bodies[4] and shows 140-fold change in phosphorylation[5]	Yes	2 of 2 p-sites conserved
SCD6	Localises into p-bodies	Yes	No p-sites
XRNA	Localises to novel foci and also in heat shock granules, degradative enzyme	Yes	No p-sites
HSP100	Effector of heat shock, mRNA increases 6-fold	Yes	No p-sites
PABP2	Localises in to heat shock granules, chaperones mRNAs	Yes	4 of 4 p-sites conserved

T. congolense responds differently to heat shock than *T. brucei*



T. brucei experiences heat shock at 41°C for 1 hour, some cell death is seen and there is a lag in growth for 24 hours afterwards as cells recover. At 42 °C for 1 hour there is significant cell death.

T. congolense does not undergo such significant changes at 41°C, appear to be more resistant to increased temperatures. They experience heat shock at 42°C.

These differences are of interest as *T. congolense* is cultured at a lower temperature than *T. brucei* and has a longer division time.

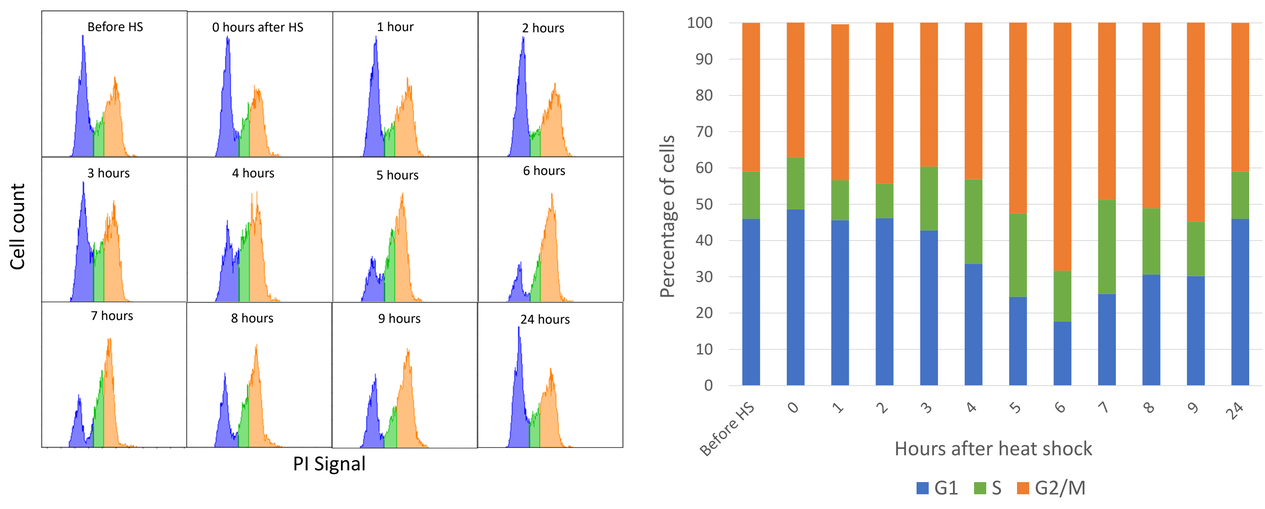
Figure 2. Growth curves of *T. brucei* and *T. congolense* when subjected to heat shock at (a) 41°C and (b) 42°C for 1 hour. Cells were heated in a water bath and counted using a haemocytometer.

T. brucei shows a growth arrest 6 hours after heat shock

5-6 hours after heat shock *T. brucei* experiences an arrest in G2/M, then the cell cycle appears to restart by 8 hours and cells move into G1. This corresponds to the lag in growth seen after heat shock.

Experiments will be repeated with *T. congolense* and this will be further investigated using DAPI staining to observe effects on cell morphology and division.

Figure 3. Cell cycle stages of *T. brucei* cells taken at 1 hour timepoints for 9 hours after heat shock (41°C for 1 hour), where 0 hours is the end of exposure to heat shock. Cells were stained with PI then (a) flow cytometry was performed, (b) cumulative frequency graph shows the percentage of cells in each cell cycle phase.



References: 1. Shaw et al. (2014) Preventative veterinary medicine. 113, 197-210. 2. Awuah-Mensah et al. (2021) PLoS Pathogens. 17. e1009224 3. Droll et al. (2013) PLoS pathogens. 9, e1003286 4. Kramer et al. (2008) Journal of cell science. 121. 3002-3014 5. Ooi et al. (2020) Journal of proteomics. 219, 102725.