Tempered transcripts: the response to iron deprivation in *Toxoplasma gondii*

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Obligate parasite *Toxoplasma gondii* can infect and scavenge essential nutrients from a diverse range of host cells. The availability of these nutrients can vary wildly from host to host, as such regulation of genes associated with nutrient sensing, scavenging and storage are critical to the success of this parasite. Iron is one of the most essential, and highly variable, nutrients which must be scavenged by *Toxoplasma*. Despite its importance, iron must be carefully regulated to maintain homeostasis due to its toxicity at high levels.

Regulation of parasite iron is complex and likely occurs at multiple levels. We observed a 40% increase in total RNA per parasite, and a bias towards upregulated transcripts in iron deprived parasites (RNA-sequencing). This contrasted a significant reduction in translation in iron deprivation; and suggested post-transcriptional regulation is important in low iron environments. This was the case for the parasite surface Zn/Fe transporter, ZFT (Tg261720). We identified a stem-loop structure in the 3' untranslated region of ZFT important for the stabilisation of ZFT transcripts. The importance of this region is evidenced by a significantly reduced survival rate in deletion mutants when cultured in low iron.

Beyond ZFT, a further 400 transcripts showed altered stability during iron deprivation. Most did not contain similar ZFT-like stem-loops - raising questions as the mechanism of changes in transcript stability. Many organisms regulate mRNA stability using RNA-binding proteins. To identify potential iron-responsive mRNA-binding proteins (mRBPs), we examined how the mRNA-bound proteome changes in low iron conditions using RNA-interactome capture. These experiments provide the first *Toxoplasma* 'mRBP-ome' of over 300 proteins. Observed iron-dependant changes to RNA-occupancy hinted at changes to splicing, composition of translation initiation complexes and formation of stress granules. To further investigate the formation of iron deprivation induced stress granules, we epitope tagged the *Toxoplasma* homologue for human stress granule initiator G3BP1 (Tg243960). We observed that Tg243960 forms stress granule-like aggregates in the parasites during iron deprivation. Work is now ongoing to validate identified mRNA-binding proteins and further investigate the formation and composition of iron starvation induced stress granules in these parasites.

Together, our data demonstrate the existence of iron-mediated post-transcriptional regulation in *Toxoplasma* for the first time and opens a new vista of mRNA-binding proteins in *Toxoplasma*.