Post-transcriptional iron regulatory mechanisms in *Trypanosoma brucei*

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Iron is essential for many enzymatic reactions but also potentially toxic when in excess. Consequently, cells exert a tight control of intracellular iron levels. Despite its central role in the virulence of protozoan parasites, our understanding of iron homeostasis in T. brucei remains limited.

Our transcriptomic analyses of bloodstream form (BSF) T. brucei identified a cohort of iron regulated mRNAs that include the main iron carrier protein (the transferrin receptor, TfR), an RNA binding protein (RBP5), genes involved endocytosis (Phosphatidic Acid Phosphatase, PAP2), ESAG3, and a variety of membrane proteins. RBP5 and TfR are upregulated within one hour of iron depletion, suggesting that they are part of the primary starvation response. In contrast, PAP2 upregulation begins after RBP5 levels have nearly peaked. We propose that PAP2 upregulation is a secondary response to iron deficiency that may be mediated by RBP5. In support of this notion, ectopic overexpression of RBP5 results in upregulation of PAP2 transcripts while knockdown of RBP5 reduces PAP2 levels. In addition, RNA immunoprecipitation (RIP) analysis indicates that RBP5 binds to PAP2 mRNA. Current efforts are focused on identifying sequence element(s) in the PAP2 RNA bound by RBP5 and determining the full cohort of mRNAs bound and potentially regulated by RBP5.

Interestingly, RIP shows that RBP5 also binds its own mRNA but not to TfR mRNAs, suggesting that RBP5 does not mediate the primary response to iron depletion. To gain insights into the factors directing the initial response to iron depletion, we have begun to define the cis-acting elements in the RBP5 mRNA that regulate RBP5 expression. By employing a dual luciferase system and deletion analyses we defined a 94-nucleotide iron responsive element (IRE) within the RBP 3'UTR that represses expression under iron replete conditions. A second 99 nucleotide element appears to constitutively down-modulate RBP5 expression, though it does not appear to be essential for iron-responsive regulation. We are in the process of refining the boundaries of the IRE to define the minimal sequences required for iron responsiveness, with the long-term goal of identifying associating RBP(s).

In support of the biological relevance of RBP5 regulation by iron levels, available transcriptome data show that RBP5 is upregulated in relatively iron-poor niches: in salivary glands relative to midgut, in adipose tissues relative to blood, and in metabolically quiescent stumpy forms. We propose a model in which early upregulation of RBP5 upon iron starvation facilitates the subsequent regulation of a variety of iron responsive genes. Insights gained from these studies will help to reveal the complexities of iron homeostasis in BSF T. brucei.