

Zinc-based regulation of the trypanosomatid ZIP transporters.

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Transition metals, such as zinc, are crucial micronutrients to trypanosomatids, yet deleterious if in excess. To thrive and cause disease, it is imperative that pathogens rapidly sense host-induced changes in metal bioavailability and translate these into adaptive responses.

In *Leishmania*, the expression of the high-affinity zinc transporter *LiZIP3* (Carvalho et al, *Mol. Microbiol*, 2015) is tightly regulated by zinc. It has been proposed that a short-lived negative regulatory and zinc-responsive RNA-binding protein interacts with a *cis*-acting element in the ZIP3 3'UTR, but this factor has not been identified. Here we show that the transcript levels of the *Trypanosoma brucei* ortholog ZIP proteins (*TbZIPs*) increase when parasites are cultured in zinc-limiting conditions (with the zinc chelator TPEN), this being reverted by addition of the metal. The fact that parallel assays with an iron chelator (DFO), Fe(II) and Fe(III) show no effect on *TbZIP* transcript levels indicates that the modulation of these transporters is zinc-specific. Additionally, analysis of mutated *TbZIP* 3'UTRs allowed the mapping of a zinc-sensing element to a 75-bp fragment.

To gather more insights on *T. brucei* ZIP transporter regulation, we generated a reporter strain where a Neo resistance gene is under the control of a *TbZIP* 3'UTR. The resulting Neo-reporter was confirmed to respond to zinc limiting and surplus conditions as expected. This strain was subjected to a genome-scale RNAi (RIT-seq) screen where gain of fitness under Neo pressure should identify zinc-responsive *trans*-acting negative regulators. *TbZIPs* are themselves strong hits in this screen, indicating that the Neo-reporter is upregulated in zinc-limiting conditions that arise due to *TbZIP* knockdown. Furthermore, we identified a nuclear RNA-binding protein with several zinc knuckle motifs and a putative nuclease domain. We suggest that this is a conserved mediator of the trypanosomatid response to environmental zinc availability.