Comparative study of the role of *Tc*HRG in the transport of hemin and hemoglobin in *Trypanosoma cruzi*

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Introduction

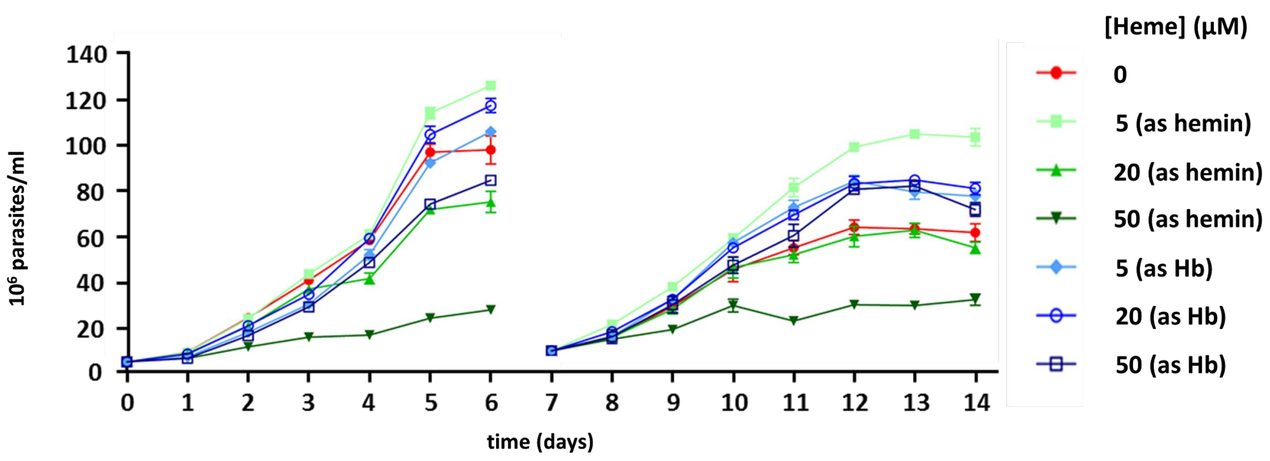
Trypanosoma cruzi is a heme auxotroph, therefore it must scavenge heme (as free heme or as hemoglobin) from their hosts. The membrane protein *Tc*HRG (*T. cruzi* Heme Responsive Gene) is involved in the uptake of this cofactor[1]. Recently, we proposed a model in which *T. cruzi* is able to sense intracellular heme and therefore modulates *Tc*HRG expression according to it[2]. Considering that, conversely to other trypanosomatids, no hemoglobin (Hb) receptor has been described yet in *T. cruzi*, we analyzed if *Tc*HRG may be involved also in Hb uptake.

Aims

- Analyze the use of Hb as a heme source
- Investigate the role of *Tc*HRG in free heme and Hb uptake

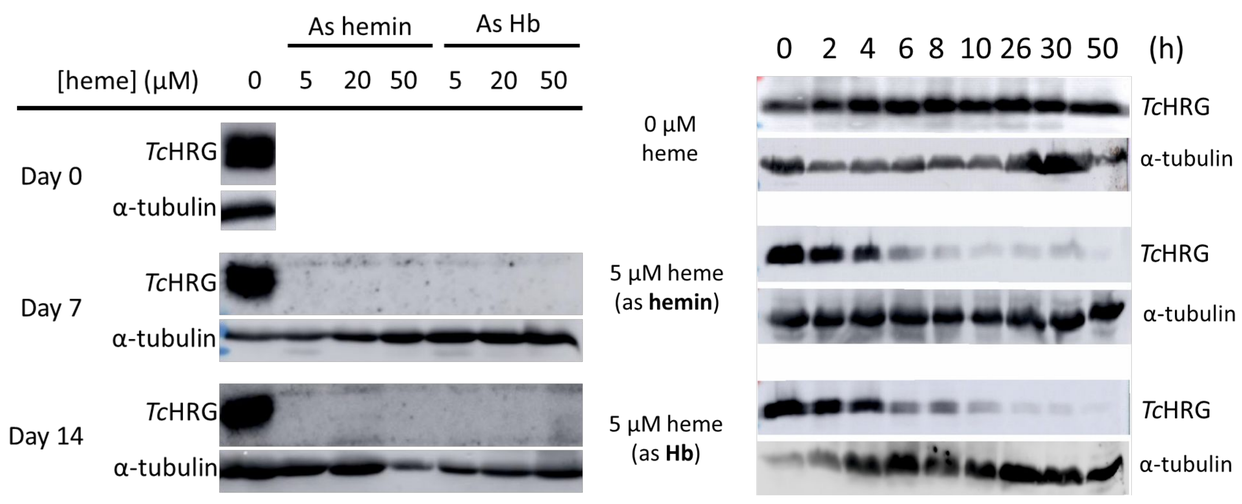
Results

Epimastigotes tolerate high Hb concentrations



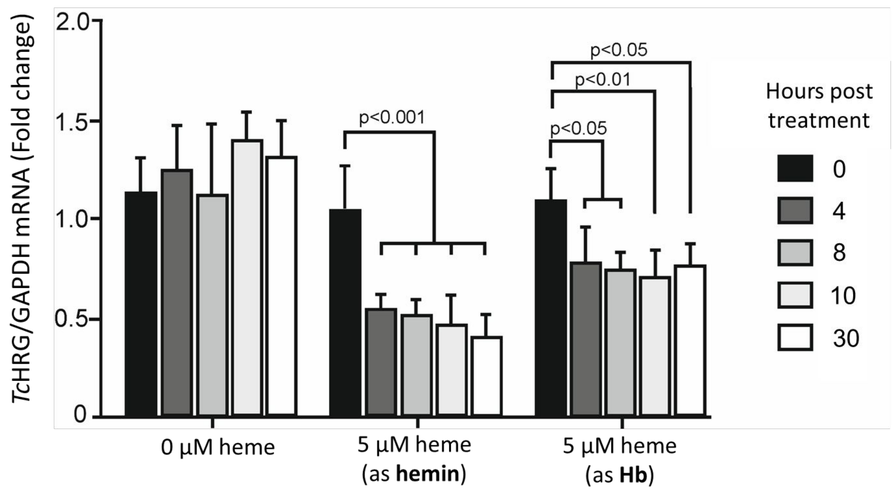
Growth curve of Dm28c Wild Type (WT) epimastigotes. Parasites were grown during 14 days in LIT (Liver Infusion Tryptose) + 10% FBS (Fetal Bovine Serum) supplemented with different equivalent concentrations of hemin and Hb (equivalent to 0, 5, 20 and 50 µM of heme). A dilution to the initial parasite concentration was performed at the 7th day, keeping the same the culture condition. Previously, the parasites were challenged to a "heme starvation" during 48 h. Each condition was performed by triplicate and each point of the curves corresponds to the mean of the three replicates ± SD. Standard growth condition is LIT + 10% FBS + 5 µM hemin.

Endogenous *Tc*HRG rapidly responds to both heme sources



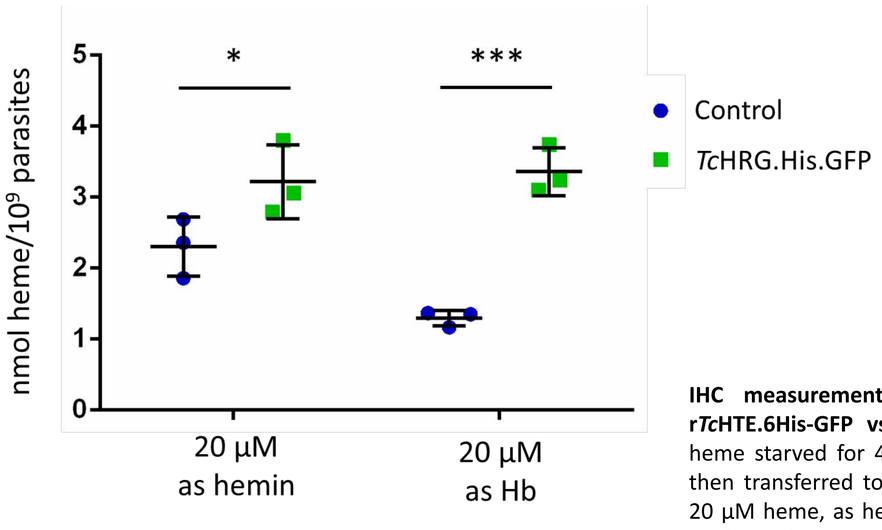
Western Blot assay in total extract of Dm28c (WT) epimastigotes. A) Samples from growth curve were taken on the 3rd, 7th and 14th day. B) Parasites were heme-starved during 48 h and then transferred to LIT + 10% FBS supplemented with 0 or 5 µM heme as hemin or Hb. Samples were taken in the first 50 h post treatment. Primary Abs: a-*Tc*HRG. Secondary Abs: a-IgG-HRP.

*Tc*HRG mRNA rapidly responds to to both heme sources



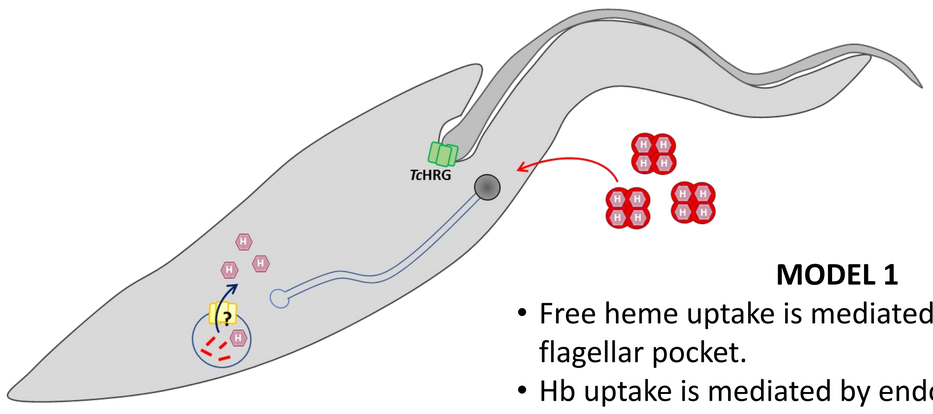
Quantitative real-time PCR (qRT-PCR). Epimastigotes were subjected to heme starvation. After 48 h, parasites were resuspended in fresh media supplemented with 0 or 5 µM heme as hemin or hemoglobin. Samples were collected for RNA isolation at 0, 4, 8, 10, 30 hours post treatment. *Tc*HRG mRNA amounts were constant in heme-starved epimastigotes. Conversely, a reduction of ≈ 50% and ≈ 25% in mRNA levels was detected 4 h after treatment with hemin and Hb, respectively.

*Tc*HRG.His.GFP over-expression enhances heme uptake



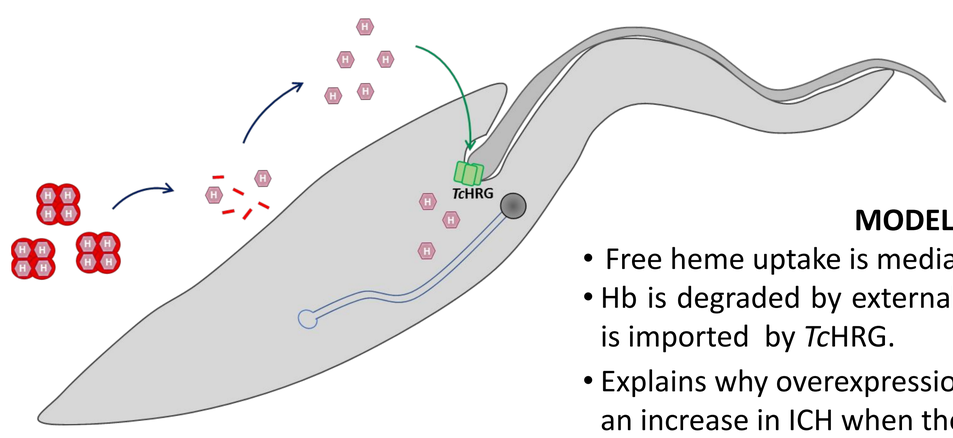
IHC measurements of parasites that overexpress r*Tc*HTE.6His-GFP vs. control parasites. Epimastigotes heme starved for 48 h were cultured during 48 h and then transferred to fresh medium supplemented with 20 µM heme, as hemin or Hb. ICH measurements were performed using Basic Pyridine Method[3]. Data is expressed as mean of the three replicates ± SD.

Proposed models for heme and Hb uptake in epimastigotes of *T. cruzi*



MODEL 1

- Free heme uptake is mediated by *Tc*HRG in the flagellar pocket.
- Hb uptake is mediated by endocytosis in the cytostome-cytopharynx complex.
- ***Tc*HRG expression is regulated by intracellular heme levels.**



MODEL 2

- Free heme uptake is mediated by *Tc*HRG.
- Hb is degraded by external proteases, free heme is imported by *Tc*HRG.
- Explains why overexpression of r*Tc*HRG generates an increase in ICH when the heme source is Hb.
- ***Tc*HRG expression is regulated by intracellular heme levels.**

Concluding remarks

- Excess of Hb does not affects growth and morphology of epimastigotes.
- At level protein and RNA level, *Tc*HRG rapidly responds to both heme sources.
- We are able to expand our models by proposing two complementary ways of Hb uptake.

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References

1. Merli, *et al.*, 2016. DOI: 10.1371/journal.pntd.0004359
2. Pagura, *et al*, 2020. DOI: 10.1074/jbc.RA120.014574
3. Trumpower and Berry, 1989. DOI: 10.1016/0003-2697(87)90643-9