Investigating RNA binding proteins as potential drug targets in *Leishmania* and *T. cruzi*

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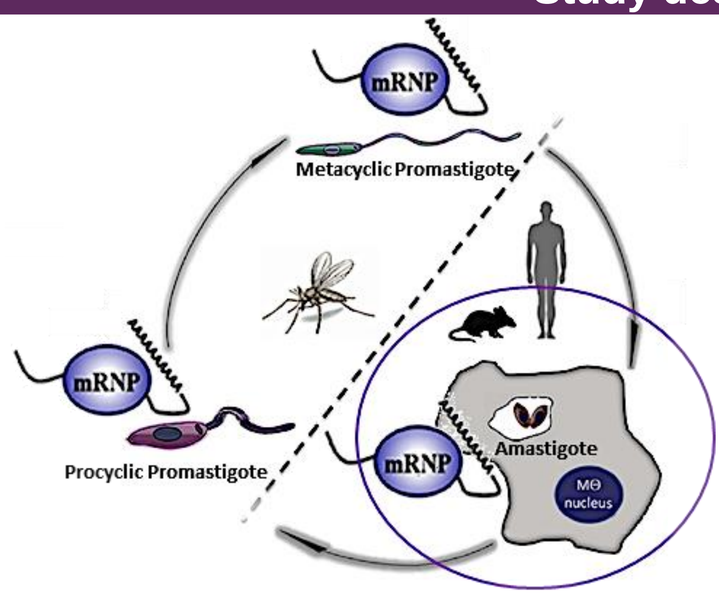
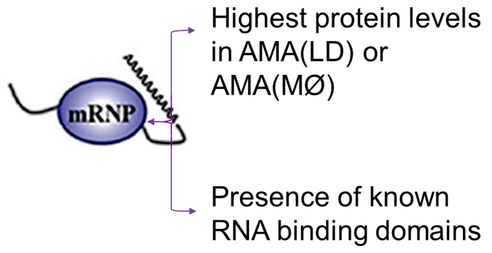
Abstract

Treatments for Leishmaniasis and Chagas disease are still a challenged and vaccines are not available. The causative agents present peculiar gene expression regulation, dominated by *trans*-regulatory mRNP ribonucleoprotein complexes. We seek to molecularly characterize RBP-driven pathways essential for parasite survival and pathology. 10 amastigote-enriched RBPs were selected from a comprehensive *L. mexicana* RBPome (*Pablos et al, MCP, 2019*) and KnockOut (KO) lines were generated using optimised CRISPR/cas9 technologies (*Baker et al, Nat Comm, 2021*). 3 RBP candidates appear essential even to promastigote stages. 5 RBPs not essential to promastigote stage cells are being examined for relevance to amastigogenesis, infectivity and viability. 4 RBP candidates are endogenously tagged to verify amastigote-relevant expression and isolate target transcripts. We hope to extend this investigation into orthologs in *T. cruzi* amastigotes; examining conserved RBP function and potential for dual combative strategies.

Study design



Essential regulators for parasite viability and pathology



Results

Table 1: Selected mRNA binding proteins candidates.

RBP	Lifecycle Stage	KO Status	End. Tagging
CCCH Zn Fngr	AMA(MØ) XL	KO (clone)	HA-tagged
XPRTase	AMA(LD) XL	KO (clone)	in process
NF-X1	AMA(LD) XL	KO (clone)	in process
NOP47	AMA(LD) XL	KO (multiclonal)	HA-tagged
DEAH helicase	META WC	+/- (multiclonal)	in process
SRP72	AMA(MØ) XL	3 attempts	HA-tagged
COPG	AMA(LD) XL	3 attempts	HA-tagged
DExD helicase	AMA(LD) XL	2 attempts	HA-tagged

Colours represent cell lines suitable for phenotypic analysis (green), inducible destabilization domain strategy (red) and RIPs (blue). AMA(LD) XL & AMA(MØ) XL: Amastigote RBPomes from mouse lesions and cultured macrophages. META WC: Metacyclic promastigote Whole Cell proteome.

Protein destabilization strategy

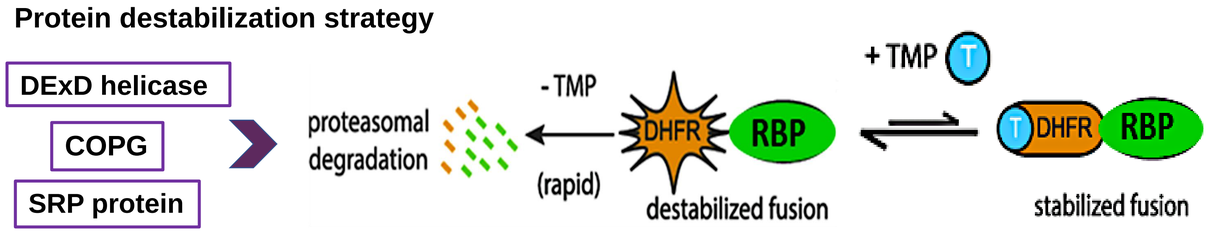


Figure 1: Inducible system for essential candidates. Failed knockout attempts suggest a possible gene essentiality of 3 RBPs candidates. As a strategy, a protein destabilization associated to an inducible stabilization system has been optimized for to generate conditional depletions.

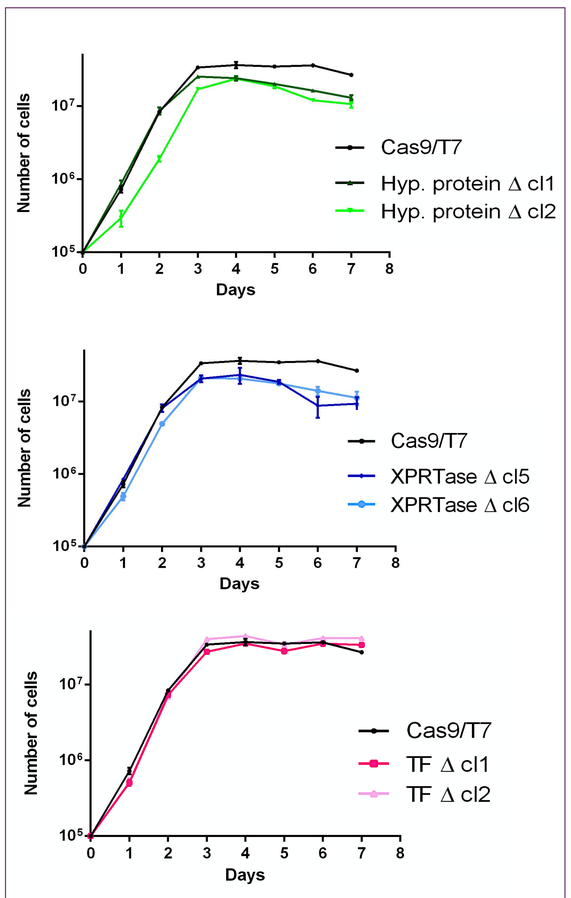


Figure 2: RBP KO cell lines show normal promastigote growth dynamics. Coloured lines show cultured growth of 2 clones per KO compared to parental line. ± SD, n = 3. Two-way ANOVA, in combination with Bonferroni's test, $p \leq 0.05$.

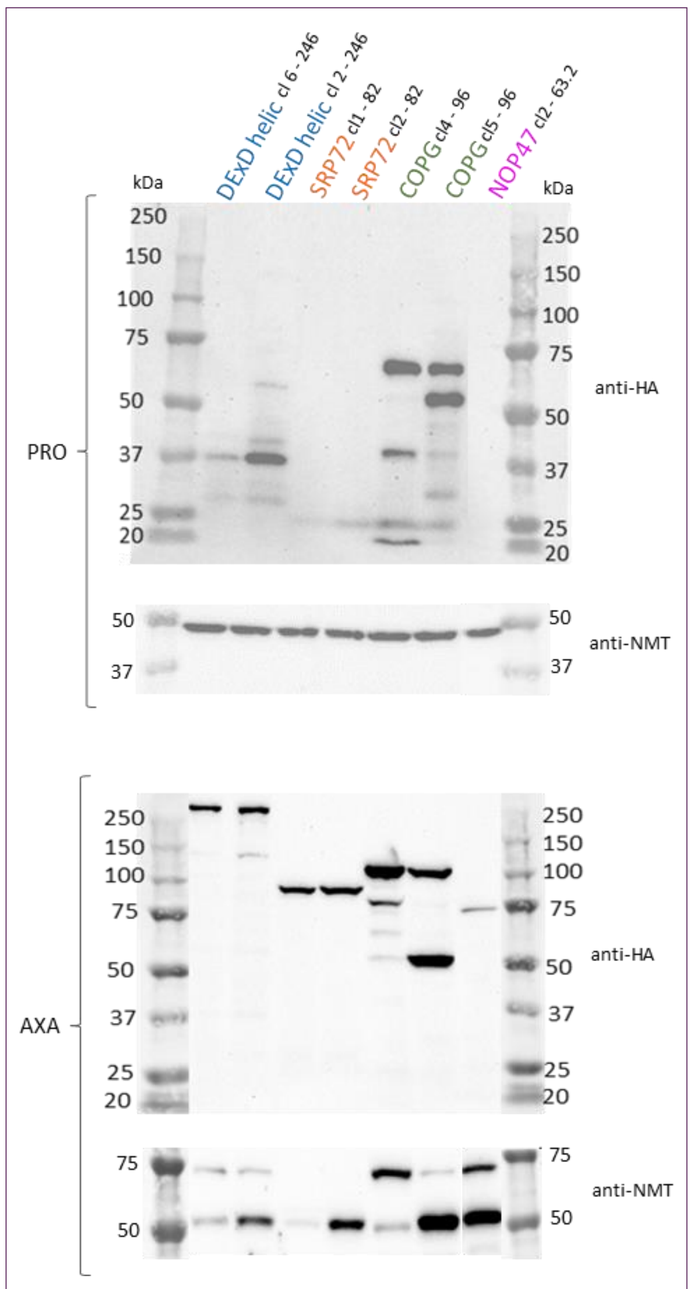


Figure 3: **RBPs Expression**. Western blots (anti-HA) of clonal transfectants (cl) for 4 endogenously-tagged RBPs expressed in the insect and infective parasite life stages. Different profiles of the same RBP are shown comparing procyclic promastigote (upper) and axenic amastigotes (down). The expected protein sizes (depicted on the top of each protein ID) were found in the amastigote stage. N-Myristoyltransferase has been used as a load control (~47 kDa).

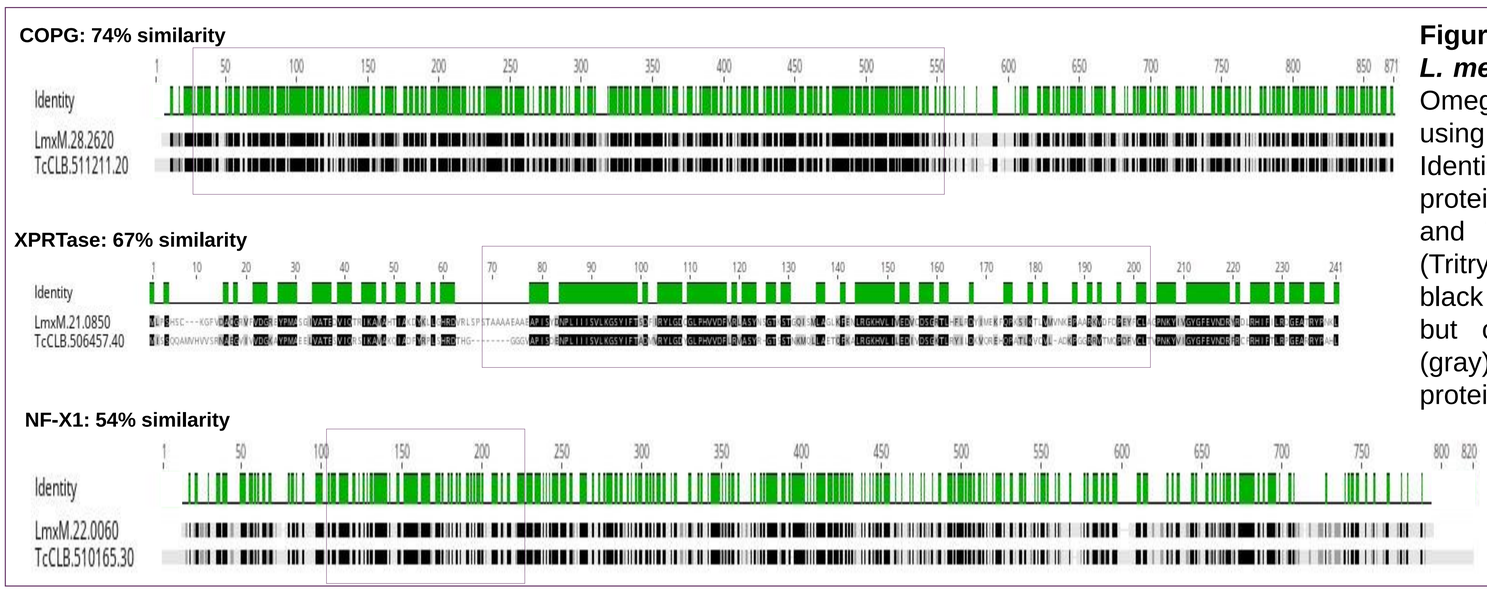


Figure 4: **RBPs conservation in *L. mexicana* and *T. cruzi***. Clustal Omega alignments generated using the Geneious software. Identical residues between RBP protein sequences of *L.mexicana* and *T. cruzi* CL Brener strains (Tritrypdb.org) are highlighted in black and green. Non-identical, but chemically similar residues (gray). Boxes depict location of protein RNA binding domains.

Conclusion

Preliminary results suggest at least 3 amastigote-enriched *trans*-regulators essential to parasite fitness with 5 further under investigation, focusing upon evaluation of amastigote stage viability and virulence in the absence or reduction of RBP levels.

Investigation in Process

- Evaluate infection capacity of KO parasite cell lines
- Development of inducible protein destabilization to investigate essential RBPs
- Identify and explore the transcript targets as potential virulence factors
- Extend significant outcomes of this investigation into RBP orthologs in *T. cruzi* amastigotes; examine potential for common combative strategies against conserved gene regulatory pathways

Acknowledgements

A Global Network for Neglected Tropical Diseases
GCRF Global Challenges Research Fund
University of York
MRC Medical Research Council