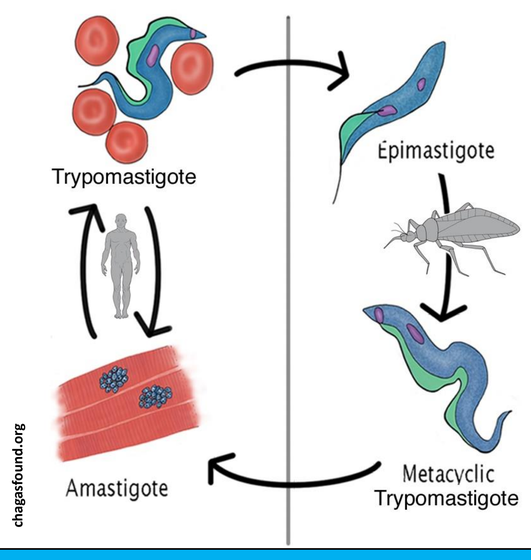
Functional analysis of *Trypanosoma cruzi* spliceosome proteins

Arthur Tributino Menezes[1], Janaína de Freitas Nascimento[2], Ariel Mariano Silber[2], Patricia Pereira Coltri[1]
[1]Institute of Biomedical Sciences – University of Sao Paulo, Department of Cell and Developmental Biology, Sao Paulo - Brazil.
[2]Institute of Biomedical Sciences – University of Sao Paulo, Department of Parasitology, Sao Paulo - Brazil.
Email: arthur.menezes@usp.br

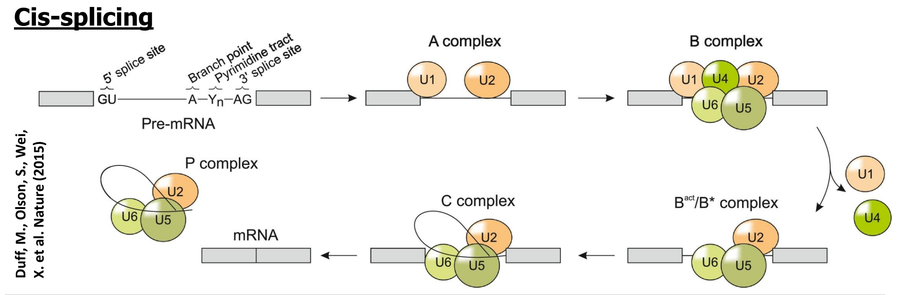
Abstract

Trypanosoma cruzi currently infects 6 to 7 million people worldwide, causing Chagas disease, which, when symptomatic, is characterized by cardiomyopathy, digestive system failure, or both. Trypanosomatids' genes are transcribed as polycistronic pre-mRNAs, which are individualized by SL trans-splicing, by the addition of a spliced leader sequence (SL-RNA) to each ORF in these pre-mRNAs. SL trans-splicing is carried out by the spliceosome, which is composed of a core of 5 ribonucleoproteins (RNPs) and several regulatory proteins conserved among trypanosomes and other eukaryotes. However, details on the function and activity of specific proteins in trypanosomatids' spliceosomes remain largely unexplored. Previously, we analyzed the *T. cruzi* spliceosome by mass spectrometry. The present work intends to characterize two of the detected proteins, TcRRM (TcCLB.510143.80) and TcHEL67 (TcCLB.506213.120), which are orthologous to the human SF3b4 and DDX3X proteins, respectively. In humans, these proteins participate in several cellular processes, including pre-mRNA splicing regulation. We overexpressed the TcRRM and TcHEL67 proteins in *T. cruzi* epimastigotes, and used CRISPR-Cas9 to edit their coding sequences. We analyzed the variations in splicing efficiency of endogenous α-tubulin, GAPDH and Poly-A polymerase transcripts by RT-qPCR. Using immunofluorescence, we confirmed that the TcRRM and TcHEL67 proteins localize within the parasite's nucleus. Besides, immunoprecipitation assays showed that TcRRM seems to be associated to U2, U5 and U6 spliceosome snRNAs, while TcHEL67 do not show any strong association. Our findings suggest that the TcRRM and TcHEL67 proteins may have a role in regulating splicing in *T. cruzi* epimastigotes. We intend to further explore the results by in vitro splicing assays, and use CLIP-seq to check for RNA association for these proteins.

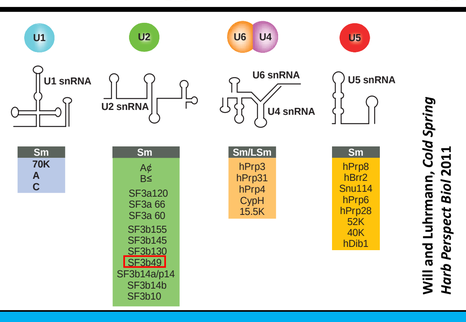
Trypanosoma cruzi life cycle



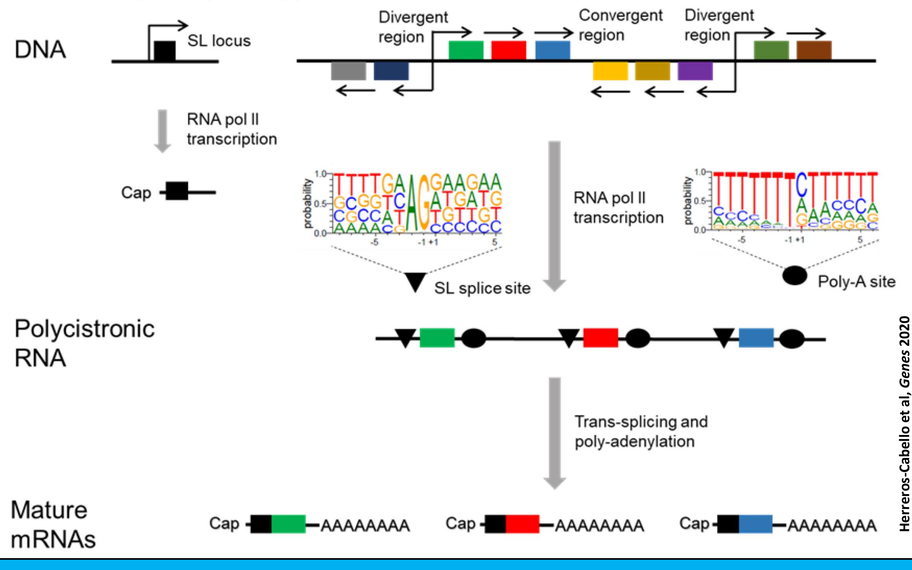
Cis-splicing



Spliceosome snRNPs (snRNAs+proteins) (human)



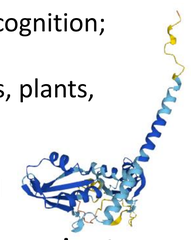
SL trans-splicing in Trypanosomatids



Methods and Results

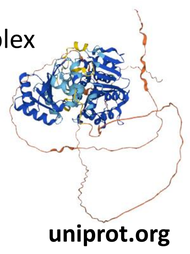
TcRRM (similar to human Sf3b4)

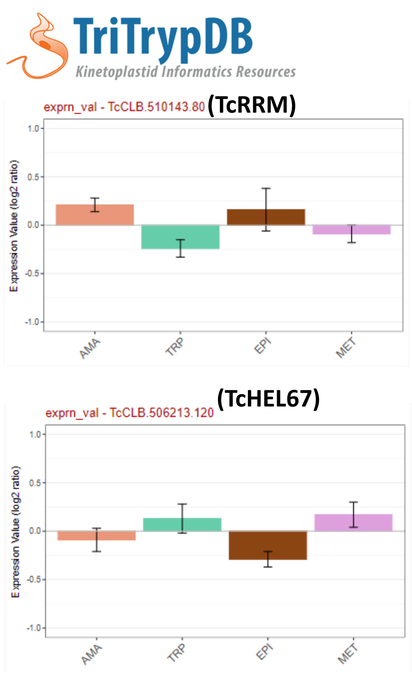
- U2 branchpoint recognition;
- Homologs: humans, plants, mice, Xenopus;
- 2 consecutive RRM domains.

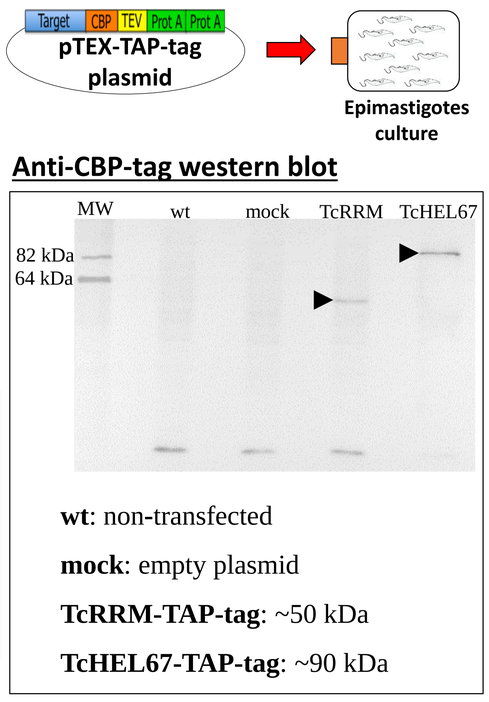


TcHEL67 (similar to human DDX3X)

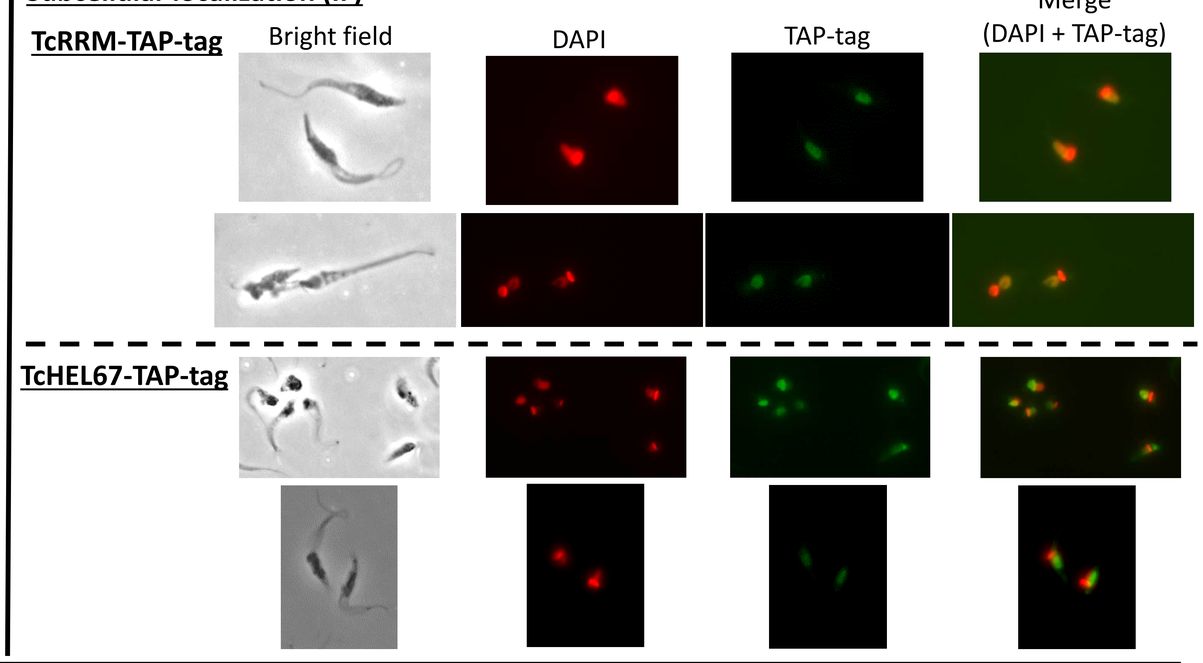
- DEAD-box helicase;
- RNA-protein complex remodeling;
- Similar motifs composition.





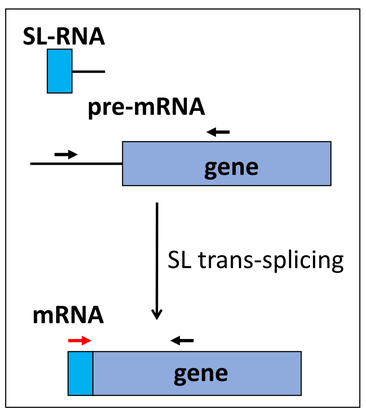


Subcellular localization (IF)



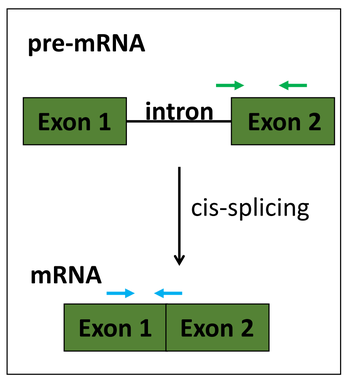
Quantitative RT-PCR (splicing efficiency)

SL trans-splicing (alpha tubulin; GAPDH)



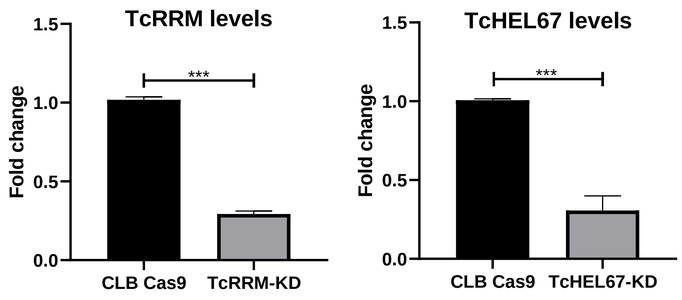
arrows: qPCR primers

Cis-splicing (Poly(A) polymerase (PAP))

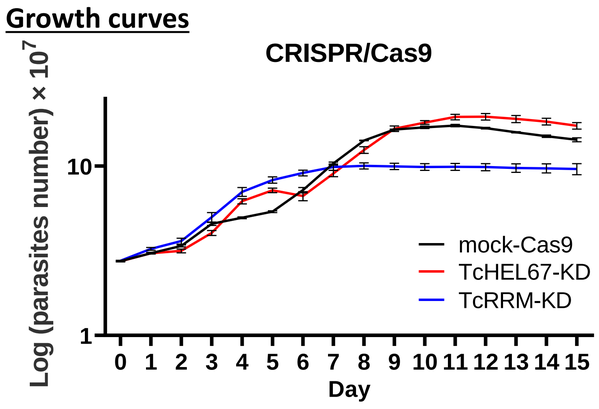


Splicing efficiency (TAP-tag groups)

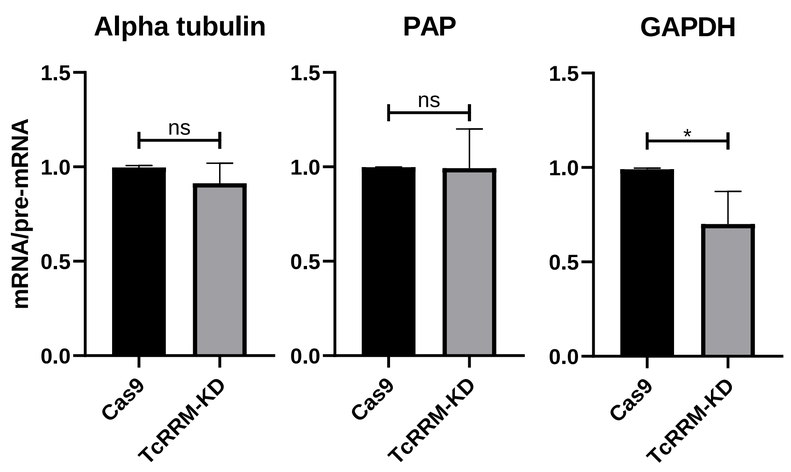
Knockdown by CRISPR/Cas9



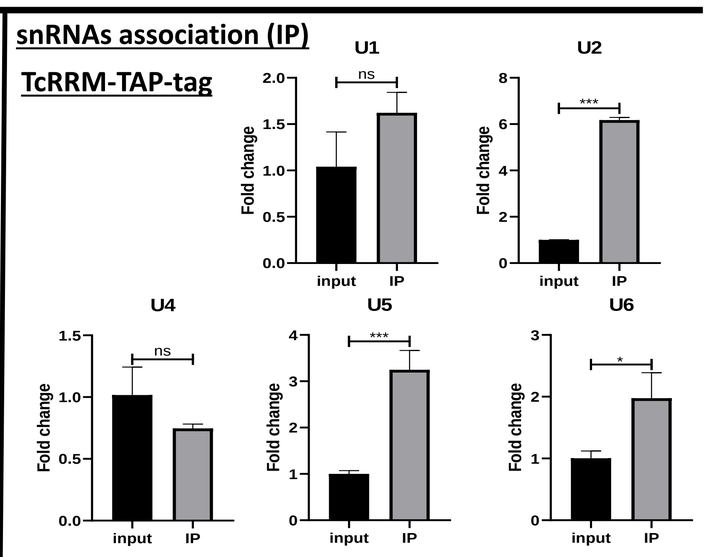
Growth curves

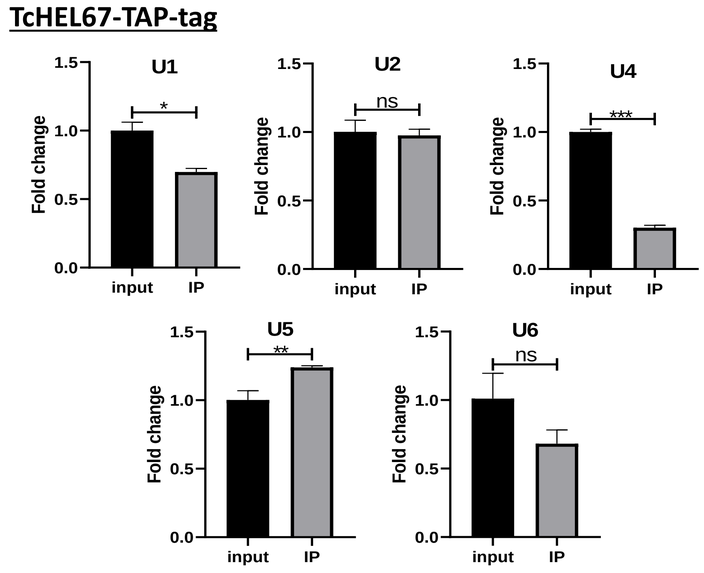


Splicing efficiency (TcRRM-KD)



snRNAs association (IP)





Perspectives:

- ❑ In vitro splicing assays;
- ❑ CLIP-seq (RNAs association).

Support: CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico; FAPESP Fundação de Amparo à Pesquisa do Estado de São Paulo; CAPES