Leishmania (Viannia) braziliensis long non-coding RNAs are enrolled in parasite fitness and interact with proteins in a structure-dependent manner.

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During its life cycle *Leishmania* parasites alternate between the phlebotomine vector and the mammalian host facing dramatic environmental changes which requires a rapid shift in gene expression to survive. Recently, the Cruz laboratory sequenced the transcriptomes of the three main life stages of *Leishmania braziliensis*, the main causative agent of tegumentary leishmaniasis in Brazil. These revealed differences not only in the expression of messenger RNAs but also of non-coding RNAs (ncRNAs). This observation raised the hypothesis that these ncRNAs could play a role in gene expression regulation in these different morphologies. To test this hypothesis, we selected 10 differentially expressed long (>200nt) presumably intergenic ncRNAs (lncRNAs) as targets. Using CRISPR/Cas9 we successfully generated knockout of 6 lncRNAs and tested their fitness in experiments mimicking key steps of the *Leishmania* life cycle. Four of these mutants presented significant differences in fitness compared to the parental line. For *ΔlncRNA45* and *ΔlncRNA66* a reduction in growth rates was observed for procyclic promastigotes in culture. For *ΔlncRNA52* a reduction in metacyclogenesis rates was observed. For *ΔlncRNA31* a reduction in axenic amastigotes duplication rate as well as a reduction in lesion sizes in BALB/c mice was observed. The existence and size of these four targets, precise start and end sequences as well as the presence or absence of Spliced Leader RNA (SL-RNA) and poly(A) tail were confirmed by northern blotting and circularization assays. While *lncRNA45*, *lncRNA66* and *lncRNA52* presented poly(A) tails of variable sizes, only *lncRNA52* presented the SL-RNA sequence at the 5' UTR. No poly(A) tail nor SL sequence were detected for *lncRNA31*. Mutations that can cause loss of secondary RNA structure were predicted *in silico* for *lncRNA45* and *lncRNA66* based on conservation. Pulldown assays using the aptamer S1m were performed using both the original and the mutated sequences of *lncRNA45*. These revealed distinct protein profiles interacting with WT vs mutant transcripts suggesting the secondary structure is essential for these lncRNA activity in *L*. *braziliensis*. We obtained the add back lines of these lncRNAs and they will be compared with knockout and parental fitness, to check if the parental phenotype is restored. For *lncRNA45* and *lncRNA66* we also generated add back lines with mutated sequence to evaluate if the loss of secondary structure can impair the fitness recovery which would strengthen the hypothesis that these structures are essential for ncRNA activity. Our results show that lncRNAs exist and are implicit in the regulation of biological processes in *L. braziliensis* parasites.

Keywords: LEISHMANIA BRAZILIENSIS, NON-CODING RNA, GENE EXPRESSION, CRISPR/CAS9, FITNESS

Financing: FAPESP/MRC via Joint Centre Partnership in Leishmaniasis, CAPES and CNPq.