VALIDATION OF A QUANTITATIVE RIBO-PROFILING APPROACH FOR THE STUDY OF *LEISHMANIA* TRANSLATIONAL REGULATION

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Protozoan parasites of genus *Leishmania* show a remarkable level of phenotypic diversity with respect to clinical symptoms, disease outcome, and drug susceptibility, which challenges disease management. Such diversity is surprising given the largely constitutive gene expression in these parasites that lack classical, promoter-driven control, raising essential questions on how *Leishmania* adapts and evolves in response to environmental change. Using an experimental evolution approach, we recently uncovered a series of regulatory mechanisms that govern *Leishmania* fitness gain in culture, including frequent gene dosage changes caused by the parasite's intrinsic genome instability, and compensatory post-transcriptional responses. We further correlated fitness gain to changes in snoRNA abundance and the modifications they guide on ribosomal (r) RNA. These data suggest the presence of fitness-adapted ribosomes that may support parasite adaptability through translational regulation, potentially filtering harmful from useful gene dosage effects. To investigate this hypothesis, we explore here the adaptive changes in mRNA translatability by profiling active ribosomes using the RiboLace method (Immagina Biotechnology). We first evaluated the quantitative use of this method by developing *L. donovani* transgenic parasites that express low and high levels of GFP (respectively termed as LdGFP_L and LdGFP_H) based on changes in the Kozak sequences that affect mRNA/ribosome interaction. Flow cytometry analysis indeed showed a 4-fold reduced mean fluorescence intensity in LdGFP_L compared to LdGFP_H parasites. Using the RiboLace kit, we isolated active ribosomes from both parasite strains and revealed by RTqPCR a 2.74-fold lower recovery of GFP mRNA in LdGFP_L compared to LdGFP_H parasites, thus validating the applicability of RiboLace to quantify differences in translatability. We are currently applying this method to assess the role of translational control and the presence of fitness-adapted ribosomes in *Leishmania* during adaptation to *in vitro* culture.

Keywords: *Leishmania*; translation; adaptation; ribossome; Kozak sequences

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