Queuosine-tRNA modification as a means for gene expression regulation in *Leishmania mexicana*

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All kinetoplastid parasites including leishmania have polycistronic transcription, hence regulation of gene expression is mediated mostly by post-transcriptional mechanisms. One of the post-transcriptional steps is represented by tRNA modifications that directly regulate translation by modulating codon–anticodon interactions. The Queuosine (Q) modification is found at the wobble position 34 of tRNAs containing the GUN anticodon, leading to the change of GUN into QUN anticodon. Consequently, the efficiency of the translation of NAU and NAC codons by Q-tRNA is symmetrical.

Here, we examine the role of the Q-tRNA modification in *L. mexicana* differentiation and infectivity. Increased abundance of Q-tRNAs in the amastigote stage, when compared to the insect promastigote stage, suggests an important role for this modification in the mammalian-infective stage. The production of Q-tRNAs is catalysed by a highly conserved heterodimeric enzyme, termed tRNA guanine transglycosylase (TGT1/2). To get a deeper insight we employed CRISPR/Cas9 to generate a gene knock-out (KO) for TGT2 subunit in *L. mexicana*, which resulted in the depletion of Q-tRNAs as expected. We did not observe any growth phenotype in TGT2 KO when cultured as promastigotes, or when differentiating promastigotes into amastigotes. Although the TGT2 KO amastigote stage did not show any growth defect in culture, the KO cells exhibited reduced infectivity in macrophages *in vitro* as compared to WT. Most importantly, mice infected with *L. mexicana* TGT2 KO developed significantly smaller lesions than with WT. The decreased infectivity could be a consequence of alteration in the host's immune response. However, the immune analysis of the infected mice showed no difference in the levels of IgG1 and other markers, but a decrease in IgG2a, which corresponds with a lower parasite abundance in the lymph nodes after TGT2 deletion.

In order to explain the observed phenotypes, we performed a proteomic analysis and assessed the abundance of the NAU codons, decoded by Q-tRNAs, in genes encoding the depleted proteins in the KO strain. Overall, TGT2 depletion resulted in a reduction of several proteins, which genes nevertheless mostly did not contain a higher proportion of NAU anticodons. However, a few outstanding hits emerged. Namely, the metalloprotease Gp63, an important virulence factor of leishmania, was significantly reduced in the KO proteome. Interestingly, Gp63 contains a higher proportion of NAU. The arginyl-tRNA synthetase, encoded by a gene with a high proportion of NAU, was also reduced in the TGT2 KO providing a plausible explanation for reduced levels of other proteins, independent of NAU codon frequency.

Here, we conclude that the Q modification is required for *L. mexicana* infectivity. Most likely, the defect is primarily not due to interference with the immune response of the host, but a consequence of altered protein expression in leishmania. Alltogether, the Q modification represents another way how the parasite can regulate the gene expression, and adapt to different hosts and conditions.