

Master Project Proposal

Title: The role of RNA modifications in poly(A) tails

Synopsis:

Epitranscriptomics is a novel field that studies how RNA modifications regulate gene expression. The most prevalent RNA modification is N6-methyladenosine (m⁶A), which consists of methylation of the adenosine base at the nitrogen-6 position. In several eukaryotes, m⁶A affects several aspects of RNA biology, including function, stability and translation. Recently, our lab identified m⁶A in the poly(A) tail of mRNA transcripts in *Trypanosoma brucei* (the causative agent of sleeping sickness). In this study, we further showed that m⁶A stabilizes transcripts by inhibiting poly(A) tail degradation, a process that is mediated by the CAF1 deadenylase enzyme (Viegas *et al*, Nature 2022). Although this work revealed a completely novel epitranscriptomic mechanism to regulate gene expression in eukaryotes, it remains unknown how m⁶A inhibits poly(A) tail degradation. In this master's project, the student will investigate how poly(A) tail degradation is inhibited by m⁶A. Our hypothesis is that the poly(A) tail is protected by an m⁶A-binding protein. Putative m⁶A-binding proteins have been previously identified. This project will consist in testing the function of such proteins in poly(A) tail protection. For that, the student will use a combination of genetic and biochemical tools, which will include (i) generation of CRISPR parasite mutants, to assess parasite viability and mRNA stability by quantitative reverse transcription PCR and (ii) *in vitro* electrophoretic mobility shift assays to measure protein:RNA binding, for which we will purify protein candidates and incubate them with methylated RNA. This project will shine light on this novel molecular mechanism by which RNA modifications control gene expression.

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Bibliography (facultative):

Zaccara S, Ries RJ, Jaffrey SR. Reading, writing and erasing mRNA methylation. *Nat Rev Mol Cell Biol.* 2019;20(10):608-624. doi:10.1038/s41580-019-0168-5

Viegas IJ, de Macedo JP, Serra L, et al. N6-methyladenosine in poly(A) tails stabilize VSG transcripts. *Nature.* 2022 Apr;604(7905):362-370. doi: 10.1038/s41586-022-04544-0.