Regulatory potential of ribosome-associated short RNAs (rancRNAs) in *Saccharomyces cerevisiae* under abiotic stress conditions

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Non-coding RNAs play a crucial role in the regulation of gene expression at multiple stages of cell life. In 2012, a novel mechanism of protein biosynthesis regulation was discovered, involving direct binding of small RNAs to ribosomes in *Saccharomyces cerevisiae*. These small RNAs, termed rancRNAs (ribosome-associated noncoding RNAs), are generated under stress conditions through the cleavage of known noncoding RNAs such as snoRNA, rRNA, and tRNA.

The aim of the studies undertaken in this dissertation was to deepen the understanding of rancRNAs, with a particular focus on two classes of rancRNAs: tRFs (tRNA-derived fragments) and sdRNAs (snoRNA-derived RNAs). The dissertation analyzed the interactions of tRFs and sdRNAs with ribosomal fractions in *S. cerevisiae*. To investigate the impact of modifications, present in rancRNAs on their accumulation levels, AlkB/AlkB-D135S proteins were utilized.

In the first stage of the study, we focused on identifying the broadest possible spectrum of rancRNAs interacting with *S. cerevisiae* ribosomes under moderate abiotic stress conditions using high-throughput sequencing. Initially, yeast was cultured in three conditions: optimal (control) and two stress conditions where the highest ribosome heterogeneity was observed, i.e., hyperosmotic shock and sugar starvation. To "freeze" the translation machinery, cells were incubated with cycloheximide, an inhibitor of protein biosynthesis in eukaryotic organisms, and then lysed under low temperature protective conditions. Lysates were subjected to ultracentrifugation in a sucrose gradient (polysome profiling), during which three ribosomal fractions were obtained: polysomes, monosomes, subunits, and one non-ribosomal fraction (free RNA). RNA was isolated from each fraction, and then size selection was performed using polyacrylamide gel electrophoresis and subsequent elution to obtain short RNAs of approximately 20-50 nt in length. Short RNAs were divided into two fractions, one of which was subjected to demethylation using tRNA demethylases AlkB (the AlkB enzyme was produced in a bacterial expression system). The prepared RNA was qualitatively and quantitatively assessed using capillary electrophoresis and then subjected to high-throughput sequencing on the Illumina platform. A total of 48 rancRNA sequencing libraries were prepared (rancRNA-seq): 2 biological

replicates x 4 fractions from the polysome profile x 3 yeast culture conditions x 2 demethylation conditions = 48 cDNA libraries. Subsequently, a standard bioinformatic analysis of the sequencing results was performed. This allowed us to identify 2028 different tRFs and 961 different sdRNAs. In the next stage, we investigated whether stress affects the association of tRFs and sdRNAs with ribosomal fractions: ribosomal subunits, monosomes, and polysomes. A detailed analysis of ribosomal fractions under abiotic stress compared to optimal conditions enabled us to demonstrate changes in the accumulation of tRFs and sdRNAs in individual fractions.

The next step involved investigating the impact of m^3C, m^1A, m^1G, and m^3T modifications on the detected levels of tRFs and sdRNAs in RNA libraries. This allowed for the identification of 1231 tRFs and 456 sdRNAs in AlkB+ libraries and 954 tRFs and 493 sdRNAs in AlkB- libraries. A heterogeneous effect of AlkB/AlkB-D135S proteins on individual rancRNAs was demonstrated.

The results presented in this doctoral dissertation have been published in one peer-reviewed scientific article.