Exploring Cytoplasmic Polyadenylation: Regulatory Mechanisms Affecting Stability of Endogenous and Therapeutic mRNAs in Hematopoietic Cells

The core aspect of every messenger RNA (mRNA) is to deliver a template for protein production in a time-dependent manner. The time dependency of these molecules is highlighted by the interplay of multiple mechanisms regulating mRNA decay and stability, which provides liquidity for cellular homeostasis.

Cytoplasmic polyadenylation, also known as re-adenylation, mediated by the TENT5 family of non-canonical poly(A) polymerases (ncPAPs) is an important mechanism for stabilizing mRNAs in many biological systems of higher eukaryotes. This PhD dissertation is devoted to the functional analysis of the selected biological functions of TENT5 ncPAPs in hematological cells, namely macrophages, in the context of mRNA vaccines, and late erythrocyte progenitors in the context of hemoglobin production.

In the case of therapeutic mRNA metabolism, where transcription is not taken into consideration, TENT5A-mediated re-adenylation of anti-COVID-19 mRNA vaccines stabilization is shown by us to be crucial for extending the half-life of these mRNAs, contributing to efficient translation, which correlates with the production of antibodies against the encoded antigen. Analysis of immune cells infiltrating the muscle post intramuscular administration, using initially immunophenotyping and later single-cell RNA sequencing, allowed me to precisely identify the major macrophage subtype able to retain therapeutic mRNA, which opens new perspectives for vaccine mRNA development.

In the context of erythropoiesis, initial hematological profiling performed in our laboratory revealed that TENT5C knockout mice display a microcytic anemia phenotype. Analyses performed by me led to a rather surprising discovery that in erythroblasts, TENT5C polyadenylates globin mRNA, which resides in the cytoplasm, contrary to all previously identified substrates of TENT5 family members, representing endoplasmic reticulum (ER) targeted transcripts. Further functional analysis enabled me to propose an ER-independent mechanisms of action that apply to TENT5C in the case of erythropoiesis. The beneficial effect of TENT5A on hemoglobin production is especially important for erythroid cells with low or completely diminished transcriptional activity.

Collectively, this dissertation highlights the importance of cytoplasmic polyadenylation in distinct biological systems where transcription does not matter and points to novel intracellular mechanisms regarding endogenous transcript metabolism as well as unexplored cellular responses in the case of exogenous transcript uptake.