Abstract

Gene expression is a multi-step process during which genetic information encoded in DNA is used as a template for protein production. For mRNA molecules involved in this process, the poly(A) tail is particularly crucial, as it influences their stability and the efficiency of translation. The poly(A) tail can be added both in the nucleus and in the cytoplasm. Cytoplasmic polyadenylation is catalyzed by non-canonical poly(A) polymerases belonging to the TENT family, which have been increasingly recognized for their role in gene expression regulation. Among them, the relatively recently discovered TENT5 proteins stand out. Previous studies in mouse models have demonstrated that TENT5 proteins play significant roles in various physiological processes, such as the immune response, bone formation, and gametogenesis. Moreover, mutations in the *TENT5C* gene are among the most common in multiple myeloma patients, highlighting the importance of these proteins in maintaining organismal homeostasis.

To deepen our understanding of the mechanism underlying TENT5 proteins' function, our research group analyzed the role of TENT-5 (PQN-44) – the only homolog of this family in the nematode *Caenorhabditis elegans*. *C. elegans* is a widely used model organism in molecular biology, valued for its simplicity, short life cycle, and ease of genetic manipulation. Our studies revealed that the TENT-5 poly(A) polymerase in *C. elegans* plays a key role in regulating the innate immune response by stabilizing transcripts involved in this process through the extension of their poly(A) tails. We observed that most TENT-5 substrates encode proteins secreted through the endoplasmic reticulum (ER). Additionally, we demonstrated that TENT-5 partially localizes to the ER, explaining the observed substrate preference.

The aim of my doctoral project was to expand on these findings and gain a more comprehensive understanding of cytoplasmic polyadenylation. First, I examined the differences in polyadenylation between the two nematode sexes. My analyses revealed significant differences in gene expression and poly(A) tail lengths between males and hermaphrodites. I also observed that TENT-5 is abundantly expressed in male-specific tissues, regulating components of the seminal fluid.

The next step was to investigate the potential redundancy between TENT-5 and other poly(A) polymerases: GLD-2 and GLD-4. My results excluded functional overlap between these proteins, showing that they regulate distinct groups of transcripts.

I also focused on elucidating the mechanisms directing TENT-5 to the ER. I examined the influence of three potential regulators – LARP-5, ATX-2, and C34F6.10 – on poly(A) tail profiles. Among these, I demonstrated that LARP-5 is the most likely factor regulating TENT-5 activity.

Finally, I studied the genes whose mRNAs are the most prominent substrates of TENT-5, the nematode specific NSPC genes. We discovered that these proteins localize exclusively

to a single cell – the excretory gland cell. My analyses revealed that this cell is primarily responsible for the production of NSPC proteins, which are partially involved in regulating the insulin signaling pathway in *C. elegans*.

Concluding, the results presented in my doctoral thesis provide new insights into the mechanisms of cytoplasmic polyadenylation and the role of TENT-5 in gene expression regulation. Moreover, my findings form a great foundation for further studies in this area.