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ARE-BPs that affect mRNA stability in the maternal to zygotic transition

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Animal embryonic development begins with the maternal to zygotic transition (MZT) (Otuska et al, 2019). Following fertilization, mRNAs deposited in the oocyte by the mother (maternal mRNAs) direct the first stages of embryogenesis (Otuska et al, 2019). The maternal mRNA is then rapidly degraded as new zygotic mRNAs are being transcribed from the embryo's genome (Otuska et al, 2019). The sequence UAUUUAU (AU-rich element or ARE) has been found to be present at a higher level in these unstable mRNAs than would be expected by chance (Tadros & Lipshitz, 2009), suggesting that AREs destabilize mRNAs during the MZT. Previous work has shown that AREs can either stabilize or destabilize mRNAs through the binding of different ARE-binding proteins (ARE-BPs) and associated recruitment of additional factors (Tadros & Lipshitz, 2009). For example, KSRP, an ARE- BP, has been shown to play a role in the degradation of inducible nitric oxide (iNOS) mRNA, but also stabilize target mRNAs in T cells. These contradictory results leave unanswered questions about when KSRP will stabilize or destabilize and mRNA.

This study aimed to assess the potential role of KSRP as a regulator of maternal mRNA stability during MZT. Zebrafish were used as the model organism due to their nearly transparent eggs, quick reproduction time, external development, and large number of embryos that can be collected in one morning. In zebrafish, KHSRP has been found to be both homologous and analogous to human KSRP (U.S. National Library of Medicine, 2021). The most similar proteins in zebrafish to human KSRP were determined by phylogenetic analysis using Ensembl. The most closely related proteins were found to be FUBP1 and FUBP3. They were also determined to be highly expressed during the MZT through analysis of previously published RNA-seq data (Lee et al, 2018), suggesting a potential role in maternal mRNA stability.

I hypothesized that KHSRP destabilizes ARE-containing mRNAs during the MZT. To test this, KHSRP and its paralogs (FUBP1 and FUBP3) were fully knocked down in the embryo using CRISPR/Cas13. Cas13 works by targeting specific RNA sequences using complementary guide RNAs (gRNAs), attaching to the specific RNA sequence, then cutting up the strand of RNA (Kushawah et al, 2020). To ensure the loss of KHSRP activity, four different gDNAs targeting all three KSRP homologs were created. Two of the gDNAs were the most similar sequences between all three proteins being targeted, one was a sequence in KHSRP, and the last was a sequence most similar between FUBP1 and FUBP3. The gDNAs and Cas13 plasmid underwent *in vitro* transcription to obtain the RNAs needed for injection. The first steps involved injecting a mix of gRNAs and Cas13 into the one-cell stage of zebrafish embryos. To train for this step, mRNA encoding GFP was first injected, allowing for easy visualization and assessment of injection efficiency. After this step was optimized, the injection of Cas13 and gRNAs will take place. After the injection of Cas13 and gRNAs, qPCR analysis will be done to determine degree of knockdown. Further, phenotypic analysis and assessment of mRNA stability will be done to determine whether KHSRP, and/or one if its paralogs, is stabilizing, destabilizing or has no effect on mRNA in the MZT.

The results of this research informs our understanding of the earliest events during embryogenesis. It was found that FUBP3, FUBP1, and KHSRP are the most closely related proteins to human KSRP within zebrafish and they are expressed during the MZT. Later steps will confirm whether they are stabilizing or destabilizing during the MZT. Further work will reveal how sequence determinants affect mRNA stability more broadly, and the knowledge gained can be used in the design of different RNA-based medicines, including RNA-based vaccines.

References

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