Influence of 3'UTR variants on germ cell-specific gene expression in Atlantic salmon

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mRNA - What do we know?

Potential applications using mRNA are increasing and could be used for vaccines, protein and antibody delivery in aquaculture. Depending on the application, the cell specifity, the location and duration of the gene expression can be of great importance. mRNA stability, translational efficiency and localization are to a large extent determined by the 5` and 3` untranslated regions (UTRs). Additionally, mRNA can be regulated through miRNA by translational inhibition and destabilization.
The 3'UTRs of genes specifically expressed in primordial germ cells (PGCs) have critical roles for the stabilization and location of these mRNAs. However, the 3'UTRs are not well conserved in the different PGC-specific genes.

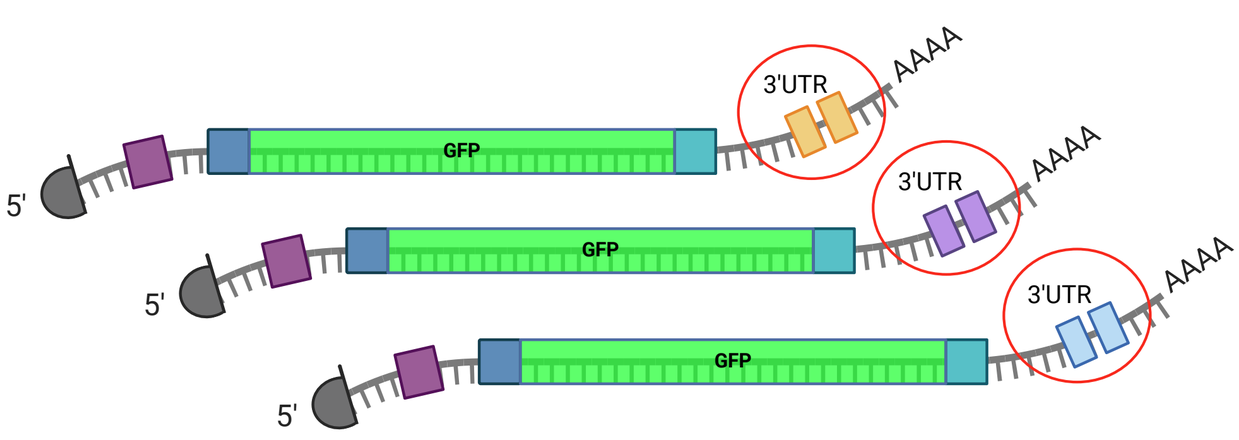
The idea

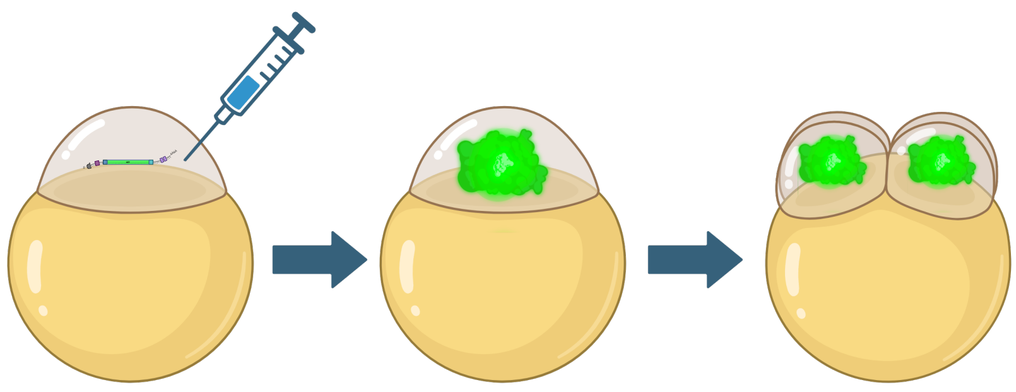
We will investigate how different 3'UTR sequences originating from salmon germ plasm genes can confer and improve localization, stability and translation of in vitro transcribed mRNAs in the germ cells.

So what did we do?

We designed multiple mRNAs containing 3'UTR domains from several genes involved in germ cell development in Atlantic salmon: *dazl*, *ddx4*, *dnd*, *nanos3 and nanos1* with or without miRNA binding sequences. All mRNA contained 3'UTR, identical 5'UTR and GFP constructs. As a control we used the 3'UTR from zebrafish nanos3 (Skugor et al. 2014).

The salmon eggs were fertilized and mRNA was injected into the blastodisc. The volume was visually adjusted to ~5% of the blastodisc volume. The GFP-expression was visually examined with a fluorescence stereo microscope.

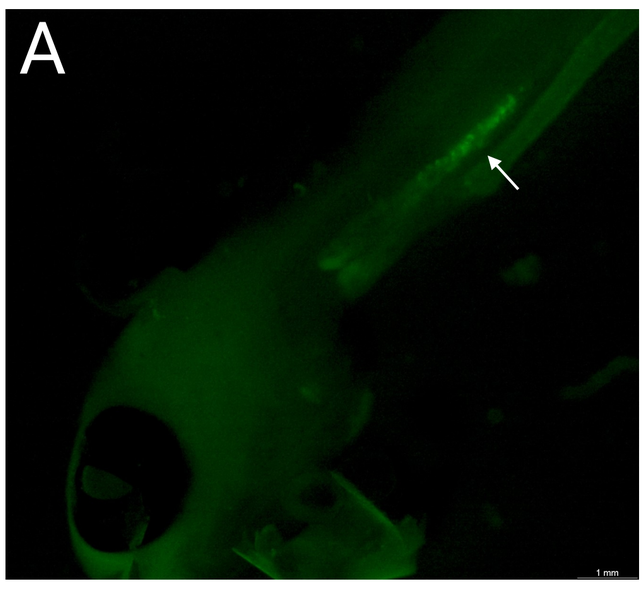


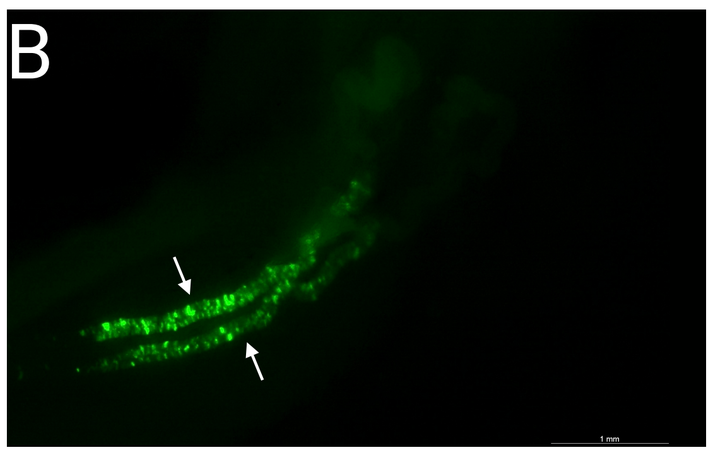


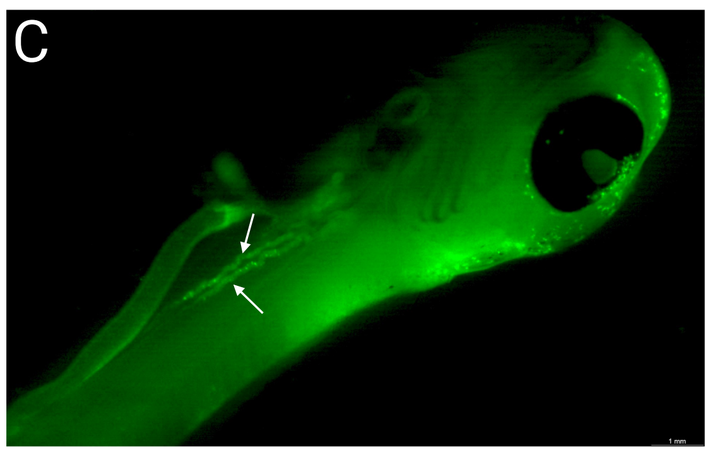
Our results

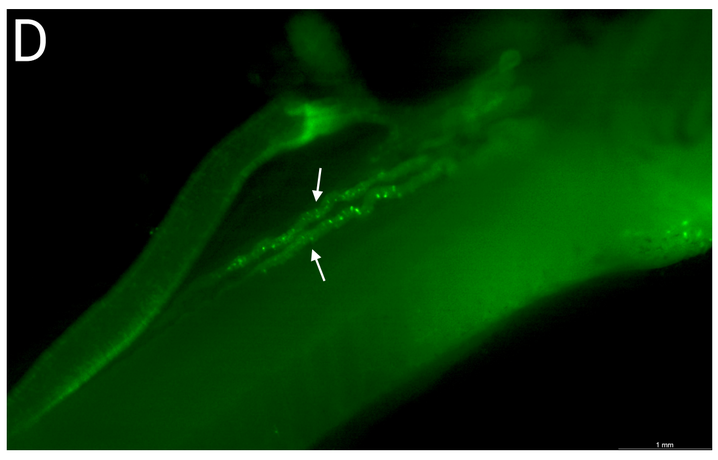
Germ cell specific GFP expression was documented using the zebrafish nanos3 (Fig. A,B) and salmon *nanos1* without miRNA binding site (Fig. C, D). The other 3'UTRs resulted in partly or complete extragonadal expression (Fig. E).

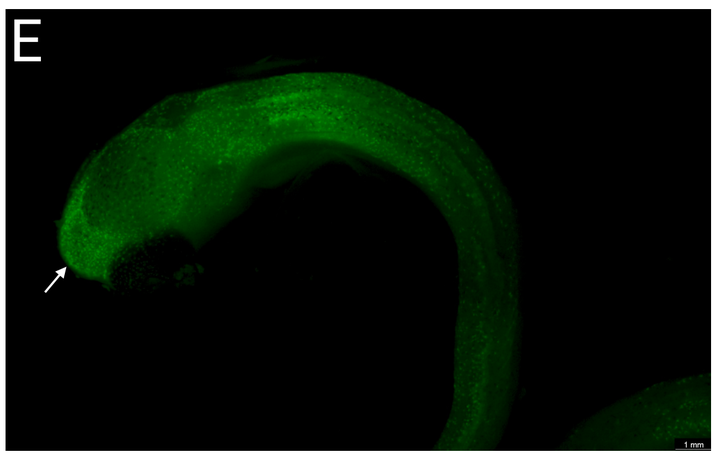
The pictures below are examples of the mRNA expression at ~450 DD.
A & B: zebrafish *nanos1*
C & D: salmon *nanos1* without miRNA target site
E: extragonadal expression of salmon *nanos3*











What's next?

The zebrafish *nanos1* and salmon *nanos1* without miRNA target site constructs resulted in germ cell specific expression. However, we need to repeat the experiment and analyse the expression at earlier life stages and also the expression duration.
Afterwards, we will test different 5'UTR to improve the translation efficiency .

Thanks to

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Skugor, A., Slanchev, K., Torgersen, J. S., Tveiten, H., & Andersen, O. (2014). Conserved mechanisms for germ cell-specific localization of nanos3 transcripts in teleost species with aquaculture significance. *Mar Biotechnol (NY), 16*(3), 256-264.