

# Analysis of short Argonaute isoforms from mouse oocytes

## **Abstract:**

Argonaute proteins carrying small RNAs form the conserved core of RNA silencing mechanisms, which repress viruses, mobile genetic elements, and genes in a sequence specific manner. The microRNA (miRNA) pathway is a dominant mammalian RNA silencing mechanism in somatic cells, which post-transcriptionally regulates large fraction of genes and thereby adjusts protein levels. miRNA-guided Argonautes inhibit translation and induce deadenylation of complementary mRNAs, ultimately resulting in their decay. In contrast to RNA interference (RNAi), which employs Argonaute slicer activity to directly cleave perfectly complementary RNAs, an effective miRNA-mediated mRNA repression requires multiple Argonaute-associated protein factors and enzymes. The miRNA pathway has been implicated in many complex biological processes ranging from organogenesis, stress-response to haematopoiesis or cancer. Surprisingly, canonical miRNAs are not essential for oocytes and early embryonic development in mice. Even the most abundant miRNAs present in mouse oocytes are unable to effectively repress target genes. However, RNAi, which shares key enzymes with the miRNA pathway, is highly active in oocytes and early embryos. The cause of miRNA inactivity in mouse oocytes remains unknown. This thesis is focused on short Argonaute isoforms encompassing Argonaute's N-terminal domain, which were discovered during a deep-sequencing analysis of oocyte's transcriptome. Thus, during my master thesis research, I cloned these isoforms from mouse oocytes and analysed their impact on miRNA and RNAi-like pathways in cultured cells. Using luciferase reporter system, I have found out that ectopically expressed short Argonautes do not affect miRNA and RNAi-like pathways suggesting that they are not functioning as dominant negative inhibitors of RNA silencing.

## **Keywords:**

Argonaute, RNA silencing, miRNA, siRNA, RNA interference, oocyte, isoform, RISC