Abstract (English)

Oocyte-to-embryo transition (OET) is one of the most complex developmental events where a differentiated oocyte gives rise to a totipotent zygote. During the growth phase an oocyte prepares for fertilization and progression to zygotic genome activation. It does so by transcribing and storing the necessary mRNAs till a fully-grown oocyte attains transcriptional quiescence. Therefore, transcriptome regulation in a fully-grown oocyte is of utmost importance. Study of post-transcriptional regulatory pathways revealed that the small-RNA mediated regulatory pathways exist in a unique conformation in mouse oocytes. Endogenous RNAi pathway is essential for mouse female germline while miRNA pathway which is ubiquitously present in most cell types is dispensable for oocyte maturation and fertilization.

My PhD project was aimed at understanding the constraints of the miRNA pathway in the oocyte which makes it non-functional. As a fully-grown oocyte is a huge cell with a proportionally large maternal transcriptome we analysed the miRNA: mRNA stoichiometry changes that occur from growing to the fully-grown mouse oocyte. Inability of miRNAs to accumulate during oocyte growth phase leads to their dilution in fully-grown oocyte rendering them inactive. Low miRNA concentrations were also observed in rat, hamster, porcine, and bovine oocytes, arguing that miRNA inactivity is not mouse-specific but a common mammalian oocyte feature. Injection of miRNA mimic molecules was sufficient to restore reporter repression, suggesting that miRNA inactivity stems from low miRNA abundance and not from active suppression of the pathway. Exceptionally abundant miRNAs were shown to be active in pig and bovine oocytes. Furthermore, while studying the associated proteins of the miRNA pathway, novel adaptations of AGO2 protein were discovered in mouse oocytes. The active MT element cluster in *Ago2* locus in mouse oocytes gives rise to a truncated AGO2 isoform which effectively reduces the expression of the full-length AGO2 protein. However, deletion of the MT-cluster failed to restore the expression of full-length AGO2 as the MT cluster acts an enhancer for the *Ago2* locus. AGO2 also has an alternate N-terminal in oocytes and this maternal AGO2 isoform has reduced catalytic activity. This points towards adaptations of *Ago2* to regulate the endogenous RNAi pathway in mouse oocytes.

Altogether, this thesis addresses the long standing question of why miRNAs are non-functional in fully-grown oocytes and also shows miRNA pathway inactivity to be a mammalian feature not restricted only to mouse oocytes. Two adaptations of *Ago2*- key effector protein in miRNA and RNAi pathway were also discovered and characterized.