Imaging miRNA biogenesis pathway and cellular localization of RNA interference triggers

Regulation of gene expression can be observed on many levels. It begins with regulation of transcriptional activity of the gene through regulatory sequences, continue in posttranscriptional regulation of primary transcript processing, translation inhibition, increased degradation of the transcript or selective activation or deactivation of proteins. Very important mechanism of gene expression regulation on mRNA level is RNA interference in which short 20 nt long RNAs interact with transcript leading to translation inhibition with or without degradation of the mRNA.

miRNAs are formed in multistep miRNA biogenesis process that requires involvement of many proteins. Primary miRNA transcript (pri-miRNA) undergoes cleavage by RNase Drosha inside the nucleus. Cleavage product, about 60 nt long secondary precursor pre-miRNA is exported by exportin-5 to cytoplasm. In the cytoplasm pre-miRNA undergoes second main step of miRNA biogenesis which is cleavage by RNase Dicer to about 20 nt long double-stranded miRNA/miRNA* duplex. Mature miRNA included in miRISC complex interact with target transcripts.

On the basis of miRNA biogenesis pathway RNA interference (RNAi) technology was developed to trigger selective gene expression regulation both in basic research to explore gene functions and in genetic therapies. In RNAi technology various types of reagents are used, siRNA resembling miRNA, shRNA – pre-miRNA and sh-miRNA – pri-miRNA.To investigate miRNA biogenesis process and RNA interference technology we will use microscopic methods. This approach will enable to add localization and dynamic aspects to the two main steps of miRNA biogenesis, RNase Drosha cleavage within nucleoplasm and RNase Dicer cleavage in cytoplasm.

The results obtained in this project will provide answers to questions regarding localization and dynamics of the miRNA biogenesis process and differences between endogenous miRNA precursors and exogenous RNA interference triggers: Where miRNA precursors localize within the cell? Are all the miRNA precursors captured by proteins involved in miRNA biogenesis? What are the dynamics of pri-miRNA interactions with DGCR8, Drosha and protein partners? How pri-miRNA structure influence its localization and processing efficiency? How expression level of precursors influence miRNA biogenesis? How delivery method define pre-miRNA recognition and fate in cell? What are the main differences in cellular localization between endogenous precursors and exogenous RNA interference triggers? The project will also provide new experimental and bioinformatic tools to investigate miRNA biogenesis and RNA interference technology. In future perspective new data regarding structure and expression level can be used in further development of RNA interference technology and more rational and systematic design of RNAi triggers.