

MicroRNAs (miRNAs) are short, non-coding RNA engaged in the regulation of gene expression. miRNA is transcribed by RNA Polymerase II (RNAPII) as a pri-miRNA which are next processed to mature miRNA by multi-subunits complex called Microprocessor. The plant complex engaged in miRNA biogenesis contains endonuclease DCL1 and additional proteins like SERRATE and HYPOPLASTIC LEAVES 1 (HYL1). In our laboratory was confirmed the role of SERRATE in miRNA biogenesis. In *se-1* mutant the level of pri-miRNA is elevated and mature miRNA decreased respectively. The role of SERRATE in alternative splicing was also shown. Recently we have discovered nuclear bodies containing pri-miRNA163 by using Fluorescence *in situ* Hybridization. In this project we plan to study additional components of this structures and decipher its role in the transcription, splicing and degradation of pri-miRNA. Our preliminary results proved that observed nuclear bodies are not dicing bodies (D-bodies), which are well known structures engaged in miRNA biogenesis. D-bodies are also site of accumulation SERRATE, DCL1 and HYL1. Despite it we have also confirmed that discovered by us nuclear structures are not Cajal bodies. In order to better understand the structure of this nuclear bodies we want to perform miRNA hybridization with the immunolocalization of other component D-bodies: SERRATE and DCL1. We plan also to study the difference in the number of nuclear bodies in mutants: *hyl1*, *se-1*, *se-2*, *dcl1-7*. It allows us to find the correlation between nuclear bodies formation and the expression of SERRATE, DCL1 and HYL1. Our previous results suggest that nuclear bodies containing pri-miRNA163 are not Cajal bodies. However we have found the difference in the accumulation of pri-miRNA163 in mutant *pcb-1*, where coilin is overexpressed. In this case pri-miRNA163 is localized always close to the nucleolus. This is why we plan to perform pri-miRNA hybridization in other coilin mutants: *pcb-2* and *ncb* to find the connection between Cajal bodies and the structures discovered by us. Despite that we want to focus on the other nuclear bodies engaged in RNA processing: nuclear speckles and paraspeckles.

To elucidate a putative role of pri-miRNA-containing nuclear bodies in transcription of *MIRs* we plan to combine FISH with the localization of RNA Polymerase II phosphorylated at Serine 5 in CTD (the modification typical for transcription initiation) and Serine 2 in CTD (transcription elongation). We will also inhibit the transcription by using α -amanitin and assess the number of nuclear bodies. We want to also perform hybridization in Mediator mutants: *med3*, *med5*, *med7*, *med9*, *med17* and *med18* and *med20a* to find the connection with this complex. Mediator is the complex involved in the recruitment of RNA Pol II to the gene promoter during transcription initiation.

Next we plan to study the role of discovered nuclear bodies in pri-miRNA splicing. We will apply miRNA hybridization with immunolabeling of proteins engaged in splicing. We will also inhibit splicing by herboxidiene and staurosporin and assess the changes in the number of nuclear bodies. The important point of this project will be also hybridization of miRNA in mutants of SR proteins: *rs31-1*, *sr34-1*, *sr35-1*, *sr45-1*, *sc130a-1*.

Despite the transcription and splicing we want to decipher the role of discovered nuclear bodies in pri-miRNA degradation or its fragments. To obtain it we will localize proteins engaged in RNA degradation like proteins removing cap structure at 5' end, subunits of Exosome complex and proteins of NEXT complex. Exosome is the complex degrading RNA from 5' end and is recruited to RNA molecule by NEXT. In this project we will also perform hybridization of miRNA in the mutants of the RNA degradation pathway: *xrn2-3*, *xrn3-3*, *xrn4-3*, *dcp1*, *hen2* and assess the number and morphology of the nuclear bodies studied in each mutant tested.

To check the hypothesis, that other miRNA also localize in nuclear bodies we will apply the localization of pri-miRNA156a, pri-miRNA158a and pri-miRNA160a, which structures are similar to miRNA163. We will also localize pri-miRNA157a and pri-165a, which haven't intron in its structure.

The results of this experiments allow us to understand the role of discovered nuclear bodies in miRNA transcription, splicing and degradation. It will also expand the current knowledge of miRNA biogenesis in plant.