The path from gene to protein in eukaryotic cells is long and complicated. A gene (DNA sequence) is transcribed into a primary transcript (pre-mRNA), which then undergoes modification and maturation to become matrix RNA (mRNA). The resulting mRNA is transported to the cytoplasm, where it is transcribed into protein. However, the amount and type of proteins created in the body change all the time, depending on ever-changing needs. For this reason, it is extremely important to precisely and tightly regulate gene expression, which in eukaryotic cells can be regulated at many levels. One possible regulation is to turn on the transcription of a given gene from DNA to pre-mRNA, leading to the formation of the needed protein. A second possibility is to stop the transcription of a gene from DNA to pre-mRNA, causing the protein encoded by that gene not to be formed. Another type of regulation is the degradation of an existing mRNA by short (21-nucleotide) single-stranded RNA molecules, called microRNAs (miRNAs), which have a sequence complementary to the particular mRNA. Due to this complementarity, miRNAs bind to a given mRNA and lead to its degradation. It is worth adding how important the regulation of gene expression by miRNAs is for the organism. For plants, complete deactivation of the pathway responsible for their formation results in the death of the plant at the embryonic stage. In contrast, partial deregulation of this process results in defects in numerous plant organs, as well as often leading to a lack of fertility.

Mature miRNAs are short (20-22nt), but cut in 2 steps by Dicer-like protein 1 (DCL1) from long primary precursors (pri-miRNAs). The pri-miRNA contains a hairpin structure in which the miRNA sequence is embedded. DCL1 needs other factors to efficiently and precisely cut pri-miRNAs, the most important of which are the HYPONASTIC LEAVES 1 (HYL1) and SERRATE (SE) proteins. Common knowledge suggests that SE and HYL1 are important for maintaining secondary RNA structures in miRNA precursors to maintain high cutting activity by DCL1. However, under certain conditions (plants grown at lower temperatures), DCL1 may be more independent of SE and HYL1. Apart from the fact that these proteins are important for the precision of DCL1 activity, there are no data on the role of SE and HYL1 in forming the secondary structure of the miRNA precursor.

The question of how these proteins affect the secondary structure of precursors is important for understanding the entire process of miRNA biogenesis. This is even more intriguing in the case of the SE protein, since in the two most common *serrate* mutants, *se-1* and *se-2*, which produce SE proteins truncated by 27 and 40 amino acids (aa), respectively, an overall reduction in miRNA and abnormal truncation by DCL1 has been observed. However, the SE formed in these mutants can still interact with HYL1, DCL1 and RNA, allowing us to ask questions: Is SE interaction with HYL1 and DCL1 important for miRNA biogenesis? What is the role of the last 27aa of SE (the most conserved part of the protein) on the secondary structure of pri-miRNAs? Our previous results showed that the THO/TREX complex is strongly associated with SERRATE in vivo. Results from other groups have also shown that THO may be important for the stabilization of pri-miRNAs secondary structure and the binding of HYL1 protein to pri-miRNAs. To date, the role of the THO/TREX complex in miRNA biogenesis is unclear, and the role of the interaction between SE and THO/TREX in miRNA biogenesis has never been confirmed. **The main goal of this project is to understand the role of SERRATE in changing the secondary structures of miRNA precursors and the impact of these changes on DCL1 activity.** To better understand this process, we would like to understand the role of the conserved part of the SE (the last 27aa) and the role of the THO/TREX complex on pri-miRNAs structure and DCL1 protein activity in miRNA biogenesis.

In this project, we would like to propose a series of experiments to analyze the in vivo secondary structure of pri-miRNAs, as well as to test the difference between the secondary structures of miRNA precursors efficiently and inefficiently cut by DCL1. To study the secondary structure of pri-miRNAs, we will use the DMS-MaPseq method. DMS only modifies nucleotides located in single-stranded regions of RNA. Combining this reagent with a special method of preparing material for next-generation sequencing, we can determine single- and double-stranded regions in the RNAs under study. We will also ask how the secondary structures of miRNA precursors change in *hyl1* and *se* mutants under normal growth conditions (22ºC), but also when plants are grown at lower temperatures (16ºC), as well as at higher temperatures (28ºC). We will also test the importance of the interaction SE with HYL1 and DCL1. Using a molecular docking method, we predicted which residues are important for the interaction between SE and HYL1/DCL1. Using this information, we will create transgenic plants in which an SE incapable of interacting with HYL1/DCL1 is formed and check the efficiency of miRNA biogenesis in these plants. In addition, we will check what role the last 27 highly conserved amino acids of SE play for miRNA precursor secondary structure and interactions with other proteins. In the third part of the project, we plan to test the role of SE and THO/TREX interactions for miRNA biogenesis, as well as in stabilizing the secondary structure of miRNA precursors.