The goal of this project is to characterize one of less understood step of microRNA (miRNA) biogenesis in plants considering unwinding of miRNA/miRNA* duplex and formation of RNA-induced silencing complex (RISC).

MicroRNAs are short, usually 19-24 nt length RNAs, that regulate gene expression on transcriptional and posttranscriptional level. Genes encoding miRNAs are transcribed by RNA polymerase II (RNAPII). RNAPII produces primary transcripts (pri-miRNAs) with a hairpin structure which contain miRNA/miRNA* duplex. The duplex is cut out from pri-miRNA in two-step process driven by DICER LIKE1 (DCL1) enzyme. After the cleavages the duplex is methylated to protect it from degradation. For years it was though that methylated duplex is next transported from nucleus to cytoplasm by HASTY protein. In cytoplasm one of the duplex strands is loaded onto ARGONAUTE1 protein to form functional RISC. However, the latest data showed an alternative pathway of the duplex export and RISC assembly. According to that new report, AGO1 is able to shuttle between cytoplasm and nucleus where one of the duplex strands is incorporated onto AGO1. Next, both molecules leave nucleus in a form of an AGO1:miRNA complex. Despite this results, the mechanism of RISC formation remains unclear. **In this project we propose a model in which DRH1 helicase unwind the miRNA/miRNA* duplex**, possibly helping in strand incorporation onto AGO1, **and facilitate AGO1:miRNA complex export.** DRH1 is a helicase belonging to the DEAD-box family. The name of the family originate from a conservative amino acid sequence consisnting of aspartic acid (D), glutamic acid (E), alanine (A) and aspartic acid (D). DRH1 is involved in rRNA biogenesis, mRNA export and in mRNA surveillance pathway called Nonsense-Mediated-Decay (NMD). Our preliminary results showed that DRH1 directly interact with AGO1. This results leads us to formulation of a hypotesis about involvement of DRH1 in miRNA/miRNA* duplex unwinding and RISC formation. To verify this assumptions, we designed series of *in vivo* and *in vitro* experiments.

In this project we plan to test involvement of DRH1 in duplex unwinding and RISC formation. First, we plan to use high throughput sequencing of small RNA to examine distribution of mature miRNA and miRNA* in nuclear and cytoplasmic fractions of helicases mutant. This approach will allow us to study the involvement of DRH1 in miRNA export. Additionally, we will test cellular localization of AGO1 in helicases mutant using immunolocalization and Western blot assay to gain information about role of DRH1 in AGO1:miRNA export. Next, we will perform high throughput sequencing of small RNA bound with AGO1 from nuclear and cytoplasmic fractions. Using this method we will get the information about the role of DRH1 in the duplex unwinding. To investigate the unwinding activity of DRH1 we will also perform an electrophoretic mobility shift assay (EMSA).

The results obtained during this project will let us to broaden our knowledge about one of the basic process occuring in eukaryotic cells. Moreover, collected data enable us to characterize the mechanism of action of poorly described DRH1 helicase.