Aim of the project is to explain how the microRNA (miRNA) molecules emerge from the RNA molecules called precursors, in plants. I am going to achieve this mainly with the use of the structural biology tools - the techniques that allow creation of a model of three-dimensional structure of a studied molecule or a complex composed of molecules. Certain of these techniques enable us to study also the dynamics of molecules, that is, their movements and structural changes that are a result of their flexibility.

The first precursor RNA molecule, coming from the transcription of a miRNA gene, is called pri-miRNA. This molecule is cleaved to create a pre-miRNA molecule. Within it, two or more additional cuts are required to release a mature miRNA molecule. All these cuts are performed by the DCL1 protein, but in order for them to happen in the correct places within the RNA, a help from HYL1 and SERRATE proteins is crucial. Three mentioned proteins together with a miRNA precursor interact physically with each other. Given that, they constitute the miRNA biogenesis complex in a plant that serves as a model organism for that study – the mouse-ear cress (*Arabidopsis thaliana*). The main hypothesis of this project states that HYL1 and SERRATE proteins, due to binding to specific elements in RNA structure, create a kind of scaffold for the DCL1 protein, guiding it to the correct cleavage sites within precursor RNAs. To verify that hypothesis, I am going to measure the small-angle X-ray scattering (SAXS) by the molecules and their complexes in solution in the first place. Obtained scattering pattern will allow me to obtain some important information. These include the size and the degree of flexibility of a studied object (molecule or complex) as well as the number of components that form the complex. Based on the scattering pattern, a three-dimensional model of the structure of a studied object can be build, albeit of a low resolution. I anticipate that the SAXS method will enable me to determine how many copies of HYL1 and SERRATE proteins associate with a miRNA precursor molecule and with which parts of it. This technique is also particularly useful for studying structural changes within molecules.

In order to make the results obtained from the SAXS technique more reliable, I am going to conduct the biochemical experiments termed “footprinting” or “toeprinting”. They allow to learn which region of RNA are protected from by enzymatic reaction by proteins bound to them. I will also attempt to obtain the complexes composed of a miRNA precursor and mentioned proteins in the form of a crystal. That would enable building of a model with resolution that makes it possible to distinguish individual atoms, thus to study the architecture of complexes in a very deep detail. To grow such a crystal is a pretty challenging task, though.

Results of the studies described in the project will enable better understanding of the bases of one of the most important gene expression (so protein and RNA levels) regulation mechanism in plants. They may aid in explaining, what some abnormalities of this process come from. Finally, they can contribute to our ability to influence the miRNA biogenesis and action.

The miRNA biogenesis complex performs a complicated task with almost no errors. Moreover, it does that in a dynamical environment, where all the molecules undergo random movements. By understanding how it is possible, we will enrich our knowledge about the physical and chemical rudiments of cellular processes. This understanding may also help nanotechnology in designing and constructing artificial molecular machines.