Apart from the canonical ribonucleotides – adenine, uracil, guanine and cytidine – their chemically modified versions can also be found in RNA molecules. Over 170 RNA modifications have been described up until now, with pseudouridine (Ψ) being the first. From a chemical standpoint, pseudouridine is an isomer of uridine. The isomerisation reaction is catalysed by pseudouridine synthase enzymes (PUS), which can work on their own or in ribonucleoprotein complexes with a specific snoRNA and helper proteins.

The role of pseudouridine has been extensively researched in some classes of RNA, such as tRNA (transporting RNA), rRNA (ribosomal RNA) or snRNA (small nuclear RNA), where it is present in high abundance. Unfortunately, technical shortcomings didn't allow for searching for pseudouridine in different RNA classes, such as mRNA (messenger RNA) or short non-coding RNAs, like micro RNAs. In recent years, we could observe fast development of high throughput next generation sequencing methods, which allows us to revisit modified ribonucleotides field, including Ψ. Now, it was possible to detect pseudouridine in yeast, plant and animal mRNAs, where it might have a role in maintaining stability of the transcript, its transportation and splicing. Recently, pseudouridine was also identified in a pool of miRNAs, both animal and plant, as well as in some of their precursors.

Micro RNAs are the most abundant class of short, non-coding RNAs. They can be found in all eukaryotes, where they are one of the most important regulators of gene expression. They work together with proteins from ARGONAUTE (AGO) family in a complex known as RISC (RNA-induced silencing complex), blocking the translation or cleaving the targeted transcripts. MiRNA biogenesis is a complicated, multi-step process, both in plants and animals – fist, *MIR* genes are transcribed by RNA Polymerase II, which produces primary transcripts (pri-miRNAs), that have to undergo processing and maturation to pre-miRNA precursors, and, finally, create a mature, functional miRNA molecule. This process is regulated on many levels, and now it is more and more evident that chemical RNA modifications, such as N-6- methyladenosine, may also be involved.

The goal of this project, which is an extension of already ongoing research at our laboratory at Department of Gene Expression, Institute of Molecular Biology and Biotechnology at Adam Mickiewicz University, **is to decipher if and how pseudouridine can influence the biogenesis and action of miRNAs**.

To achieve this, we planned a series of experiments, that will allow us to: 1) verify the list of pseudouridylated miRNAs with the use of an alternative method; 2) look into the basics of the mechanism of small miRNA pseudouridylation; 3) describe the influence of pseudouridine on processing of miRNA precursors; 4) check the interactions between proteins involved in pseuouridylation and proteins of miRNA microprocessor; and 5) understand the influence of pseudouridine and PAUSED protein on the intercellular and intercompartmental transportation of miRNAs.

This project will not also significantly broaden the knowledge in the field of epitranscriptomics, but will also describe the new regulatory levels of the biogenesis and action of miRNAs.