Genetic information must be protected, maintained and copied from cell to daughter cells, from generation to generation. In plants, most of the cells contain complete genetic information, and many of these cells can regenerate to a whole new plant. Such a feature leads to the need for precise control of which genes will be active and which not because in growth and differentiation, only the activity of specific genes for the individual cells, tissues, organs are required.

One of the mechanisms controlling the gene activity is RNA interference (RNAi), which down-regulates or blocks the expression of specific genes at the transcriptional or post-transcriptional level. The crucial part of the RNAi is guiding the RNAi machinery to the target. It is mediated via sequence complementarity of the target with a small RNA (sRNA), which is diced from a double-stranded RNA (dsRNA) precursor. The molecular mechanism of dsRNA and sRNA formation and also the target origin predestinates the subsequent silencing pathway.

In transcriptional gene silencing (TGS), the gene expression is regulated through chromatin epigenetic modifications. One of the epigenetic marks is cytosine methylation, which is established mainly by RNA-directed DNA-methylation (RdDM) pathway. Although the protein machinery was relatively well-described, little was known about the dynamics of the key initial phase of this process. Thus, we developed a system that allowed us to study this phenomenon in detail. We used a homogeneously responding tobacco BY-2 cell line, and in combination with an inducible system for controlled production of sRNA, we were triggering *de novo* RdDM. Our results show that the TGS-related DNA methylation was faster than previously reported, progressively increased and gradually inhibited the promoter activity within two days in proliferating cell culture. In our study focused on silencing at the post-transcriptional level, we observed that the distinct origin of dsRNA strongly affected sRNAs production, leading to unexpected variations in the dynamics and reversibility of the silencing and also in the extent of accompanying RdDM.

The epigenetic modifications naturally differ within the genome according to the need for regulation in the given locus. Besides their effect on chromatin regulation, they may also influence genome editing tools, such as the widely used engineered programmable endonuclease CRISPR/Cas9. As little was known about the effect of epigenetic modifications on its efficacy and mutagenesis, especially in plants, we developed an experimental system based on *Nicotiana benthamiana* where we described how the DNA cytosine methylation mark indirectly impaired Cas9 activity and also affected the follow-up DNA repair.