All organisms on Earth store their genetic information in the form of DNA with specific nucleotide sequence. This information determines how the organism will develop, function and react to environmental stimuli. However, not all genes are active at certain time and place and precise regulation of their activity is crucial for all organisms. Eukaryotic organisms have DNA packed by special proteins called histones into structure of chromatin. Not only does it allow to accomodate and spatially organize genetic material, but also, by changes of chromatin condensation, enables to regulate its accessibility to different processes. It is true also for the machinery which activates genes (carry out their expression) - histones may expose DNA sequence in decondensed chromatin or cover it in condensed chromatin; as a consequence, the machinery may be able or not to bind this sequence, respectively. Changes in interactions between DNA and histones may be caused by addition of specific chemical groups to the latter. Acetylation is one of such important modifications. Special enzymatic complexes called histone acetyltransferases add acetyl groups to particular histone lysines. Generally, acetylation results in chromatin decondensation and makes DNA accessible for other proteins. Hence, it is often correlated with gene activation and expression. Acetylation, its effect on gene expression and acetyltransferases are conserved in living organisms, even if species are not related. This fact confirms their importance.

Such a very important acetyltransferase complex in yeast (a model organism in biology) is the NuA4 complex, consisting of 13 proteins. It acetylates genes and enables their activation. Studying the composition of such complexes and the roles played by their particular subunits is difficult and laborious, but we know that NuA4 has a counterpart in human, too. So far, not much is known about the plant version of NuA4 - whether its composition, catalyzed reactions and effect on DNA-dependent processes are similar. only counterparts of three NuA4 subunits were found in Arabidopsis (amodel plant) and, indeed, they are connected with histone acetylation. Recently, we discovered and described the next one, which we named AtEAF1 because we think it is the equivalent to yeast EAF1 protein. In yeast EAF1 plays an important role - it serves as a platform, to which other subunits bind, forming the full comlex. EAF1 is the most important for gene regions called promoters; remaining gene regions can be bound by the other parts of the complex. Importantly, promoters are crucial for gene activity because they recruit proteins responsible for gene expression.

The question I would like to answer in this projects is: are the EAF1 functions in gene activation the same as in its plant counterpart? Is AtEAF1 responsible for proper histone acetylation at gene promoters and, consequently, does it regulate their activity? Answers will not only enrich our knowledge about plant molecular mechanisms, but also let us compare how gene expression is “managed” in different organisms and how these “methods” of gene control evolved.

In Arabidopsis there are two almost identical genes AtEAF1A and AtEAF1B. To study the particular gene function it is very common to analyze the effects of its inactivation. There are many available Arabidopsis lines with mutations which deactivate particular genes, but none has two AtEAF1 genes mutated. Moreover, it would not be possible to obtain such line by crosses commonly used in genetics.
Therefore, I am going to use an advanced technique based on the system called CRISPR/Cas9 for directed mutation of both genes at the same time. The protein Cas9 is introduced to plants and guided by specific RNA fragments to complementary gene sequences, which it cleaves. As a result, it leads to irreversible gene mutation and inactivation. We almost managed to obtain such plants - they are severely changed in comparison to wild-type plants. They are small, grow very slowly, have very pale leaves and sterile pollen. I would like to find out which genes are deregulated there, in other words: which of all genes have higher expression than wild-type ones and which have lower expression. It will be possible owing to the technique called RNA-seq, which shows expression levels of all genes simultaneously. I expect to find a group of deregulated genes, which will explain phenotypic changes (changes in plant appearance and development). Then, I am planning to analyze changes in histone H4 acetylation for a number of deregulated genes, especially in promoter region. For this purpose, using the antibody specifically recognizing acetylated histone H4, I am going to fish out all DNA fragments that bind to this modified protein (this method is called chromatin immunoprecipitation) and thereafter compare them quantitatively in mutant and wild-type lines. Collected results should allow to correlate the lack of active AtEAF1A/B proteins with promoter acetylation status and gene expression. Furthermore, I have also mutant lines for counterparts of other NuA4 subunits with similar but escalated to different extent phenotypic changes. I would like to check if they have similar genes deregulated and similarly changed acetylation status. Some differences may indicate functional differentiation of plant NuA4 subunits.