

Posudek disertační práce Mgr. Adély Příbylové

Dynamics of *de novo* DNA methylation and its impact on transgene expression and CRISPR/Cas9 mutagenesis

The thesis addresses important issues in current plant epigenetics: initiation, onset and time course of transcriptional gene silencing and posttranscriptional gene silencing. To do so, the author team had to develop an experimental system allowing for an inducible production of sRNAs in tobacco BY-2 cells. The decrease in mRNA levels has started already 6 hours after induction, and progressed till the day 2.

A variable response has been observed in posttranscriptional gene silencing – its dynamics, maintenance and extent. The response was dependent on the origin of the dsRNA silencer.

These results are demonstrated in two published papers (Příbylová et al., *Epigenetics Chromatin* 2019) and Čermák et al, *BBA-Gene Regul. Mech* 2020).

The last and very interesting part of the Dissertation deals with the effect of chromatin state (namely of DNA methylation) on CRISPR/Cas9 editing frequency and the mechanism of the repair of induced DNA damage. In this part, *Nicotiana benthamiana* leaves and via virus-induced gene silencing through the induction DNA methylation in the CRISPR/Cas9 target region. While the DNA methylation in the promoter region decreased the efficiency of Cas9 activity, DNA methylation inside the coding region had no effect. Based on an abundance of single-nucleotide homology-mediated DNA repair and indications of its repair through 5' nucleotide overhang on the PAM DNA strand, the authors speculate on a novel repair pathway rather than MMEJ repair.

Overall, the dissertation has been processed at a very good level and provides original results in the attractive research domain of plant epigenetics.

I have the following comments, questions and criticisms:

p13: “but in non-gene sequences, they are often very stable and inherited from generation to generation” – it is not obvious whether generations of cells or individuals are meant here.

At numerous places, the author uses the verb „could“, although she does not want to express the past or condition.

An incorrect syntax complicates the intelligibility of the text at several places, e.g.:

p.21: „Whether the c-NHEJ repair process involves annealing of short microhomologies (MHs) differ among literature.“

p.22 „For SSA around 20-100 bp MH is necessary“.

I am asking for an explanation and a closer substantiation of the conclusion written in p 26:

„Although siRNAs patterns along the sequence differed in the compared studies (Figure 2), in the end, it seems that involved RNAi pathways could consist of similar proteins and in the comparable ratio.“

p. 32: „Those changes could lead, for example, to tighten the DNA around the nucleosomes, which could lead to the spatial rotation (torsion) of the DNA and thus different exposure of the target site to the Cas9 nuclease domains.“ – In contrast to the other parts of the Discussion, this explanation seems not to be correct in my view. DNA wounded on a histone octamer has quite

clearly defined length and positions of its coils with respect to basic amino acids of histones. Therefore, DNA binding to histone octamer may be temporarily weakened (breathing), namely at the nucleosome DNA entry and exit (these coils interact with H2A-H2B dimers). Or, because of the H1 presence, the protected region of nucleosomal DNA can be longer. Also, the translational or even the rotational position of the nucleosome may possibly change but always this is a shift. The radius of DNA supercoils on the nucleosome surface does not change – contrary to the suggested explanation. **I would like to ask for the author's statement** – maybe I got it wrong. However, the next conclusion of the author is already correct – to answer this question, experiments are needed in which cleavage sites will be designed to defined positions with respect to the experimentally determined nucleosome positions. Eventually, nucleosome positions could be, at least additionally determined. **What methods could be used to do so?**

Overall, I find the dissertation thesis of Mgr. Adéla Přibylová to be of above-average level, meeting all the standards of Ph.D. thesis. Therefore, I recommend to award her with the Ph.D. title after a successful defense.

In Brno, Nov. 19, 2021

Prof. RNDr. Jiří Fajkus, CSc.