

Living organisms have evolved proteins that facilitate the reading, copying, and repair of genetic material. These proteins, including flap endonuclease 1 (FEN1), play crucial roles in DNA replication and repair in eukaryotic cells. FEN1 is a nuclear protein found in yeast, animal, and plant cells. The lethal effect of FEN1 deficiency in animals and plants provide strong evidence supporting the importance of this protein for eukaryotes. Precise regulation of FEN1 functions is essential for the undisturbed functioning of both animal and plant cells. Studies of human and yeast FEN1 indicate that phosphorylation is one of the post-translational modifications regulating the functions of this protein. Despite the crucial role of FEN1, the limited information on the regulation of its functioning in plants is surprising. In our ongoing research, we are attempting to fill this gap. Our previous study led to the identification of the first Arabidopsis nuclear kinase, an enzyme responsible for the transfer of the phosphate group to the specific substrate, which regulates the activity of FEN1. Moreover, it seems that other nuclear kinases and factors may also contribute to the regulation of FEN1 phosphorylation status. Currently, we are at the beginning of understanding the role of phospho-signaling in the regulation of FEN1 functioning at different levels in plant cells. It is known that the addition of a phosphate group can change many protein properties, including function, activity, stability, interaction with their partners and subcellular localization. Which of these mechanisms are active in the case of plant FEN1 remains an open question. The goal of this project is to broaden our knowledge about (i) plant nuclear kinases involved in the modulation of plant FEN1 functions, (ii) nuclear factors engaged in the regulation of plant FEN1 phosphorylation, and (iii) the effects of FEN1 phosphorylation in the context of DNA replication and the plant cell response to DNA replication stress. Among many experimental techniques we plan to use during the project is cryo-electron microscopy (cryo-EM). This technique allows the imaging of the protein samples with high resolution. In our research, cryo-EM will be applied to study the structure of the complexes formed between FEN1 and its partners. In addition to *in vitro* studies, we also plan to investigate the effects of modified versions of FEN1 functioning *in planta*. To accomplish this, specified versions of FEN1 will be introduced into Arabidopsis mutants lacking this protein. The phenotype and the response to conditions that disturb DNA replication of these plants and wild type ones will be compared. The results of this project will contribute to the development of knowledge in particular on the role of phosphorylation of plant FEN1 protein in DNA metabolism. Acquired knowledge will also deepen our understanding of the mechanisms regulating plant response to DNA replication stress.