Seed funding use – results

We used the seed funding provided in the INTEG-RNA project to establish collaborations with project partners on the following topics:

1. To identify protein interactors of plant TR to elucidate plant telomerase RNP composition (*collaboration with Falk Butter*, IMB Mainz)
2. To map sites of TR-protein interactions (*collaboration with Julian Koenig*, IMB Mainz)
3. To characterize post-transcriptional modifications of TR (*collaboration with Mark Helm* JGU Mainz)

Topic 1: Our Ph.D. student, Mgr. Alžbeta Kusová, prepared and purified plant nuclear extracts from seedlings of *Arabidopsis thaliana*, which were then used (during Alžbeta's stay in Mainz in 2022) for a subsequent analysis using mass spectrometry in F. Butter's laboratory. These experiments were performed as a part of our common mini-project in WP1 - Characterisation of plant telomerase RNA-binding proteins. In parallel, extracts prepared earlier independently by the Falk Butter group and Jiří Fajkus group were also used for MS identification of RNA-binding proteins. These *in vitro* experiments resulted in identification of three putative binding proteins. The mini-project then continued with preparation of constructs for identification of *in vivo* RNA-binding proteins. Currently, plants transformed with constructs expressing AtTR-MS2 and MCP-TurboID are available. In addition, we use in parallel the technique ChIRP-MS - Comprehensive identification of RNA-binding proteins by mass spectrometry that was successfully optimized in our laboratory.

Topic 2: In collaboration with the laboratory of Prof. Julian Koenig, IMB Mainz, our Ph.D. student, Mgr. Anna Rudolfová, optimized iCLIP method (individual-nucleotide resolution crosslinking and immunoprecipitation) and showed that iCLIP can ve applied for a high-resolution mapping of binding of AtTR protein interactors. However, this approach is efficient only for sequence-specific interactors and as the next step following the less demanding analyses as EMSA, RIP, CLIP.

Topic 3: This topic was solved during the secondment of Dr. Lucie Bozděchová, a postdoctoral fellow in Jiří Fajkus lab, in Prof. Mark Helm's laboratory, JGU Mainz in 2022, and recent internship (2023) of Mgr. Jiří Rudolf, a new Ph.D. student in J. Fajkus's laboratory, in the same host lab. The aim of the internship (postdoctoral secondment) was to prepare plant telomerase RNA samples for analysis of RNA modifications by sequencing or LC-MS. Since the method for determining of modifications depends on the amount of prepared purified material, this approach was challenging in case of telomerase RNA, as it is present in very small amounts in cells. During the L. Bozdechova's stay, purification procedure was established and RNA fraction corresponding in size to the telomerase RNA was obtained. The prepared amount of material, however, was not sufficient for successful downstream analysis. Therefore, a more robust approach was designed during the stay of J. Rudolf in 2023, and its efficiency was validated. Currently, the material is a subject of follow-up analyses.