1 Characterisation of plant telomerase RNA-binding proteins

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1.1 SUMMARY

This collaborative project aims to identify proteins that interact with the RNA subunit of Arabidopsis thaliana telomerase (AtTR). We will apply in vitro and in vivo pull down experiments to purify TR-protein complexes and subsequent mass spectrometry identification of candidate protein interactors. The genuine involvement of the candidate proteins in telomerase ribonucleoprotein (RNP) complex structure and function will be characterised using reverse genetics, microscopy and molecular biology experiments. The project uses the expertise of the Laboratory of Falk Butter (IMB Mainz, Germany) in mass spectrometry and characterisation of specific RNA- and DNA-binding proteins (including telomere-binding proteins), to advance the existing expertise of the Laboratory of Jiří Fajkus, (CEITEC MU, Czech Republic) in diverse aspects of telomere biology, including structure, function and evolution of plant telomeres and telomerases.

1.2 SCIENTIFIC BACKGROUND

Telomerase is RNA-protein complex that compensates for the incomplete replication of chromosome ends using the reverse-transcriptase activity of its catalytic protein subunit (telomerase reverse transcriptase, TERT). Telomere synthesis is templated by a short region of the telomerase RNA subunit, TR. In addition to its templating role, TR provides a scaffold to assemble the whole telomerase ribonucleoprotein complex. Recently (Fajkus P et al., NAR 2019) we described TR in land plants. Contrary to previous reports and presumptions based on the existing knowledge in animal and yeast telomerases, plant TRs are monophyletic and transcribed with RNA polymerase III. The biogenesis and composition of plant telomerase RNP complex is, however, largely unknown.

1.3 AIMS OF THE RESEARCH PROJECT

The proposed project aims at identification of AtTR-associated proteins. Besides TERT and dyskerin, our preliminary experiments did not confirm plant orthologs of the known mammalian TR-binding proteins as bona fide binding partners and functional components of plant TR. In addition, differences in composition and structure of plant RNP are presumably underlying specific features of telomerase regulation in plant cells, namely its highly flexible and reversible regulation in response to developmental and regeneration processes, phytohormone levels and environmental conditions (Fajkus et al., MGG 1998). Therefore, we set to determine AtTR-asociated proteins using a de novo RNA-centric approach.

1.4 WORK PROGRAMME, METHODOLOGY AND DIVISION OF TASKS

We will follow three independent strategies to identify subunits of the telomerase complex by mass spectrometry.

Task 1

First, we will perform in vitro RNA-pulldowns using cell extract of actively growing plant cells (seedlings, suspension culture cells or flower buds) with a validated telomerase activity and in vitro transcribed telomerase RNA from Arabidopsis thaliana fused with the minimal S1-aptamer tag (Butter et al., 2009). This strategy can easily be done in the Butter group as the workflow is applied routinely in diverse projects and requires only the shipment of Arabidopsis cell extract containing a minimum of 2 mg proteins. The proteins bound to AtTR will be identified using quantitative mass spectrometry in the Butter group. As a negative control, reverse complementary sequence of AtTR with the same tag will be used. A comparison between the telomerase-positive, and telomerase-negative extract will also be done (using the extract from fully mature leafs) to assess the potential involvement of identified candidates in active telomerase RNP.

Jiří Fajkus group: preparation of plant material in a lyophilized form, preparation of AtTR construct suitable for in vitro transcription of AtTR and the negative control

Falk Butter group: preparation of tagged AtTR and control version, preparation of cell and tissue extracts, pull down experiment and MS analysis, evaluation of results.

Timing: April – July 2021

Task 2

Secondly, the Fajkus group will establish transgenic plants carrying a tagged-version of AtTR that can be purified from a protein extract. The tag will either be the widely used MS2 aptamer (3x) or the minimal S1 aptamer used for in vitro experiments (Task 1). Both tag versions can be tried in parallel (it only requires small modifications of the transformation vector by PCR). The AtTR version to be used in the in vivo assay will correspond to the version which has been successfully used in our previous AtTR complementation experiments (Fajkus et al, NAR 2019). Homozygous attr mutants complemented with the tagged version of AtTR, and negative control plant lines will be generated and genotyped. Seeds from well established lines will be used to grow seedlings on Petri dishes and plants. Then, pull down experiments will be performed with seedling and plant leaf samples in analogy to Task 1.

Jiří Fajkus group: Preparation of AtTR and control rc-AtTR constructs, plant transformation, selection and genotyping.

Transportation of plant material to FB laboratory and preparation of extracts (a postdoc from JF lab – funded by a secondment from INTEG-RNA project, not a part of a budget of this project).

Falk Butter group: performance of in vivo pull-down experiment, MS and its evaluation under a presence of a postdoc from JF lab during the secondment.

Timing: June 2021- May 2023

Task 3 (optional, a back-up approach)

In the third approach, the Fajkus lab will aim to fuse a tag to the catalytic protein subunit AtTERT or its fragments to purify the telomerase RNP complex by IP experiments. Constructs are available in JF laboratory.

Jiří Fajkus group: Generation of transformed A. thaliana plants.

Falk Butter group: Preparation of telomerase extracts, in vivo pull down, MS identification, AtTR detection (under a presence of a postdoc from JF group).

Timing: October 2022 – May 2023

Expected publication outputs: Since Oct 2021, preparation of publication outputs will start and continue till the end of the INTEG-RNA project.

1.4.1 Benefits of this research project and future prospects of collaboration

The project will result not only for generation of the novel knowledge in the attractive field of plant telomerase biogenesis but at the same time also in advancing the know-how of JF laboratory in RNA-centric methods for the analysis of RNA-binding proteins. The postdoc secondment planned in association to this project (but not included in its budget) will also enhace future career prospects of the postdocs, enriching the collaboration network of the postdoc and the JF laboratory as a whole.

The results obtained in this collaborative project will be further expanded with subsequent molecular biology and functional testing of identitfied candidate proteins using three hybrid experiments, IP-TRAP, and IP-RTqPCR. Variants of fusion version of the candidate interactors will be prepared to examine their in situ and in vivo localisation and co-localisation with telomerase. Further, knock out mutants in genes coding for the identified TR-binding proteins will be ordered from mutant repositories and their phenotype features, telomere stability and telomerase activity examined in at least three subsequent generations.

1.5 BUDGET

Material and services costs

JF laboratory: 4 500 Eur - molecular biology kits, enzymes for genetic manipulation, common chemicals, primers, sequencing, cultivation and manipulation of plants and tissue cultures.

FB laboratory: 4 500 Eur - enzymes for genetic manipulation, kits and reagents for pull down experiments, contribution to costs of proteomic analysis.

PostDoc Secondment (not a part of this project budget):

4 weeks in FB laboratory (travel including transport of plant material (by car), accommodation, daily allowance). Presumed in 2022 to perform the in vivo pull-down experiments.