1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title:

Function of m6A (N6-methyladenosine) in stress granules (SGs) assembly and translation control in plants subjected to hypoxia stress

1.1. Project goals

The research problem of this project concerns the molecular mechanisms of plant responses to abiotic stress. Recently, a new level of gene expression regulation has been discovered, and it is a chemical modification of nucleotides in RNA. This phenomenon has been termed epitranscriptomic regulation gene expression. Transcriptome-wide mapping of various nucleotide modifications of mRNA in Arabidopsis has demonstrated that this epitranscriptomic regulation is an essential and widespread molecular mechanism underlying key plant developmental processes, including embryo development, trichome morphogenesis, root development andresponse to abiotic stress (Zheng et al. 2020). Among the diverse modifications found on mRNAs, N6-methyladenosine (m6A) is the most prevalent modification in both plants and animals (Liu and Pan, 2016). Extreme environmental conditions require exceptional responses at the genome level that increase adaptation tostress. Plants are sessile organisms that are permanently restricted to their site of germination to require extensive
stress response capabilities. One of the important mechanisms of regulation of gene under istranslation fine-tuning (Merchante et al. 2017). Recently, it has been shown that one of the of the regulation of protein synthesis in adverse conditions in plants is the spatial organization the transcriptome in cells. The accumulation of transcripts in cytoplasmic stress granules (SGs) regulates mRNA availability to ribosomes (Niedojadło et al. 2014, 2016; Sorenson and Bailey-Serres 2014). Studies in a nonplant model have shown that m6A affects the transport of mRNA from the nucleus to the cytoplasm and their localization in cytoplasmic structures and. However, to date, despite premises, there is no information regarding the role of m6A in functionally localization of mRNA in the cell in adverse environmental conditions in plants.

The project aims to understand the function of m6A in SGs assembly and translation control in plants subjected to hypoxia stress.

1.2. Outline

The consequence of climate change is the increase in extreme weather events such as: storms, and flooding, which are responsible for huge losses in agriculture and all its sectors: crops, animal husbandry and forestry. With the advent of the new millennium, the frequency of natural disasters has increased sharply and has occurred at a consistently high rate over the past 20 years. Floods, caused by excessive rain, reduce the oxygen concentration available to plants, leading to hypoxia. This primarily reduces ATP synthesis. Limited energy production leads to an excessive accumulation of toxins such as alcohols and aldehydes in the tissues. Then there are metabolic changes, including

the transition to anaerobic respiration leading to an energy crisis of cells.

The research problem of this project concerns the molecular mechanisms of plant responses to hypoxia stress. Recently, a novel mechanism for regulating gene expression has been discovered involving chemical modifications of the nucleotides in RNA. Among the diverse modifications found on mRNAs, N6-methyladenosine (m6A) is the most prevalent modification in both plants and animals.

In response to stress, there is, inter alia, a strong reduction in the level of protein synthesis because this process consumes approximately 40% of cellular energy. One of the supposed mechanisms to control this process is the accumulation of mRNA in cytoplasmic stress granules (SGs). Specific proteins and RNAs accumulate in SGs, isolating them from ribosomes, thus providing another step gene regulation that can directly affect cell survival. Therefore, the interplay between SGs and translationally active ribosomes needs to be clarified. However, the mechanism of this phenomenon is not fully understood in plants.

In the project we will use the following methods: microscopic techniques (immunolocalization of proteins and FISH RNA using Stellaris probes), molecular biology (RNA sequencing, nanopore RNA sequencing, immunoprecipitation of SGs, ribosomes and RNP (TRAP method) to study: 1. the function of m6A in mRNA accumulation in SGs; 2. the role of m6A and SGs in the regulation of translation; 3. nuclear RNAs contribute to the biogenesis of SGs.

Understanding new mechanisms of regulating the response to hypoxia stress in the model Arabidopsis thaliana plant in the future will allow for the development of cultivars with desired properties, including increased tolerance to hypoxic stress and protection against crop loss.

1.3. Work plan

- Cultivation of A. thaliana seedlings in in vitro cultures under physiological conditions and hypoxic stress.
- Isolation and analysis of the SG transcriptome (RIP techniques, RNA-seq).
- Tracking and identification of nuclear transcripts in SGs (techniques involving BrU, RIP, BrU-RNA-seq).
- Intracellular localization of transcripts present in SGs and the nucleus (in situ hybridization).
- Investigation of the nuclear transcriptome under stress conditions in plants treated and untreated with nuclear transport inhibitors (isolation of cell nuclei using the INTACT technique and RNA-seq).
- Analysis of stress granule presence in A. thaliana mutants with knockout genes encoding RNA "seeds" (FISH technique).
- Identification of RNA-associated proteins remaining in the nucleus and transported from the nucleus to SGs during stress (isolation of selected RNPs and protein identification using bottom-up LC-MS/MS technique).

1.4. Literature *(max. 10 listed, as a suggestion for a PhD candidate)*

- Maruri-López et al. (2021) Front Plant Sci. 12:722643;
- Niedojadło et al. (2016) RNA Biol. 13:531-543;

- Parker et al. (2020) eLife 9:e49658;
- Guzikowski et al. (2019) Wiley Interdiscip.
- Sorenson and Bailey-Serres (2014) Proc. Natl. Acad. Sci. USA 111:2373-2378;
- Kubiak et al. bioRxiv*: https://doi.org/10.1101/2023.12.14.569339;*

1.5. Required initial knowledge and skills of the PhD candidate

The candidate must hold a Master's degree in molecular biology, biotechnology, biology, or related fields. The candidate should be prepared for new challenges and approach them with enthusiasm. The doctoral candidate should be able to handle minor setbacks in scientific work and consistently strive to achieve set goals. The individual applying for this position should demonstrate commitment to scientific work and be available for travel related to research. Additionally, skills in cell and molecular biology are desirable, including techniques such as RNA work, genotyping and RNA-seq. The candidate must hold a Master's degree in molecular biology, biotechnology, biology, or related fields.

1.6. Expected development of the PhD candidate's knowledge and skills

The doctoral candidate is expected to continuously develop their knowledge and skills during the doctoral studies. The work in this position primarily requires acquiring new competencies, interpreting results, and developing new concepts within the scope of the conducted research.

In the first year of work, the doctoral candidate should master the substantive basics of their research and learn techniques for isolating cell nuclei and stress granules, extracting RNA from them, and generating expression libraries. In the following year, they should achieve initial results from the transcriptome analysis of isolated structures and the localization of specific RNA. By the end of the third year of work, the doctoral candidate should have sufficient results to write the first publication. During this time, they should also identify proteins associated with specific RNA and analyse mutants.

Throughout the doctoral work, the doctoral candidate will be under the scientific supervision of the supervisor and will have the opportunity to develop their skills through scientific trips and participation in scientific conferences.