**INTERNSHIP PROPOSAL M2 RESEARCH 2023-2024**

**TITLE:** Integrative analysis of nuclear protein quality control pathways in plants

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Address, phone, e-mail:

Protein Maturation, Cell fate and Therapeutics

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Number of PhD currently supervised: 1

Possibilty to give rise to a PhD proposal : ◼ YES ☐ NO

Direct presentation of the proposal to the students: ◼ YES ☐ NO

◼ 31/08/23 (IPS2) ☐01/09/23 (IJPB)

**NEW :** we plan to communicate, for information, the internship proposals to M1 students so that they can get an idea of the research themes of the field. Would you agree to have your proposal distributed to them?

◼ YES ☐ NO

INTRODUCTION, SCIENTIFIC CONTEXT : Plants are sessile organisms, and their ability to adapt to biotic and abiotic stresses is crucial for survival in natural environments. Temperatures above the normal optimum are sensed as heat stress (HS) by all living organisms. HS is an increasingly prevalent environmental constraint for plants as global warming has increased the frequency of extreme high temperature events and is expected to be particularly detrimental1. HS is a major abiotic stress that limits the growth of plants and is a serious threat to crop production worldwide including cereals, legumes, and oil crops.

Proteotoxic stress caused by heat-induced protein misfolding is a major challenge to the survival of all organisms. Therefore, cells invest in an extensive protein quality control network of well-conserved components and stress response pathways that act to lower the amount of cellular aberrant proteins2. This includes the protein degradation and synthesis machineries as well as molecular chaperones ensuring conformational maintenance. For plants, understanding these protective responses has important agronomic value for maintaining yield in the face of unpredictable daily temperature fluctuations and the anticipated impacts of elevated temperatures due to global climate change.

Many of these pathways have been described in the cytoplasm and the chloroplast. However, little is known about mechanisms that protect the nuclear proteome upon adverse conditions. Recently, a new nuclear pathway has been discovered in mammalian cells and highlights a new protein quality control principle3,4. We have demonstrated that the nucleolus acts as a liquid-liquid phase separated protein quality control compartment that is essential for maintaining nuclear protein homeostasis (proteostasis). Particularly, an integral part of the cellular management of nuclear misfolded proteins is their reversible sequestration within the nucleolus3. Despite agronomical interest, whether this pathway exists in plants is unknown.

RESEARCH PROPOSAL : For this M2 project, we propose to pioneer the field the nucleolar protein quality control in plant. In particular, the potential role of the nucleolus will be investigated. We will make use of a model protein which is substrate of the pathway. This system is based on the metastable firefly luciferase fused to the GFP and targeted to the nucleus. The project will include the description and use of Arabidopsis thaliana lines stably expressing this protein and its variants. Furthermore, the fate of this model in various tissues and upon various stress conditions will be investigate. Live and fixed confocal microscopy analysis will be used to assess the localization of the sensor in these different conditions. Cellular biochemistry analysis will be engaged to measure the folding status of the reporter and its accumulation during stress. Finally, we will engage in quantitative mass spectrometry approaches to depict the nucleolar proteome in condition in various stress conditions. Beyond high-end mass spectrometry analysis this will also make use of novel cytometry tools. Thus, this project offers various approaches ranging from plant biology, and state-of-the-art proteomics to exquisite microscopy to address an innovative question of agronomical relevance.

METHODOLOGIES: The student will learn molecular biology and cellular biochemistry technics and plant biology aspects. The team employs various models including human cells and plant. Therefore, the student will also benefit from a very rich and stimulating scientific environment. Live and fixed confocal microscopy imaging will be used to localize the sensor protein in different stress conditions in combination with various markers of nuclear compartments. Cellular biochemistry will be used to characterize the status of the protein, such as its folding state and aggregation. Finally, to discover new protein quality control effectors the student will perform the isolation of intact nucleoli to decipher their proteomes using cutting-edge quantitative mass spectrometry. Therefore, the project offer a well-rounded methodological package and a rich environment to improve the learning experience. The team is located at the I2BC with easy access to state of the art facilities in particular for microscopes and mass spectrometers with available expert on site. The team is scientific support up of the PROTEOMIQUE-GIF mass spectrometry facility.

REFERENCES (maximum 5)

1. Battisti, D. S. & Naylor, R. L. Historical warnings of future food insecurity with unprecedented seasonal heat. Science (80-. ). 323, 240–244 (2009).

2. Balchin, D., Hayer-Hartl, M. & Hartl, F. U. In vivo aspects of protein folding and quality control. Science vol. 353 aac4354 (2016).

3. Frottin, F. et al. The nucleolus functions as a phase-separated protein quality control compartment. Science (80-. ). 365, 342–347 (2019).

4. Azkanaz, M. et al. Protein quality control in the nucleolus safeguards recovery of epigenetic regulators after heat shock. Elife 8, (2019).