Funded PhD project in Integrated Structural & Cell Biology in Grenoble, France

Title of the PhD project:
PhaSET: Phase Separation of EARLY FLOWERING 3 and Plant Thermoresponse

PhD supervisors:
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Host laboratory:
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Project summary:
Increased average temperatures due to global warming have already altered plant phenology for both wild and domesticated species. Plants are able to perceive temperature and subsequently reprogram their growth and development for optimal reproduction and survival under warmer climatic conditions. The conserved plant protein, EARLY FLOWERING 3 (ELF3), plays a central role in both the circadian regulated growth and environmental response signalling pathways. ELF3 acts as a scaffold for complex formation with transcription factors such as LUX ARRYTHMO, EARLY FLOWERING 4 and PHYTOCHROME INTERACTING FACTOR 4, all known to play roles in circadian regulated growth and temperature response. In Arabidopsis thaliana and other Brassicaceae, ELF3 possess a low complexity prion-like domain (PrD) containing poly-glutamine (polyQ) repetitions of varying lengths. The number of polyQs varies across the different accessions of Arabidopsis and within the Brassicacaea family, and modifies plant thermoresponse. Recent studies have demonstrated that the ELF3 PrD triggers liquid-liquid phase separation (LLPS) in vitro and in vivo in a pH and temperature-sensitive manner and LLPS is required for plant thermoresponsiveness. However, the molecular determinants of phase separation are poorly understood and the effects of polyQ variation on the structure and dynamics of the phase separated state are unknown. The proposed project will determine the structure and dynamics of ELF3 PrDs using a combination of biochemical assays, fluorescence microscopy and small angle X-ray scattering and correlate this with in planta function. The project will provide a biophysical and molecular understanding of how pH and temperature triggers phase separation of ELF3 and the effect on ELF3 activity. In the long term, these results will allow us to modulate ELF3 phase states for tuning of plant temperature response.

Required skills:
Experience in protein biochemistry (expression, purification) and molecular biology are required. Experience in small angle scattering techniques and/or plant biology are desired but not required.

Student role:
The student will perform site directed mutagenesis, protein expression, purification and characterisation using turbidity assays, fluorescence microscopy and FRAP. Structural characterisation will be performed using small angle x-ray and neutron scattering. The student will examine the phase separation properties of ELF3 in vitro as a function of temperature and pH. Rationally designed mutants with altered phase separation characteristics will be designed and tested in vitro and in planta.

Keywords:
Liquid-liquid phase separation, thermosensing, Arabidopsis thaliana, fluorescence microscopy, small angle X-ray scattering

Relevant publications of the team:

- Jung, J.-H.; Barbosa, A. D.; Hutin, S.; Kumita, J. R.; Gao, M.; Derwort, D.; Silva, C. S.; Lai, X.; Pierre, E.; Geng, F.; Kim, S.-B.; Baek, S.; Zubieta, C.; Jaeger, K. E.; Wigge, P. A. A Prion-like Domain in ELF3 Functions as a Thermosensor in Arabidopsis. Nature 2020, 585 (7824), 256–260. https://doi.org/10.1038/s41586-020-2644-7.
- Silva, C. S.; Nayak, A.; Lai, X.; Hutin, S.; Hugouvieux, V.; Jung, J.-H.; López-Vidriero, I.; Franco-Zorrilla, J. M.; Panigrahi, K. C. S.; Nanao, M. H.; Wigge, P. A.; Zubieta, C. Molecular Mechanisms of Evening Complex Activity in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (12), 6901–6909. https://doi.org/10.1073/pnas.1920972117.
- Ezer, D., J. H. Jung, H. Lan, S. Biswas, L. Gregoire, M. S. Box, V. Charoensawan, S. Cortijo, X. Lai, D. Stockle, C. Zubieta, K. E. Jaeger and P. A. Wigge (2017). The evening complex coordinates environmental and endogenous signals in Arabidopsis. Nat Plants 3: 17087.