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Title of the M2 research internship:

Structural characterization of LFY transcription factor

Project summary:

LEAFY (LFY) is a key transcription factor (TF), initiating the formation of the floral meristem and regulating the genes involved in the formation of floral organs. LFY contains 3 domains, a SAM N-terminal oligomerization domain, a disordered intermediate sequence and a C-terminal DNA binding domain. The protein binds DNA as a dimer but is also able to oligomerize on low-affinity DNA binding sites. To date, its functional and structural study has been limited to its DNA-binding domain and its oligomerization domain. The 3D crystallographic structure of the complete LFY protein remains a challenge due to its modular organization, the small quantities of protein produced, its instability and its heterogeneity. The aim of this internship is to obtain the structure of the complete LEAFY protein in complex with DNA by cryo-EM. The complete protein is difficult to purify on gel filtration but give a homogenous dimeric form on gel-shift assay providing a way to obtain a homogeneous protein/DNA for cryo-EM studies. To increase the success of the project, whole LFY proteins from different species will be tested. Mutants affected in the SAM oligomerization domain will also be tested to ensure that the protein does not polymerize but assembles into a stable dimer on DNA. The purification steps will include production of whole proteins (in bacterial or insect cell systems), affinity purification followed by migration of these complexes onto electrophoresis gels and then extraction. To compensate for the low concentrations of extracted proteins in the gel, we will be using new-generation grids. After optimization, the grids will be screened on the IBS cryo-EM platform. These results will enable us to determine the interactions between the three domains of LFY and their impact on the binding of the protein to DNA.

Keywords:
transcription Factor, structural biochemistry, cryo-EM

Relevant publications of the team:

-Sayou C, Nanao M, Jamin M, Pose D, Thévenon E, Grégoire L, Tichtinsky G, Denay G , Ott F, Peirats Llobet M, Schmid M, Dumas R & Parcy F (2016) A SAM oligomerization domain shapes the genomic binding landscape of the LEAFY transcription factor. Nat Commun. 7, 11222.

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-Rieu P, Turchi L, Thévenon E, Zarkadas E, Nanao M, Chahtane H, Tichtinsky G, Lucas J, Blanc-Mathieu R, Zubieta C, Schoehn G, Parcy F. (2023) The F-box protein UFO controls flower development by redirecting the master transcription factor LEAFY to new cis-elements. Nat Plants. 9(2), 315-329.

-Rieu P, Beretta VM, Caselli F, Thevenon E, Lucas J, Rizk M, Franchini E, Nanao M, Kater MM, Dumas R, Zubieta C, Parcy F & Gregis V (2024). The ALOG domain defines a new family of plant-specific transcription factors acting during Arabidopsis flower development. PNAS121, 10

-Zhang M, Jungblut A, Kunert F, Hauptmann L, Hoffmann T, Kolesnikova O, Metzner F, Moldt M, Weis F, DiMaio F, Hopfner KP, Eustermann S. (2023) Hexasome-INO80 complex reveals structural basis of noncanonical nucleosome remodeling. Science 381(6655).