UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2024-2025

Laboratory/Institute: CEA/IRIG/BGE
Director: Marie-Odile Fauvarque
Team: Genetics & Chemogenomics
Head of the team: Marie-Odile Fauvarque

Name and status of the scientist in charge of the project: Camille Sayou, CEA researcher
HDR: yes ☐ no x
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Program of the Master's degree in Biology:

☐ Microbiology, Infectious Diseases and Immunology ☐ Structural Biology of Pathogens
X Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology

Title of the project: Exploring the role of deubiquitinases in transcriptional regulation

Objectives

The general aim of the project is to identify deubiquitinases with a direct role in gene transcription using an original large-scale genetic screen. The specific goal of this M2 internship is to implement cutting-hedge molecular and cellular tools that will be necessary to perform the screen.

Abstract

Gene transcription is a point of integration for many stimuli, determining their normal or pathological development. Like most cellular functions, transcription is regulated by the ubiquitin system, which consists of the reversible addition of ubiquitin groups to proteins and can modulate their properties. This complex system is orchestrated by the antagonistic activities of more than 600 ubiquitin ligases and 100 deubiquitinases. Deubiquitinases are of particular interest as a source of therapeutic targets in cancer. Although some deubiquitinases have been shown to be involved in the transcriptional process, a systematic investigation is lacking.
In response to this gap, this project will contribute to set up a genetic screen. It will be divided into 2 parallel tasks: [A] Establishment of a versatile cell line for conditional depletion of deubiquitinases, based on a CRISPR/Cas strategy. Here, we will target one deubiquitinase candidate known to regulate transcription. [B] Implementation of a cell-based assay to measure live transcription. The incorporation of 5-ethynyl uridine into nascent transcripts will be monitored using high-throughput, high-content imaging. In future, the deubiquitinase depletion system and the transcription-monitoring assay will be combined and applied to other deubiquitinases in a large-scale screen.

Methods

Establishing a stable cell line involves molecular biology, genetic engineering and cellular biology. Cells will express an inactive Cas9 fused to a transcriptional repressor, and guide RNAs targeting a deubiquitinase. In addition, microscopy and image analysis methodologies will be applied to monitor nascent transcription.

Up to 3 relevant publications of the team

-Milligan L, Sayou C, Tuck A, Auchynnikava T, Reid JEA, Alexander R, De Lima Alves F, Allshire R, Spanos C, Rappsilber J, Beggs JD, Kudla G, Tollervey D. RNA polymerase II stalling at pre-mRNA splice sites is enforced by ubiquitination of the catalytic subunit. eLife (2017); 10.7554/ELIFE.27082.
-Franco G, Taillebourg E, Delfino E, Homolka D, Gueguen N, Brasset E, Pandey RR, Pillai RS, Fauvarque MO. The catalytic-dead Pcif1 regulates gene expression and fertility in Drosophila. RNA (2023) 10.1261/rna.079192.122.
-Thevenon D, Seffouh I, Pillet C, Crespo-Yanez X, Fauvarque MO, Taillebourg E. A Nucleolar Isoform of the Drosophila Ubiquitin Specific Protease dUSP36 Regulates MYC-Dependent Cell Growth. Front Cell Dev Biol. (2020); 10.3389/fcell.2020.00506.

Requested domains of expertise

Molecular and cellular biology skills are necessary. Cellular imaging skills would be a plus.