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Host laboratory:
Lab : BGE (https://www.bge-lab.fr/en)

Host group/team:
Genetics and Chemogenomics (Gen&Chem)

Title of the M2 research internship:
Investigating non-catalytic functions of an essential deubiquitinase

Project summary:
Background
Ubiquitination is a reversible post-translational modification that regulates protein stability and function through the addition of ubiquitin groups. This process is tightly controlled by the opposing actions of ubiquitin ligases and deubiquitinases (DUBs). Among these, DUBs are of particular interest as potential therapeutic targets due to their deregulation in certain cancers and their susceptibility to chemical inhibition. Our research group investigates DUBs in both physiological and pathological contexts using mammalian cell culture and *Drosophila* as model systems. We and others have demonstrated that the DUB USP36 is essential for cell growth in both human and fly. USP36 is primarily localised in the nucleolus, where it plays a crucial role in ribosome biogenesis by deubiquitinating multiple substrate proteins and facilitating nucleolar transcription.
Rationale and Hypothesis
Unpublished findings from our group have revealed an intriguing discrepancy: while USP36 knockout is lethal in *Drosophila*, inactivation of its catalytic activity results in only mild growth defects. This suggests that the essential function of USP36 in *Drosophila* relies predominantly on non-catalytic properties rather than its enzymatic activity. Given the evolutionary conservation of USP36, we hypothesise that a similar mechanism may exist in human cells.
Objectives and Experimental Approach
The goal of this internship is to assess whether human USP36 also possesses non-catalytic functions that contribute to its role in nucleolar homeostasis and cell growth. To achieve this, an inducible CRISPR-Cas9 system will be developed to deplete USP36 in human cells. A catalytically inactive USP36 variant will be expressed to assess its ability to rescue the effects of knockdown. The impact of USP36 depletion and mutant rescue will be evaluated at both cellular and molecular levels. Cell viability, proliferation, and nucleolar integrity will be assessed. Additionally, western blot and immunofluorescence assays will monitor the stabilisation of known USP36 substrate proteins, while nucleolar transcription will be measured using a 5-EU assay. This study will clarify whether USP36 function extends beyond its enzymatic activity, providing new insights into its role in nucleolar homeostasis and potential therapeutic relevance.

Keywords:
Ubiquitin system, deubiquitinases, cellular growth, transcription, molecular and cellular engineering

Relevant publications of the team:

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 Jun 19;8:506. doi: 10.3389/fcell.2020.00506. PMID: 32637412; PMCID: PMC7316882.

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Pandey RR, Delfino E, Homolka D, Roithova A, Chen KM, Li L, Franco G, Vågbø CB, Taillebourg E, Fauvarque MO, Pillai RS. The Mammalian Cap-Specific m6Am RNA Methyltransferase PCIF1 Regulates Transcript Levels in Mouse Tissues. Cell Rep. 2020 Aug 18;32(7):108038. doi: 10.1016/j.celrep.2020.108038. PMID: 32814042.