ENS – IISER Network / BIOSANTEXC Project

Internship Proposal Form

(Discipline/Field name): Cell Biology

Internship title: Nuclear pore dynamic during the circadian cycle

Keywords related with the subject (minimum 3): Nups, STED imaging, Circadian rhythm, mouse liver, Immunoblotting

Name of the laboratory at ENS: IGFL

Name of the internship supervisor(s): K. Padmanabhan and F. Marmigère

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Prerequisites for the internship:
Requested level: Master 1 or 2

Foreseen internship dates:
April to June 2025

Internship proposal:
Circadian activities control the physiology of almost all organisms on Earth. A feed-forward loop orchestrates the oscillatory activity of "core clock genes", a set of transcriptional activators and repressors that daily regulates fluctuations of about 15% of the mammalian transcriptome. This loop is ensured by a timely nucleocytoplasmic transport of macromolecules and involves daily reorganization of chromatin architecture. Nuclear Pore Complexes (NPCs) are large protein complexes formed by the assembly Nucleoporins (NUPs). They are the only gateway for macromolecules crossing the nuclear envelop. Here, we hypothesize that during the circadian cycle, NPCs are dynamic and that this dynamic tunes nucleocytoplasmic transport. Our preliminary results show that in mouse hepatocytes, the diameter of the pore and NUPs protein levels oscillate over a 24 hour period. The objective of the project is to investigate whether these fluctuations result in a change in NPCs composition and/or density. To address this question, the internship will use a full collection of purified mouse hepatocytes already available in the lab to perform immunochemistry for different candidate NUPs, and quantify by STED imaging NPCs density and composition during the circadian cycle. In addition, he/she will participate to the analysis of a proteomic dataset generated by the lab on a time-course series of hepatocytes nuclear envelop to evaluate circadian changes in this compartment. He/she will validate by immunoblotting and/or STED microscopy the candidates identified in this screen. Finally, using CRISPR/Cas 9 gene editing, he/she will start to build HepG2 cell-lines to deplete specific Nups using the auxin-mediated degron approach and further investigate the effect of depletion on NPC density and composition by microscopy.