Laboratoire de Physico-Chimie Curie,
UMR168 - 11 rue Pierre et Marie Curie - 75 231 PARIS CEDEX 05

M2 internship proposal

High-throughput single-molecule FISH for quantitative measurements of transcriptional kinetics in developing *C. elegans* larvae

Internship project description

Gene expression is known to be highly dynamic, often stochastically fluctuating between periods of quiescence and periods of pronounced transcriptionally activity (Tunnacliffe et al. 2020). To fully understand how patterns of gene expression are established and maintained during development, for instance, studies of *in-vivo* transcription kinetics are required.

Our teams has recently developed technologies that allow us to visualize and analyze transcription dynamics of microRNA (miRNA) genes in developing *C. elegans* larva in real-time using the so-called MS2-MCP-GFP tethering system (Kinney & Sahu et al. 2023).

The goal of this internship is to **combine** of **live-imaging and high-throughput single-molecule fluorescent in situ hybridization** (FISH; smFISH) in developing *C. elegans* larvae to perform quantitative measurements of transcriptional dynamics of miRNAs. We will achieve this using a microfluidic device that allows trapping and imaging up to 100 larvae simultaneously (Figure 1A). Once the device has been tested and optimized, the student will characterize gene expression using smFISH across several developmental timepoints. We are particularly interested in precise estimates of the number of actively transcribing RNA pol II molecules at the loci of miRNAs, as well as the total number of miRNAs produced

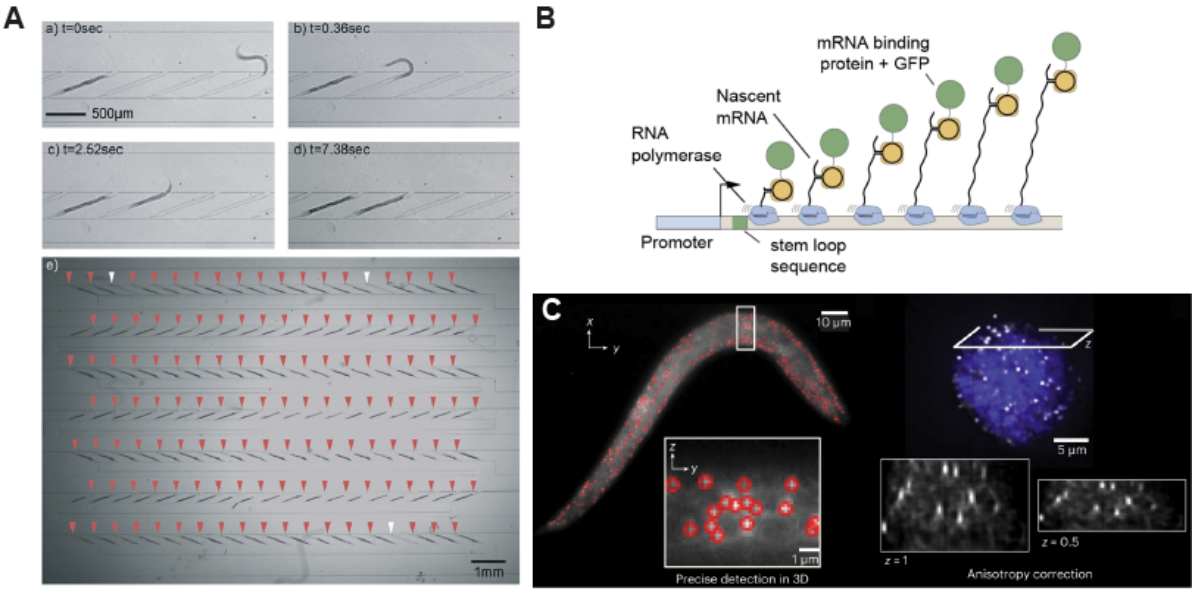

Figure 1: (A) Adult *C. elegans* roundworms can be trapped in a microfluidics chip for immobilization and fixation. High-throughput is achieved by positioning trap channels in series along a large channel in the microfluidics device. **(B)** Schematic illustration of the MS2-MCP-GFP system. Transcriptional kinetics can be visualized in real-time by tagging nascent RNAs with GFP proteins. **(C)** Imaging of single mRNA molecules labelled with smFISH. mRNA molecules can be counted all across the animal body, allowing to quantify transcriptional parameters for the gene in question.

during each transcriptional episode. Once the analysis is performed for wild-type animals, we will analyze the impact of specific mutations in regulatory sequences upstream of miRNA genes.

miRNA dysregulation influences critical molecular pathways involved in tumor progression, invasion, angiogenesis and metastasis in a wide range of cancer types. The quantitative analyses proposed in this internship are an important step towards understanding their regulation in normal development and pathology.

Requirements

Candidates for this internship should have a strong motivation for interdisciplinary research at the interface between bioengineering, microfluidics and developmental biology. Experience in live-microscopy, immunostaining or microfluidics with biological systems is a plus. Most importantly, the student should demonstrate a passion to see and study life in action. For details, please contact wolfgang.keil@curie.fr.

Environment

The Keil lab at Institut uses an interdisciplinary strategy to study transcriptional regulation, cell-fate patterning and morphogenesis in the model organism *C. elegans*. We are particularly interested in how developing system achieve robustness and precision in the face of environmental variability and molecular noise. To tackle this question, we develop and apply novel techniques for obtaining quantitative high-resolution dynamic gene expression data. We also develop theoretical approaches to conceptualise mechanisms of development and uncover general principles of developmental patterning. Check out https://curie.fr/equipe/keil for more information and feel free to contact us directly.

The Research Center at Institut Curie is a major player in the field of cancer research. It comprises a Hospital and a Research Center with over 1000 employees covering a wide range of nationalities. The Curie Institute Research Center aims to develop opened-ended science to progress in knowledge while exploiting arising possibilities to improve cancer care by stimulating synergies between research, training and innovation to support patients and serve our society.

Time frame

Start Date: January 2024
Duration: 6 months

Project references

smFISH in chips: a microfluidic-based pipeline to quantify *in situ* gene expression in whole organisms, Lab Chip (2020) 10.1039/C4LC00789A

What Is a Transcriptional Burst? Trends in Genetics (2020)
https://doi.org/ 10.1016/j.tig.2020.01.003

Team references

Circadian rhythm orthologs drive pulses of heterochronic miRNA transcription in *C. elegans*. Developmental Cell (2023); https://doi.org/10.1016/j.devcel.2023.08.006

An Epigenetic Priming Mechanism Mediated by Nutrient Sensing Regulates Transcriptional Output during C. elegans Development. Current Biology (2021) https://doi.org/10.1016/j.cub.2020.11.060

HLH-2/E2A Expression Links Stochastic and Deterministic Elements of a Cell Fate Decision during C. elegans Gonadogenesis. Current Biology (2019)
https://doi.org/10.1016/j.cub.2019.07.062

Long-Term High-Resolution Imaging of Developing *C. elegans* Larvae with Microfluidics. Developmental Cell (2017)
https://doi.org/10.1016/j.devcel.2016.11.022