Group name: ***Molecular mechanisms of neuronal identity and brain repair***
IP name: Eduardo Leyva-Díaz
Group web: https://orcid.org/0000-0001-6750-9168

Title of the MRP/TFM: Programming and reprogramming neuronal identity via CUT factors

Summary of the Project:

The complexity of the nervous system relies fundamentally on the great diversity of its basic units, the neurons. The identity of specific cell types in the nervous system is defined early in development by unique transcriptional programs that are actively maintained throughout an organism's life. Changes in neuronal identity, such as gene expression or epigenetic modifications, are crucial for the plasticity of the nervous system. These changes can modify synaptic connectivity and neuronal function, which in turn can contribute to nervous system adaptation and the ability to learn and remember information. Defects in the acquisition and maintenance of neuronal identity can result in severe neurodegenerative and neuropsychiatric conditions, including Alzheimer's disease and Parkinson's disease. Therefore, elucidating the molecular mechanisms controlling neuronal identity is essential for understanding how to maintain a healthy and functional nervous system.

In a previous work we have found that CUT homeobox genes are required for pan-neuronal gene expression and neuronal function (Leyva-Díaz and Hobert, 2022). This project aims to decipher the mechanisms through which CUT factors control neuronal identity, and apply this knowledge for brain repair. This TFM project will expose the student to several techniques in the field of molecular and cellular neurobiology as well as a strong component on genetics through the use of the animal model *C. elegans*. The specific aims are:

1. Define transcription factor networks controlling CUT gene expression.

Experimental Approach: To understand how CUT transcription factor expression is regulated and maintained from embryos to adulthood, we will analyze CUT gene reporter expression in different mutant backgrounds. CRISPR reporters for *C. elegans* CUT genes will be analyzed at different developmental stages in different groups of mutants: i) Proneural bHLH factors; ii) CUT factors (autoregulation); iii) Terminal selector transcription factors. Future experiments will include chromatin accessibility profiling (via ATAC-seq) and evaluation of transcription factor targets (via ChIP-seq) on CUT mutant animals.

2. Direct astrocyte to neuron reprogramming using CUT transcription factors.

Experimental Approach: By using different combinations of CUT and neuron type specific master regulators, we will reprogram endogenous mouse astrocytes into specific neurons *in vitro*. To directly reprogram mouse astrocytes, we will infect astrocytes with a cocktail of retroviral vectors encoding selected candidate factors. Future experiments will include transcriptional profiling of the induced neurons as well as *in vivo* reprogramming.

Methods and technology involved in the MRP/TFM Project:

Aim 1: *C. elegans* genetics and maintenance, image acquisition and analysis, generation of reporter alleles through CRISPR/Cas9 technology.

Aim 2: preparation of mouse astrocyte primary cultures, cell transfection/transduction, immunocytochemistry, image acquisition and analysis.

Member/s of the lab who will act as tutor/co-tutor of the project (if different from the group IP):

Contact: eleyva@umh.es