Proposal of Master Research Project / Proyecto fin de Master for the academic year 2023-24

Group name: Mecanismos transcripcionales y epigenéticos de la plasticidad neuronal
IP name: Angel L. Barco Guerrero
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Title of the MRP/TFM: KAT3-mediated neuronal identity modulation in neuronal primary cultures using nanobody-based auxin-degron system

Summary of the Project:

During development, neuronal cells acquired their specific cell identity by progressively refining the set of transcriptional programs that will be necessary for their particular function. This process is orchestrated by a complex chromatin remodeling thanks to the regulated expression of identity determinant transcription factors and epigenetic regulators. While still poorly understood, it is known that this identity must be actively maintained throughout all their post-mitotic lives, thanks in part to the same transcription factors that participated in the latest states of its acquisition and to the action of particular chromatin remodelers. In this sense, our laboratory recently demonstrated how the lysine acetyltransferase type 3 (KAT3) proteins CBP and p300 play a critical role in the maintenance of neuronal identity in the adult mouse brain by specifically depleting them in glutamatergic neurons of the forebrain thanks to an inducible double-floxed knockout animal (Lipinski et al., *Nat Comm*, 2020). In this TFM, the student will investigate if this process could be reversed by developing new genetic tools based on the auxin-degron and nanobodies systems, generating a tool that might allow for neuronal identity switch with interesting potential applications. The master student will participate in the design and cloning of the necessary elements to develop this tool, as well as the amplification and purification of their coding DNA and their transduction into hippocampal neuronal primary cultures. Moreover, the master student will also conduct the analysis of the effects that these manipulations have at physiological level in neuronal cells by conducting RNA expression analysis together with inmunocytological assessment of key neuronal markers that could be affected by this process. Our general objective is to better understand the mechanisms regulating neuronal identity maintenance at epigenetic level and to develop new biomedical tools based on the genetic manipulation of KAT3 proteins.

Methods and technology involved in the MRP/TFM Project:

DNA cloning and amplification, hippocampal neuronal cultures, magnetofection and viral transduction of primary neuronal cultures, RNA extraction, quantitative PCR, immunofluorescence, confocal microscopy.
Depending on the results obtained additional molecular biology techniques will be carried out such as CUT&TAG to assess the genome coverage changes of different histone modifications.

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

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