Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Identifying new regulators of LINE-1 activity using CRISPR-based genetic screens

Synopsis:

Long interspersed nuclear element-1 (LINE-1 or L1) are considered as the most successful and abundant retrotransposon family, representing 17% and 21% of the human and mouse genome respectively. Despite their abundance and recent progress, we are still lacking a full picture of the regulatory mechanisms governing LINE-1 activity and their impact on genome function are not fully elucidated. This is particularly evident concerning younger elements that still have the potential to be transcriptionally active and may not be yet fully neutralized by their host. This project aims to dissect the molecular mechanisms controlling LINE-1 regulation in the mouse genome, with a particular focus on the youngest L1MdTf and L1MdA active subfamilies. For this, we plan to use cutting-edge CRISPR-based genetic screening approaches to identify novel trans-acting regulators modulating LINE-1 expression in diverse cellular contexts.

For this Master proposal, we will generate reporter cell lines harboring endogenous L1MdTf/L1MdA elements fused to a fluorescent marker. We will then conduct CRISPR-mediated knockout screens in mouse embryonic stem cells (ESCs) and neural progenitor cells (NPCs) derived from ESCs, to uncover lineage-specific regulators of LINE-1 activity. Through this approach, we aim to elucidate the temporal and lineage-specific dynamics of LINE-1 regulation in ESCs and NPCs, shedding light on the interplay between LINE-1 elements and host factors during development and differentiation. Furthermore, we will use the same screening approaches to explore the differential regulation of LINE-1 elements on the active and inactive X chromosomes in female cells, which is potentially influenced by the distinct chromatin landscape and 3D architecture of the active versus inactive X. With this strategy, our objective is to uncover trans-acting factors governing LINE-1 activity across diverse chromatin environments.

The outcomes of this research proposal are expected to deepen our understanding of LINE-1 biology and its contributions to gene expression networks, epigenetic regulation and cellular function.

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