INVESTIGATING CRITICAL PATHWAYS THAT ACT ALONGSIDE HISTONE INHERITANCE TO MAINTAIN EPIGENETIC CELL IDENTITY

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Inheritance of cell identity depends on accurate propagation of epigenetic information during DNA replication. Parental histone recycling is a key mechanism underlying this process and is mediated in part by the replicative helicase subunit Mcm2. Disruption of symmetric parental histone recycling using a separation-of-function allele (*Mcm2-2A*) alters chromatin inheritance and impairs cell identity transitions in mouse embryonic stem cells (mESCs) [1], underscoring its importance in maintaining epigenetic cell identity. In addition to histone recycling, faithful epigenetic inheritance also relies on coordinated activity of DNA methylation and an array of chromatin-maturation pathways [2]. However, it is unclear whether all pathways that contribute to stable cell identity have been identified, and what their relative importance is in the contexts of cell division and differentiation.

To investigate this, this project aims to demonstrate and characterize synthetic genetic interactors of *Mcm2-2A* in self-renewing and differentiating mouse embryonic *Mcm2-2A* cell lines were generated of several gene candidates previously identified in a genome-wide CRISPR screen. A competition-based flow cytometry assay was established and confirmed to quantify cell fitness over time. This set up allowed relative mESC fitness to be assayed both in self-renewing conditions by cell culture in serum/LiF medium, and upon cell identity change by differentiation into Retinoic Acid (RA) medium.

Using this approach, a top-hit gene was validated as a genetic interactor of Mcm2-2A: surprisingly, knockout of this gene in wildtype cells conferred a fitness advantage in self-renewing conditions that was abrogated in the Mcm2-2A background. During differentiation in RA, the effects of these mutations became deleterious and are being further studied using complementary live-cell imaging, to temporally resolve synthetic lethal interactions during cell identity change. Ongoing experiments extend this strategy to additional candidate genes, including those with unannotated function or unestablished link to chromatin inheritance, as well as known players.

Through this framework, this project seeks to clarify how multiple epigenetic maintenance pathways cooperate with histone inheritance to appropriately preserve or alter cell identity. This may provide insight into diseases associated with epigenetic instability and cell identity aberrations, including cancer, and indicate vulnerabilities that could be therapeutically exploited.

Keywords: epigenetic inheritance, histone recycling, CRISPR screen, synthetic genetic interactions

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