Regulation of Nuclear Gene Positioning during Muscle Differentiation

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OVERVIEW

Nuclear lamins are major components of the lamina, a filamentous protein meshwork underlying the inner nuclear membrane of metazoan cells. The nuclear lamina is fundamental to maintaining chromatin architecture in the nucleus by providing anchorage sites for heterochromatic genomic regions, termed lamina-associated domains (LADs). Lamins, categorized into A- and B-type based on their biochemical properties, also interact with numerous nuclear envelope transmembrane proteins (NETs). These can, in turn, establish interactions with the genome and, together with lamins, contribute to nuclear chromatin organization. Apart from LADs, specific genes associate with the nuclear lamina in a more dynamic manner, a process that is essential for the coordinated temporal regulation of gene expression during development and differentiation. Whereas several studies have focused on understanding the establishment and anchorage of heterochromatic LADs to the lamina, the dynamic attachment of specific genes to the nuclear periphery remains poorly understood. Here, we aim to elucidate the detailed molecular mechanisms involved in nuclear positioning of specific genes during myogenic differentiation.

We developed a reporter system to easily track the localization of selected myogenic genes that have been shown to selectively undergo gain or loss of nuclear envelope association during muscle differentiation. Specifically, we inserted a short array of Lac repeats downstream of these genes using CRISPR/Cas9, to enable their microscopic visualization when bound by the Lac repressor fused to GFP. Systematic knock-out of several muscle specific nuclear envelope proteins, followed by evaluation of the peripheral positioning of the tagged loci, revealed certain NETs that contribute to peripheral tethering. Following the identification of the relevant tethers at the nuclear periphery, we plan to use the reporter cell line to gain more mechanistic insights into the regulation of the release of or binding to the nuclear envelope during muscle differentiation.

1. Working Hypothesis

2. Aim of the Project

To investigate the mechanisms involved in the regulation of nuclear positioning and expression of myogenic genes during differentiation.

3. Reporter cell system to monitor and analyze nuclear position of loci during differentiation

4. Pax7 localizes more centrally in proliferating cells and MyoD moves to the nuclear interior during differentiation

5. LA/C and LBR depletion affects heterochromatin organization and possibly positioning of MyoD locus

6. MyoD locus attachment to periphery is lost upon combined deletion of muscle specific NETs

FUTURE PROSPECTS

Determine factors and pathways contributing to the gain and loss of NE attachment

(A) Distribution of MyoD locus position in knock out cell lines displayed as violin plots of normalized radial distance to the nuclear periphery. Dashed line represents separate experiments. (B) Cells from LAC KO and LBR double knock-out lines display altered heterochromatin organization. Cells were stained for immuno-fluorescence using antibodies against H3K9me2, H3K9me3 and Hoechst to stain DNA. Representative z-stacks are shown. (C) Loss of peripheral attachment of LADs in double knock-out cell lines. Lamin B1 -CHAF of two LADs found in the same region as the MyoD and Pax7 genes, one LAD in chromosome II and an Ig control experiment. Data are expressed as percentage of the input values obtained from the experiment.

(B) Distribution of MyoD locus position in proliferating myoblasts. p value <0.0001 Right: distribution of MyoD gene in proliferating and differentiating muscle cells, p value <0.0001 (D) Distribution of MyoD locus position in proliferating myoblasts. p value <0.0001 Right: distribution of MyoD gene in proliferating and differentiating muscle cells, p value <0.0001

(C) Density curves of data shown in B.}

(D) Percentage of cells per group expressing the MyoD locus in proliferating myoblasts. p values are indicated.

(E) Flowchart of image acquisition and evaluation workflow.

(F) Calculation of shortest distance to peripheral and interior lamina points.

(G) Image segmentation and identification of nuclear periphery.

(H) Calculation of shortest distance to peripheral and interior lamina points.

(I) Heat map showing the combined results of the distance to the nuclear periphery.

(J) Heat map showing the combined results of the distance to the nuclear periphery.

(K) Heat map showing the combined results of the distance to the nuclear periphery.

(L) Heat map showing the combined results of the distance to the nuclear periphery.

(M) Heat map showing the combined results of the distance to the nuclear periphery.

(N) Heat map showing the combined results of the distance to the nuclear periphery.

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(V) Heat map showing the combined results of the distance to the nuclear periphery.

(W) Heat map showing the combined results of the distance to the nuclear periphery.

(X) Heat map showing the combined results of the distance to the nuclear periphery.

(Y) Heat map showing the combined results of the distance to the nuclear periphery.

(Z) Heat map showing the combined results of the distance to the nuclear periphery.