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Coupling between chromosome intermingling and gene regulation during cellular differentiation

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ABSTRACT

In this article, we summarize current findings for the emergence of biophysical properties such as nuclear stiffness, chromatin compaction, chromosome positioning, and chromosome intermingling during stem cell differentiation, which eventually correlated with the changes of gene expression profiles during cellular differentiation. An overview is first provided to link stem cell differentiation with alterations in nuclear architecture, chromatin compaction, along with nuclear and chromatin dynamics. Further, we highlight the recent biophysical and molecular approaches, imaging methods and computational developments in characterizing transcription-related chromosome organization especially chromosome intermingling and nano-scale chromosomal contacts. Finally, the article ends with an outlook towards the emergence of a functional roadmap in setting up chromosome positioning and intermingling in a cell type specific manner during cellular differentiation.

Contents

1. Introduction

Cellular differentiation and developmental processes are largely coupled with alterations in nuclear architecture, global changes in chromatin compaction, structural reorganization within the nucleus, which together impinges on gene expression profiles [1–4]. Embryonic stem (ES) cells have a low cytoplasmic-to-nuclear volume ratio, round and floppy nuclei [5], as well as highly dynamic chromatin [6,7]. Differentiation of ES cells into various lineages results in stiffer nuclei [8,9], condensed chromatin, cell-type specific epigenetic modifications, genome reorganization [10,11] and hence altered gene expression profiles. Recent developments in the field of genome regulation have provided early hints towards an emergence of cell type specific chromosome architecture during stem cell differentiation [11–13]. Such 3D organization of chromosome territory has been postulated to control cell-type specific gene expression and also to serve as a modular code for efficient transcriptional output.

Visualization of chromosome territories and systematic analysis of their spatial configurations are achieved by techniques such as

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fluorescence *in situ* hybridization (FISH), chromosome conformation capture (3C)-based techniques combined with various ChIP-seq approaches, as well as theoretical models [14–19]. Chromosome painting together with confocal microscopy or Structured Illumination Microscopy (SIM) has revealed the non-random three-dimensional chromosome architecture and the transcriptionally active property of chromosome surfaces [20–23]. Genome-wide 3C analysis (Hi-C) in combination with ChIP-seq and mathematical modeling, and genome-wide Chromatin Interaction Analysis with Paired-End-Tag sequencing (ChIA-PET) has uncovered intra-/ inter-chromosomal domains associated with active transcription markers and the architectural protein CCCTC-binding factor CTCF based on a large population of cells [24–27].

To interrogate chromatin architecture at a single-cell and higher-resolution level, more advanced imaging techniques have been developed. One powerful method that has been widely adopted is 'single-molecule localization microscopy'. This imaging technique involves repeated acquisition of sparse and stochastic subsets of fluorophores from a sample. The location of each fluorophore is retrieved by finding the center of their point spread function, and this location information is used to construct a super-resolution image. Using the single-molecule localization microscopy, researchers have successfully revealed the filamentous components of ~70 nm within Drosophila metaphase chromosomes [28]. In addition, such superresolution microscopy is also capable of resolving structural differences amongst active, inactive, and repressed chromatin [29]. Moreover, in a system of open chromatin spreads that bypasses the difficulties arising from the crowded environment of nucleus [30], the single-molecule localization microscopy is able to directly visualize functional chromosomal contacts formed by multiple genes, which are required for the co-expression of specific gene clusters [31].

In this article, we first summarize the current knowledge in the field of stem cell differentiation regarding alterations in nuclear architecture, chromatin compaction, along with nuclear and chromatin dynamics. Further, we highlight the recent biophysical and molecular approaches, imaging methods and computational developments in characterizing transcription-related chromosome organization especially chromosome intermingling. We summarize the current findings in the field of genome regulation during ES cell differentiation and provide future directions on understanding the molecular mechanisms underlying the differentiation-related chromosome reorganization.

2. Morphological and mechanical changes of nuclear architecture during differentiation

The process of ES cell differentiation towards fibroblast lineage is marked with decrease in nuclear height and significant increase in the volume, as revealed by simple nuclear labeling and morphological analysis [5] (Fig. 1A). In addition, live-cell confocal tracking of the nuclear lamina in ES cells shows larger nuclear area fluctuations as compared to differentiated fibroblast cells [5,32] (Fig. 1B). These results suggest that the nuclei of stem cells are more fluidic, which has further been confirmed using micropipette aspiration and scaffold stretching techniques. The micropipette aspiration and mechanical stretching techniques have been widely used to probe the mechanical property of cell nuclei, and the results exhibit that nuclei in ES cells and in Mesenchymal stem cells (MSCs) are highly deformable and stiffen more than 5-fold after differentiation [8,9].

The increase in nuclear stiffness is often accompanied with chromatin compaction during the differentiation of ES cells. Degrees of chromatin compaction have been quantitatively measured using various imaging-based techniques such as Fluorescence Anisotropy Imaging (FAI) [5,33,34] and Fluorescence Recovery after Photobleaching (FRAP) [35]. Since fluorescence anisotropy measures the rotational mobility of fluorophores, FAI of cells with a stably integrated H2B-EGFP fusion protein outputs the rotational mobility of H2B and hence of the local chromatin compaction status. Fluorophores with lower rotational mobility in more compacted chromatin have larger anisotropy values and are color-coded towards red; whereas in less compacted chromatin, fluorophores have higher rotational mobility, smaller anisotropy values and are color-coded towards blue [5]. The color-coded anisotropic maps of stem cells and differentiated cells reveals larger heterogeneity of chromatin compaction in stem cells compared to differentiated cells (Fig. 1C), indicating the synchronization of chromatin compaction status during cell differentiation [5]. FRAP analysis of core histones and other chromatin architectural proteins like HP1α, which measures the exchange rates of these chromatin-associated proteins and thus the compaction of chromatin, shows that faster recovery dominated in ES cells whereas slower recovery is more often observed in fibroblast cells [6,32]. These results suggest that as nuclear stiffening occurs during cell differentiation, the local organizations of chromatin within the nuclei are more stabilized and synchronized.

The nuclear stiffening during onset of differentiation has largely been attributed to the upregulation of a nuclear structural protein Lamin A/C that forms a protein meshwork underneath inner nuclear membrane [36,37]. ES cells have reduced levels of Lamin A/C [36], which increases during differentiation [38]. Overexpression of Lamin A/C in ES cells significantly reduces the nuclear area fluctuations [5], whereas Lamin A/C knock down studies in epithelial cells shows similar deformability as that of ES cells [8]. The nuclear shape change during differentiation is, on the other hand, influenced by the emergence of cytoskeletal network in the cytoplasm of differentiated cells [5,39,40]. Cell differentiation is concomitant with actin stress fiber formation and myosin phosphorylation, which together constitute acto-myosin contractile apparatus in cells (Fig. 1D) [5]. These acto-myosin filaments, in the form of apical stress fibers, apply significant load on nucleus and hence affect nuclear morphology and dynamics [10,41,42]. Depolymerization of these stress fibers in fibroblasts transits the fluctuation of nuclear area back to the stem cell-like state [41]. These results together point towards the role of Lamin A/C and cytoskeleton on nuclear stiffening and morphological alterations during cell differentiation, which further reorganizes transcription-related chromatin architecture. Importantly, such changes in the prestress experienced by the nucleus, the nuclear shape and heterogeneity, as well as the chromatin dynamics have also been observed during drosophila embryogenesis [10,32,43]. In the next section, we describe quantitative investigations of three-dimensional chromosome positions at different stages of differentiation.

3. Radial positioning of chromosomes in ES cells and fibroblasts

Eukaryotic DNA is packaged into chromatin fibers with different levels of organization, and are further compartmentalized into distinct chromosome territories in interphase nuclei [44–46]. The fundamental unit of chromatin fibers is nucleosome, consisting of ~150 base pairs of DNA wrapped 1.6 times around an octamer of core histones (H2A, H2B, H3 and H4) and sealed with a single linker histone (H1) [47–51]. The nucleosome chains interact with non-histone proteins and condense into coiled-coil higher order structures [49,52,53]. These higher order structures further get remodeled to form compact heterochromatin (transcriptionally silent) and loosely wound euchromatin (transcriptionally active),