Leading Edge
Minireview

Cell

Spinning the Web of Cell Fate

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Spatiotemporal changes in nuclear lamina composition underlie cell-type-specific chromatin organization and cell fate, suggesting that the lamina forms a dynamic framework critical for genome function, cellular identity, and developmental potential.

Introduction

The incredible complexity and plasticity of eukaryotic genome organization underlies the transformational ability of stem cells to become an array of diverse tissues and differentiated cell types. Interphase chromosomes are spatially arranged into dynamic structures and subcompartments that significantly influence gene activity. The nuclear lamina (NL), for example, preferentially interacts with transcriptionally silent chromatin characterized by low gene density and the absence of active histone modifications. Lamina-associated chromatin domains (LADs) are sharply defined and vary between cell types, suggesting interactions between chromatin and the NL are actively established and dynamically modified during cellular differentiation and development. Nevertheless, to what degree this nonchromatin nuclear structure actively participates in gene regulation and differentiation remains an active area of research. Recent studies by Clowney et al. (2012), Kohwi et al. (2013), and Solovei et al. (2013) provide evidence that spatiotemporal differences in lamina composition and genome architecture underlie developmental competence and differentiation, suggesting the nuclear lamina is directly involved in spinning the web of cell fate.

Chromatin at the Nuclear Lamina

The nuclear lamina is a thin proteinacious layer of highly conserved intermediate filament proteins, called lamins, which lie at the interface between interphase chromatin and the inner nuclear membrane. Lamins maintain the mechanical integrity and shape of the nucleus and serve as a platform for chromatin organization and gene regulation. Lamins are encoded by three genes in mammals and categorized as either A type (lamin A/C), or B type (lamins B1 and B2). Whereas B type lamins are expressed in essentially all mammalian cell types, A type lamins appear only in a subset of differentiated cell types and at low levels in embryonic stem cells (ESCs) (Eckersley-Maslin et al., 2013).The requirement of A type and/or B type lamins for appropriate nuclear architecture is often cell type specific, suggesting the function of lamins can be differentially utilized in a cell-type- and tissue-specific manner. The flexibility in lamin dependency may also be contingent on the presence of lamin-associated proteins, such as the Lamin-B Receptor (LBR), a nuclear envelope protein that can also anchor heterochromatin to the nuclear periphery (Solovei et al., 2013). Nevertheless, B type lamins are essential for tissue differentiation and organ development, and mutations in A type lamins and lamin-associated proteins cause a wide range of human diseases referred to as laminopathies.

Nuclear Periphery and Gene Repression

In most cell types, the nuclear periphery is associated with transcriptionally silent and late replicating chromatin, a feature that appears to be conserved from yeast to humans. Movement of genes to the nuclear periphery often coincides with gene repression, yet artificial tethering experiments suggest that perinuclear localization is sufficient for downregulation of some, but not all, genes (Burke and Stewart, 2013). The mechanisms responsible for perinuclear gene silencing and the role of lamins remain poorly defined. However, mapping of interactions between chromatin and lamins in vivo using a microarray-based approach indicates that the NL associates with large, sharply defined domains characterized by low gene expression levels (Guelen et al., 2008; Pickersgill et al., 2006). LADs identified in both *Drosophila melanogaster* and human fibroblasts contain widely spaced, coordinately expressed gene clusters, confirming earlier microscopy-based evidence that the nuclear periphery preferentially interacts with gene-poor regions. LADs are also partially enriched for repressive H3K9 and H3K27 methylation, and recent genetic screens in *Caenorhabditis elegans* demonstrate that enzymes involved in H3K9 methylation are essential for sequestering heterochromatin at the nuclear periphery (Towbin et al., 2012). Whether the formation of heterochromatin itself is sufficient to drive perinuclear anchoring is unknown. However, many genes are devoid of repressive histone modifications in human LADs (Guelen et al., 2008), suggesting that mammalian chromatin-lamina interactions are not solely dependent on H3K9 methylation. LAD organization also requires the transcriptional repressor HDAC3, a histone deacetylase targeted to the nuclear periphery by lamin-associated protein Emerin (Demmerle et al., 2012; Zullo et al., 2012), suggesting the removal and absence of active histone marks are the defining features of peripheral localization.

Understanding the mechanisms by which LADs are established and the molecular link between perinuclear localization and heterochromatin remains a priority for future research. Nevertheless, mapping of LADs in both *Drosophila* and humans provides preliminary evidence that chromatin insulators, which correlate with physical domain borders and mediate long-range interactions, are involved in peripheral compartmentalization.

For example, insulator protein CTCF delineates sharply defined lamina domain borders in human fibroblasts and mouse embryonic stem cells (Guelen et al., 2008; Handoko et al., 2011) and is essential for perinuclear positioning of the cystic fibrosis-relevant *CFTR* gene (Muck et al., 2012). Functional analysis of LAD-derived DNA sequences in murine fibroblasts further reveals an enrichment for GAGA sequences, which are bound by a transcriptional repressor, cKrox, in complex with HDAC3 and lamina-interacting protein Lap2β (Zullo et al., 2012). cKrox mediates chromatin-lamina interactions in a cell-type-specific manner, suggesting LADs may be developmentally regulated by differential recruitment of cKrox and other factors. In addition to insulator proteins, a subset of lamina-associated domain borders are enriched for H3K4me3 in the absence of CTCF (Zullo et al., 2012) and delineated by promoters oriented away from LADs (Guelen et al., 2008), suggesting genome-NL domain organization is likely specified by a complex combination of nuclear factors.

Genome-wide mapping studies of chromatin-lamina interactions cannot discriminate between perinuclear associations and interactions that occur within the nucleoplasm, which are also involved in cell proliferation and differentiation (Burke and Stewart, 2013). Interactions between chromatin and nuclear pore proteins, which are often associated with gene activation, similarly take place both in and away from the nuclear periphery, suggesting dynamic movement of perinuclear lamins and pore proteins is an important process in gene regulation. Targeting of lamins to the nucleoplasm appears cell type specific and depends on the expression of different lamin-interacting proteins, yet the dynamics of chromatin-lamina interactions in the nucleoplasm remain ill-defined.

Genome-NL Dynamics through Differentiation and Disease

Gene expression patterns underlying cellular identity must be reprogrammed in order for pluripotent stem cells to give rise to a complex system of tissues and differentiated cell types, a feat accomplished collectively by transcription factors, chromatin, and DNA modifications, and by 3D rearrangement of chromatin organization. A series of genome-wide mapping experiments carried out in mouse ESCs, sequentially derived neural precursor cells (NPCs), and differentiated astrocytes (ACs) reveal how genome-NL interactions are reorganized during lineage commitment and terminal differentiation, (Peric-Hupkes et al., 2010). LADs are surprisingly congruent across cell types, with overlap ranging between 73%–87%. In a follow-up study, cell-type-independent LADs are shown to be highly conserved between mouse and humans ESCs and characterized by high A/T content (Meuleman et al., 2013), suggesting constitutive LADs are specified by interactions between A/T sequence elements and the nuclear lamina. Cell-type invariant NL-interacting sequences are also A/T rich in ESCs, further suggesting that conserved LADs represent an inherited backbone structure for peripheral chromatin contacts. Nevertheless, localized, cell-type-specific differences in chromatin interactions indicate that some degree of LAD reorganization occurs concomitant to differentiation (Peric-Hupkes et al., 2010). Reorganization of NL interactions from ESC→NPCs→ACs is largely cumulative, i.e., gene relocation during lineage commitment is maintained during subsequent cell-type transitions. Genes that undergo repositioning are substantially different across differentiation lineages and important for cellular identity, suggesting genome-NL dynamics reflect a progressive, lineage-specific process in which factors important for maintaining pluripotency or involved in cell fate decisions are regulated by "locking," or unlocking, genes at the nuclear periphery.

Kohwi et al. (2013) provide supporting evidence that lamins indeed contribute to cell fate decisions through gene repositioning and repression at the nuclear periphery. In *Drosophila* embryonic neuroblasts, progenitor competence is lost over time, wherein sequential expression of temporal identity genes determines the cell fate of neuronal progeny. The first transcription factor expressed, Hunchback (Hb), specifies early-born U1/U2 neuronal identity within a limited early competence window. Tracking of the *hunchback* (*hb*) genomic locus in vivo reveals that the *hb* gene is gradually and synchronously repositioned to the nuclear lamina coincidentally with the end of the neuroblast early competence window (Kohwi et al., 2013). Depletion of lamin extends neuroblast competence by reducing both *hb* positioning and gene silencing, suggesting peripheral compartmentalization and repression of *hb* is an important determinant of neuronal fate specification and progenitor competence. To what extent lamins are required for developmentally regulated reorganization of other competence-relevant loci will require future exploration. However, disruption of *Drosophila* lamin also prevents peripheral compartmentalization and repression of testis-specific gene clusters in somatic cells (Shevelyov et al., 2009), supporting a general model in which the nuclear lamina imprisons developmental loci for tissue-specific gene repression.

Independent studies of laminopathies also provide insight into the function of chromatin interactions at the nuclear lamina. For example, Hutchinson-Gilford progeria syndrome (HGPS) is a premature-aging disease caused by progerin, an incompletely processed mutant form of lamin A that promotes abnormal chromatin structure and increased DNA damage. Expression of a GFP-progerin transgene in human mesenchymal stem cells (hMSCs) causes aberrant expression of general and tissue-specific differentiation markers and disrupts the cellular identity, function, and differentiation potential of hMSCs in a manner consistent with phenotypes of HGPS patients (Scaffidi and Misteli, 2008). Examination of cells from HGPS patients further revealed that SKIP, a downstream coactivator of Notch target genes normally sequestered and repressed by the nuclear lamina, loses association with this structure, suggesting aberrant Notch signaling and differentiation abnormalities result from disrupted genome-NL interactions. Indeed, recent sequencing-based mapping of lamin A/C associations reveals that heterochromatin interactions at the NL are reduced genome-wide in HGPS cells, in accordance with microscopy-based evidence (McCord et al., 2013). By integrating profiles for lamin A/C and H3K27me3 with 3D organization changes, McCord et al. (2013) also demonstrate global changes in chromatin compartmentalization in HGPS cells. Changes observed in spatial genome organization correlate with and are preceded by changes in

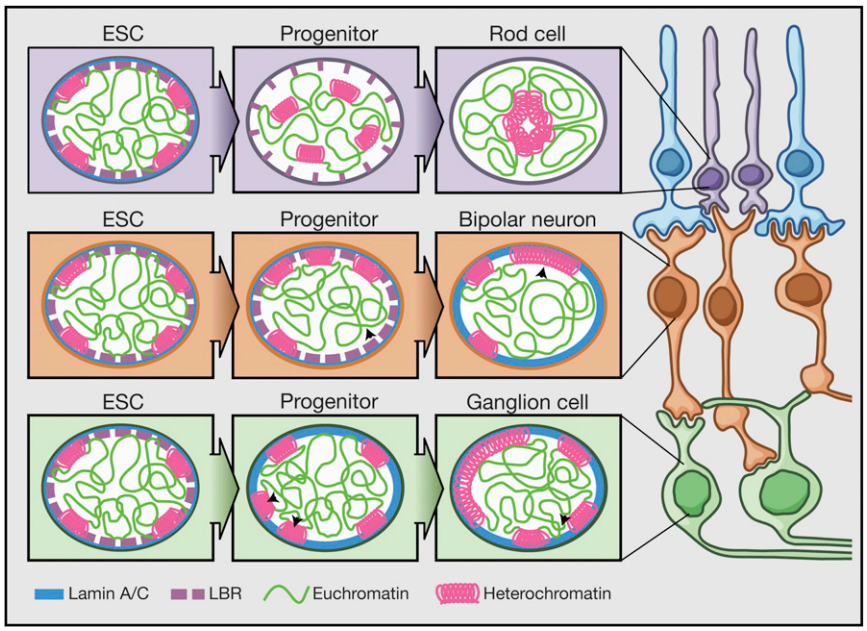

Figure 1. Retinal Cells Differentiation and Chromatin Organization

Spatiotemporal differences in the nuclear lamina composition of differentiating retinal cells (left to right) underlie tissue-specific chromatin organization and genome function. Comparison of lamina composition and genome-NL interactions in the nuclei of embryonic stem cells (ESC), progenitor cells, and differentiated retinal rod cells, bipolar neurons, and ganglion cells. Peripheral compartmentalization of heterochromatin is mediated by lamin proteins; euchromatin is largely nucleoplasmic. Restructuring of chromatin-lamina interactions is a gradual and cumulative process, wherein changes that occur during lineage commitment are often maintained in terminally differentiated cells (Peric-Hupkes et al., 2010). In most cell types, lamin A/C and the inner nuclear membrane protein LBR are consecutively transcribed, with LBR expressed early and A type lamins developmentally regulated for expression in differentiated cells (see bipolar neurons and ganglion cells; Solovei et al., 2013). However, neither LBR nor lamin A/C are transcribed in the differentiated rod photoreceptor cells of nocturnal mammals, causing inversion of nuclear architecture with implications for night vision (Solovei et al., 2009).

lamin A/C and heterochromatin, providing additional evidence that reduction of H3K27me3 and loss of heterochromatin-lamina interactions underlie changes in chromatin structure and genome function.

Additional disease-related mutations in lamins and lamin-associated proteins provide insight into the functional relevance of dynamic genome-NL interactions for tissue differentiation. Emery-Dreifuss muscular dystrophy (EDMD) is a slow progressing degenerative muscle disease caused by autosomal-dominant or X-linked mutations in *LMNA* or in the lamin-interacting protein Emerin, respectively. Recapitulation of a severe late-onset EDMD-linked lamin mutation in *C. elegans* leads to muscle-specific perinuclear retention and repression of transgene-generated heterochromatin carrying a strong muscle-specific promoter (Mattout et al., 2011). The dominant single point mutation in lamin also disrupts tissue-specific expression patterns and leads to defective muscle organization. Together, integration of basic and clinical research suggests that genome-NL interactions are an important regulatory mechanism for controlling cellular identity, differentiation potential, and maintenance of tissue integrity.

Inverted Nuclear Architecture: Learning from "Inside Out"

In an extreme twist on the relationship between nuclear organization and genome function, specific cell types exhibit an "inside-out" architecture in which genes and markers of active chromatin are found exclusively at the nuclear periphery and heterochromatin centrally positioned. Nuclear inversion occurs in the nuclei of mouse retinal rod cells (Solovei et al., 2009), wherein rearrangement of chromatin takes place during terminal differentiation of rod nuclei (Figure 1) and affects the optical properties of the retina by reducing light scattering in the outer nuclear layer. This unusual pattern of nuclear inversion also develops in the rod nuclei of several other nocturnal mammals, suggesting rearrangement of chromatin represents an adaptation for night vision. Nuclear inversion is gradually established over several weeks, and a recent follow up study (Solovei et al., 2013) suggests that changes in NL composition underlies the dynamic arrangement and maintenance of chromatin organization in the differentiating rod cells.

In most cells, lamin A/C and the inner nuclear membrane protein LBR are consecutively transcribed, with LBR expressed early and A type lamins developmentally regulated for expression in differentiated cell types. Sequential expression of LBR and lamin A/C is common across diverse cell types, and differentiated cells that do not express A type lamins often persistently express LBR. Strikingly, inverted rod nuclei express neither lamin A/C nor LBR, and transgenic expression of LBR preserves establishment of the conventional nuclear architecture in differentiated rod cells (Solovei et al., 2013). Moreover, nonrod cells that do not express lamin A/C undergo inversion in LBR null mice, and all postmitotic cells undergo inversion in double-null $LBR^{-/-}$ $LMNA^{-/-}$ mice, indicating that nuclear inversion is caused by the loss of both LBR and/or lamin A/C. Transgenic expression of lamin C alone does not prevent inversion in rod nuclei, suggesting that in contrast to LBR, A type lamins require additional lamin-associated factors for establishing heterochromatin tethers. In myoblasts, deletion of A type lamins reduced expression of muscle-related genes, whereas deletion of *LBR* had a slightly opposite effect, indicating that lamin A/C and LBR inversely regulate tissue-specific transcription patterns. Loss of lamin A/C or LBR had comparatively smaller effects on muscle-specific transcription in differentiated muscle, suggesting lamin dynamics are most critical during the early stages of myotube differentiation.

Similar evidence for the role of LBR and NL composition in development and tissue-specific gene expression comes from

recent studies in mouse olfactory neurons. Murine olfactory sensory neurons (OSN), which choose and monoallelically expresses one out of ~1,400 olfactory receptor (OR) genes, are organized into subregions of the olfactory epithelium called zones. OR genes in mice are highly similar, and previous findings suggest OR choice might be determined by dynamic reversal of repressive H3K9 and H4K20 methylation marks along OR clusters (Magklara et al., 2011). Repressed OR loci colocalize with H3K9 and H4K20 marks in differentiation-dependent and OSN-specific nuclear aggregates, which may function to maintain silencing and conceal transcription factor binding sites that might otherwise disrupt transcription of the active OR allele (Clowney et al., 2012). Silenced OR foci are established near the center of OSN nuclei and requires the downregulation and removal of LBR, reminiscent of heterochromatin remodeling in differentiating rod photoreceptor cells. Similarly, loss of LBR leads to OR aggregation in non-OSN cells, and OR foci in OSNs are disrupted by ectopic expression of LBR, which causes decompaction of OSN heterochromatin and coexpression of many OR genes. Dynamics in NL composition are therefore critical for remodeling and effective silencing of nonchosen OR alleles in olfactory neurons.

Implications for Reprogramming?

The dynamics of nuclear lamina composition during differentiation and the importance of lamins in human health have influenced our evolving view of the nuclear periphery, from a simple framework for nuclear structure to a complex system underlying genome function and development. The spatiotemporal differences in NL composition and nuclear organization also suggest that genome-NL interactions are likely to be an important and understudied feature of "dedifferentiation." Reprogramming of somatic cells into induced pluripotent stem cells remains an inefficient process, and cells that successfully acquire pluripotency do so gradually, through multiple waves of transcription and changes in chromatin and DNA modifications. The similarly slow progression in restructuring of nuclear architecture in differentiating tissues, including repositioning of the *hb* locus in differentiating neuroblasts (Kohwi et al., 2013), remodeling of heterochromatin compartmentalization in rod photoreceptor cells and olfactory sensory neurons (Clowney et al., 2012; Solovei et al., 2013), and the gradual loss of lamin A/C interactions and compartmentalization in HGPS cells (McCord et al., 2013), suggests that changes in genome-NL interactions may be the rate-limiting step for both cellular differentiation and reprogramming. The progressive, lineage-specific nature of remodeling also indicates that peripheral compartmentalization is altered in intermediate steps, perhaps relying on multiple rounds of cell division. The important role of NL composition and dynamic genome-NL interactions in early differentiation suggests that the nuclear architecture established in somatic cells may also represent a barrier to reprogramming, where factors important for maintaining pluripotency are "locked" away. It is therefore conceivable that understanding the step-wise progression of chromatin-lamina alterations and NL composition differences concomitant to lineage commitment and terminal differentiation might serve as a guide for how to find our way back.

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