The nuclear lamina guards the genome and in many ways contributes to regulating nuclear function. Increasing evidence indicates that the lamina dynamically interacts with chromatin mainly through large repressive domains, and recent data suggest that at least some of the lamin-genome contacts may be developmentally significant. In an attempt to provide an additional meaning to lamin-genome contacts, a recent study characterized the association of gene promoters with A-type lamins in progenitor and differentiated cells. Here, we discuss how A-type lamins interact with spatially defined promoter regions, and the relationship between these interactions, associated chromatin marks and gene expression outputs. We discuss the impact of A-type lamins on nucleus-wide and local chromatin organization. We also address how lamin-promoter interactions are redistributed during differentiation of adipocyte progenitors into adipocytes. Finally, we propose a model of lineage-specific “unlocking” of developmentally regulated loci and its significance in cellular differentiation.

Keywords: chromatin, differentiation, lamina-associated domain, lamin A/C, nuclear lamina, promoter

Abbreviations: ChIP, chromatin immunoprecipitation; ES cell, embryonic stem cell; LAD, lamina associated domain; LMNA, lamin A/C; Mb, megabase; NL, nuclear lamina; RNA polymerase II, TSS, transcription start site

Submitted: 09/19/2013
Revised: 10/13/2013
Accepted: 10/18/2013

http://dx.doi.org/10.4161/nucl.26865
*Correspondence to: Philippe Collas; Email: philippe.collas@medisin.uio.no
although some cell type-specificity is observed. Lamin-genome contacts can also be more spatially restricted and occur on clustered or stand-alone loci.

Lamins have been proposed to play a role in the organization of heterochromatin and to influence the 3-dimensional (3D) conformation of the genome. Lamin-genome contacts can also be more spatially restricted and occur on clustered or stand-alone loci. Lamins have been proposed to play a role in the organization of heterochromatin and to influence the 3-dimensional (3D) conformation of the genome.

It does not come as a surprise, then, that mutations in nuclear lamins and LMNA in particular, cause diseases. The co-called laminopathies present various symptoms such as partial lipodystrophies, myoﬁbrotrophies, cardio-myopathies, skeletal abnormalities, neuropathies or premature aging. The molecular mechanisms leading to laminopathies remain unclear but they may involve abnormalities in heterochromatin organization, signal transduction and autophagy.

An emerging hypothesis is that through their extensive range of interaction partners, lamins may relay signals from the cytoplasm to chromatin, and contribute to modulating gene expression and cellular functions. This implies that at least some of the lamin-genome contacts are locus-specific and regulated in a temporal and cell type-specific fashion. Recent data, including some from our laboratory, suggests that this may be the case. It is also conceivable that actively transcribing domains are pulled away from the NL toward the nuclear interior, while silent domains randomly contact the NL and become “locked” at the NL. Recent single-cell imaging of LADs argues for some stochasticity in the formation of LADs.

Here, we highlight recent insights on the association of nuclear lamins with the genome, including gene promoters. Topological maps of promoter-associated LMNA in relation to chromatin modifi- cations reﬁne the concept of LADs and point to an unsuspected modulatory role of lamins on the transcription outcome of genes they associate with. In addition, perturbations in NL composition by downregulating A-type lamins show an unsuspected inﬂuence of LMNA loss.

Figure 1. The nuclear lamina interacts with the genome through lamina-associated domains or LADs. The nuclear envelope consists of an outer and inner nuclear membrane (ONM and INM respectively) under lay by the lamina. LADs largely consist of inactive chromatin region and are bordered by CTCF proteins. Active loci preferentially locate outside LADs.
on chromatin organization at the genome-wide level and at the level of individual promoters. Lastly, differentiation redistributes lamin-promoter contacts with a degree of lineage specificity. This suggests a model of lineage-specific “unlocking” of genes from the nuclear lamina as a priming step for differentiation-induced transcriptional activation.

LADs generally reside in transcriptionally repressive chromatin domains and accordingly, they are overall gene-poor or contain genes which are silent or expressed at a low level.11,12,15,19 Yet, not all genes located within LADs or interacting with lamins are repressed,11 suggesting a sub-LAD tuning of gene expression. An examination of this issue recently prompted the question of whether lamin would interact with gene regulatory elements such as promoters, the chromatin environment of these promoters, the expression status of the corresponding genes, and whether lamin-promoter interactions are developmentally regulated.22

An interrogation of LMNA contacts with promoters by chromatin immunoprecipitation (ChIP) in human adipose stem cells (ASCs), the precursors of adipocytes, identified over 4000 promoters in contact with LMNA.22 Interacting sequences are enriched in GAGA motifs, consistent with mouse data,29 and in A/T-rich stretches. Whether these interactions are mediated by transcription factors remains unknown but is likely,35 and some of these factors may potentially link LMNA-promoter contacts to developmental regulation.37,38

A feature of LMNA, in contrast to B-type lamins, is their existence as a nucleoplasmic pool, in addition to their association with the NL.9 Accordingly, all chromosomes harbor some level of LMNA enrichment, with chromosomes 1, 11, 21, and X being highly enriched (Fig. 2a). There is however no evidence of LMNA ‘hot spots’ at or near known genomic landmarks such as pericentric or subtelomeric regions.22 However, alternating windows of contiguous LMNA-rich and LMNA-poor genes can be evidenced across the genome, consistent with a view of lamin-associated regions at the nuclear periphery as well as in the nuclear interior (Fig. 2b). Incidentally, we find a strong correlation between LMNA enriched domains identified by ChIP in ASCs22 and LMNB1 LADs identified by DamID in lung fibroblasts (Fig. 2b). This confirms the view that lamin-rich domains are in majority conserved between cell types,20 even when they are identified by different methods.

The genome-wide patterns of LADs identified by DamID for A- and B-type lamins are also similar,22 despite the exclusive peripheral localization of LMNB1 (in contrast to LMNA which is also found in the nuclear interior).
This conundrum may be solved by several observations: (1) differences do exist between A- and B-type lamins; (2) the nuclear envelope invaginates into the nuclear interior, which may result in LMNB1 contacting internal chromatin; (3) it remains formally possible that intranuclear LMNA does not significantly associate with chromatin, and that LMNA lamins are largely peripheral. Clearly, interactions of peripheral vs. internal A-type lamins with chromatin remain to be examined closely. What is the relationship between lamin enrichment and chromatin activity? In all cell types examined to date, LADs consist largely lie in repressive chromatin environments. Similarly, gene expression in a genomic “neighborhood” (e.g., a genomic bin of 1 Mb) negatively correlates with LMNA enrichment in that neighborhood (Fig. 3, continuous black line). However, in a neighborhood of LMNA (i.e., a genomic area with many genes interacting with LMNA), the expression of genes which are themselves not bound by LMNA shows no relationship to LMNA enrichment in their neighborhood (Fig. 3, dotted black line). Thus, localization of a gene in a LMNA-rich neighborhood is by itself not repressive, suggesting that a LMNA neighborhood is in itself not conducive of transcriptional repression. Moreover, a subset (~2%) of LMNA-bound promoters harbors trimethylated H3K4me3 (H4K4me3), which marks the TSS of active or potentially active genes. These genes show a wide range of expression levels arguing that a subset of LMNA-bound genes escapes transcriptional repression. So association of a promoter with LMNA is not systematically linked to transcriptional repression, implying that LMNA interaction by itself is not conducive of a repressive state. LMNA may in itself not have any repressive function but may be involved in genomic recruitment to, or stabilization within, a repressive chromatin compartment.22 Conceptually, peripheral and intranuclear lamins may be associated with distinct transcriptional functions. At the nuclear periphery, lamins may be involved in the concentration of repressive chromatin modifiers such as histone methyltransferases and deacetylases, and other co-repressors. The NL may also tether chromatin at the nuclear interior for distinct transcriptional regulatory functions, not all of which may be repressive. These speculations remain to be tested.

### Topological Landscape of LMNA on Promoters

The chromatin context of LADs begins to be well characterized, even at the single-cell level. Nonetheless the recent data topologically refine and extend the concept of LADs in several ways. First, results reveal LMNA association with spatially restricted regions on promoters, with a median size of 1.5 ± 0.3 Kb. This is consistent with, in mouse cells, LADs harboring many confined lamina-associating sequences in the Kb range, and with focal genomic domains associated with the inner nuclear membrane in *Caenorhabditis elegans*. Second, LMNA occupancy is not uniform within promoter regions: LMNA density is highest in an “upstream-distal” region, and at the TSS and 5′ end of genes; less frequently, LMNA occupies a TSS upstream-distal region (Fig. 4A). Interestingly, LMNA enrichment in the various sub-regions is linked to distinct gene ontology terms (Fig. 4B), supporting the finding that the incidence of promoters enriched in LMNA in more than one sub-region is minimal.22

These observations do not preclude LMNA enrichment beyond the relatively narrow promoter regions examined in that study. In fact, four clusters of LMNA peaks can be identified based on their topology over promoter regions (Fig. 4C): an “up-cluster” spanning the 5′ end of the region, a “within-cluster” restricted to the 4 Kb region, a “down-cluster” spanning the 3′ end of this region, and an “across-cluster” crossing both 5′ and 3′ ends. This clustering indicates that LMNA generally interacts with distinct spatially restricted promoter sub-regions.
LMNA Positioning on a Promoter Is Linked to a Gene Expression Outcome

Functional insights on this differential promoter marking by LMNA begin to emerge from expression data. Positioning of LMNA on a promoter appears to be associated with a distinct expression pattern (Fig. 5A). Upstream-distal LMNA interaction is compatible with gene expression depending on the associated histone modifications; in contrast, upstream-proximal and TSS-downstream association correlates with gene inactivity (Fig. 5B). This repression is independent of H3K4me3, H3K9me3, or DNA methylation. Notably, TSS occupancy by LMNA correlates with a silent state even when H3K4me3 marks the TSS. This may be due to inhibition of nucleosome turnover, recruitment of repressive chromatin modifiers, or prevention of TSS occupancy by RNA polymerase II (RNAPII). Consistent with this possibility, we find little overlap between LMNA enrichment and RNAPII occupancy, while depletion of LMNA from the TSS after LMNA downregulation correlates with enhanced RNAPII density at this site (Lund EG, unpublished data). This raises the possibility that LMNA may constitute a barrier to assembly of the transcription machinery at the TSS.

LMNA Influences Nucleus-wide and Local Chromatin Marks

The structural role of the NL and its interaction with the genome suggest that the lamina plays a role in organizing chromatin into domains marked by distinct epigenetic modifications. In line with this possibility, downregulation of LMNA with an shRNA impacts the nature of histone modifications on promoters, by lowering the incidence of repressive modifications (e.g., H3K9me3 or H3K27me3) and strongly increasing permissive H3K4me3 marking. Notwithstanding, loss of LMNA does not correlate with gene activation. Moreover, the large numbers of genes whose histone modification pattern is altered by LMNA loss, compared with those initially marked by LMNA, implies that these large scale chromatin changes affect loci not associated with LMNA to start with. This argues that LMNA influences chromatin composition beyond sites it interacts with.

In addition to a genome-scale influence, the effect of downregulating LMNA is also perceived locally, at the sub-promoter level. We found that LMNA downregulation, but not full depletion, results in some promoters retaining LMNA. On these promoters, LMNA shows a strong tendency to be delocalized from the TSS. This, in turn, affects the enrichment and positioning of histone modifications: whereas densities of H3K9me3 or H3K27me3 on those promoters that retain LMNA are not affected, H3K4me3 marking extends into the promoter area, over ~6 nucleosomes upstream of the TSS. This intriguingly occurs on all H3K4me3-marked promoters, i.e., not only on promoters that harbored LMNA at the TSS to start with and that lost it upon LMNA knock-down, but also on promoters that did not associate with LMNA to begin with. This observation reinforces the long-range influence of LMNA on chromatin organization at the nucleus-wide level.
Adipogenic Differentiation Remolds LMNA-Promoter Interactions

Nuclear lamins have been involved in the organization of chromosomes and cytoskeleton, and tissues but there is still little understanding of their role in these processes. The Drosophila B-type lamin regulates epidermal growth factor receptor signaling in cyst stem cells in the niche supporting keratinocyte differentiation, showcasing how changes in the nuclear envelope during differentiation have also been reported, showing how changes in the nuclear envelope contribute to positioning heterochromatin and altering gene expression and differentiation. This argues for a role of lamin-genome contacts, and particularly of changes in these contacts, during differentiation.

As a first step to addressing a putative role of lamins in differentiation, embryonic stem (ES) cell differentiation into neuronal progenitors (step 1) followed by astrocytic differentiation (step 2) revealed that loci that dissociated from the NL (B-type lamin) during step 1 had a stronger propensity to dissociate from the NL than those retained NL association. What remains unknown in that study however is whether upregulation of LMNA when ES cells exit pluripotency (step 1) plays a role in maintaining loci inactive at this stage after dissociation from B-type lamins. LMNA expression may conceivably provide an additional level of regulation of developmental gene induction: genes destined to be activated upon terminal differentiation may be handed over to LMNA at this stage after dissociation from the NL at the nuclear periphery during differentiation may be masked by changes in epigenetic and transcription factor binding states and results in profound remodeling of the nucleus. It is also accompanied by remodeling of LMNA-genome interactions: in ASCs losses of and gains of LMNA peaks occur upon terminal differentiation. Recent data argue indeed for a release of lineage-specific genes from LMNA upon terminal differentiation. Differentiation of adipocyte progenitors (ASCs) into adipocytes is initiated by changes in epigenetic and transcription factor binding states and results in profound remodeling of the nucleus. In particular, genes controlling adipogenic induction, which do not interact with LMNA in ASCs (e.g., PPARG, FABP4, LEPP), dissociate from LMNA in adipocytes (Fig. 6A). Moreover, these acquire H3K4me3 (Fig. 6A) and become expressed in adipocytes. In contrast, genes implicated in differentiation into other lineages (e.g., MYOD) or involved in pluripotency (i.e., active in ES cells but not in ASCs or adipocytes; e.g., Pou5f1), which interact with LMNA in ASCs, remain associated with LMNA in adipocytes (Fig. 6A).

Could this unlocking of loci from the NL be lineage-dependent? This would be an attractive possibility: it seems that lamin-linked loci controlling induction into a given lineage are released from the lamina while those controlling other lineages remain bound (Fig. 6B). Disengagement from nuclear lamins may create a promoter conformation enabling acquisition of permissive chromatin modifications and RNAPII loading onto the TSS. How LMNA displacement may promote RNAPII loading is not known. Interestingly, a recent report on how enhancer RNAs enable chromatin access to RNAPII at define sites, including TSSs, and apparently in a lineage-specific manner, raises the hypothesis of an interplay between LMNA and non-coding RNAs on the regulation of expression of lineage-specific genes. The advent of powerful genomic tools to investigate 3D genome architecture, together with no act with lamin in ASCs (e.g., MYOD) should rapidly enhance our understanding of the regulation of genome architecture during differentiation and development.

Disclosure of Potential Conflicts of Interest No potential conflicts of interest were disclosed.

References

1. Docke T, Grezes K, Fournier B. Lamina-independent lamins in the nuclear interior serve important functions. Cell Stress Chaperones Apr 2010; 15:333-41; PMID:20331092; http://dx.doi.org/10.1007/s12192-010-0333-1.

2. Beuret F, Crouser BS, Goldman AE, Goldman RD. Nuclear lamin functions and diseases. Trends Genet 2012; 28:444-7; PMID:22797640; http://dx.doi.org/10.1016/j.tig.2012.06.001.

3. Gaara L, Huber M. Lamin A at the crossroads of the cytoskeleton and nucleus. J Struct Biol 2010; 177:29-35; PMID:20224848; http://dx.doi.org/10.1016/j.jsb.2010.01.009.

4. Beuret F, Beuret CS. The nuclear lamina: flexibility in function. Nat Rev Mol Cell Biol 2003; 4:12-24; PMID:12821247; http://dx.doi.org/10.1038/nrm1038.

5. Steven C, Burke B. Transcriptional stress and early nuclear envelope contacts control a single major lamin polyprotein densely resembling lamin B. Cell 1987; 51:303-9; PMID:3111894; http://dx.doi.org/10.1016/0092-8674(87)90044-7.

6. Weissen HJ, Lazaridis I, Georgatos SD. Nuclear lamin heterogeneity in mammalian cells. Differential expression of the major lamin variants and variations in lamin B promoter length. J Biol Chem 2000; 275:12139-43; PMID:10839563.

7. Soler M, Wang XS, Thimmak C, Schinde CS, Kuch S, Zernick M, Callon TV, Derry D, Fournier B, Fischle W, et al. LBR and lamins A/C sequentially tether peripheral heterochromatin and strongly regulate differentiation. Cell 2012; 150:586-96; PMID:22718355; http://dx.doi.org/10.1016/j.cell.2012.01.009.

8. Delhaye E, Tranmar M, Copsey-Maun M, Gallard C, Courvalin JC, Bueno R. The truncated polypeptide closely resembling lamin B. Cell 1988; 51:383-92; PMID:3311384; http://dx.doi.org/10.1016/0092-8674(87)90634-9.

9. Schleiffer A, Gesson K, Foisner R. Lamina-independent functions. Cold Spring Harb Symp Quant Biol 2010; 75:147-54; PMID:20134888; http://dx.doi.org/10.1101/sqb.2010.75.018.

10. Ziegler G, Kalousek R, et al. Dynamic organization of human chromosome enhancer RNAs enables chromatin access to RNAPII at define sites, including TSSs, and apparently in a lineage-specific manner. EMBO J 2008; 27:548-58; PMID:18487314; http://dx.doi.org/10.1038/nature06947.

11. Gadea I, Puig L, Brandt E, Masseron F, Azevedo J. Nuclear lamins interact with LMNA in ASCs (e.g., MYOD) or involved in pluripotency (i.e., active in ES cells but not in ASCs or adipocytes; e.g., Pou5f1), which interact with LMNA in ASCs, remain associated with LMNA in adipocytes (Fig. 6A).

Could this unlocking of loci from the NL be lineage-dependent? This would be an attractive possibility: it seems that lamin-linked loci controlling induction into a given lineage are released from the lamina while those controlling other lineages remain bound (Fig. 6B). Disengagement from nuclear lamins may create a promoter conformation enabling acquisition of permissive chromatin modifications and RNAPII loading onto the TSS. How LMNA displacement may promote RNAPII loading is not known. Interestingly, a recent report on how enhancer RNAs enable chromatin access to RNAPII at define sites, including TSSs, and apparently in a lineage-specific manner, raises the hypothesis of an interplay between LMNA and non-coding RNAs on the regulation of expression of lineage-specific genes. The advent of powerful genomic tools to investigate 3D genome architecture, together with no act with lamin in ASCs (e.g., MYOD) should rapidly enhance our understanding of the regulation of genome architecture during differentiation and development.

Disclosure of Potential Conflicts of Interest No potential conflicts of interest were disclosed.
van Steensel B, Misteli T. Mapping of lamin A- and lamin B genomic binding profiles affects rearrangement of heterochromatic domains and SAHF formation of heterochromatic domains and SAHF formation. Genes Dev 2008; 22:316-25; PMID:21953297; http://dx.doi.org/10.1101/gr.159400.113

Lun D, Cao K, Aggarwala V, Cruickshanks HA, Worner N, Krebs M, Boudreau R, Giorgi G, LeGros G, Krebs J, et al. The ARID family transcription factor bright promotes developmental plasticity. Stem Cells Dev 2010; 20:2447-59; PMID:20742710; http://dx.doi.org/10.1089/scd.2010.0583

Colmenares MC, Lund EG, Oldenburg AR. Closing the nuclear envelope in C. elegans chromosome arms are anchored to the nuclear membrane via discontinuous association with LEM-2. Genome Biol 2008; 9:15-26; PMID:18059368; http://dx.doi.org/10.1186/gb-2008-9-2-15

Mikkelsen TS, Xu, Z. Zhang, X. Wang, Y. Gimbline JM, Lande ES, Bueno-Orovio AG. Comparative epigenomic analysis of mouse and human adipogenesis. Cell 2010; 143:852-67; PMID:21037795; http://dx.doi.org/10.1016/j.cell.2010.12.017

Jensen LS, Redfield RJ, Holzmann BJ, Lu Z, Phillips AC, Zhou J, et al. Developmental regulation of gene expression. Nat Rev Genet 2004; 5:94-103; PMID:14710247; http://dx.doi.org/10.1038/nrg1243

Mikkelsen TS, Xu, Z., Zhang, X., Wang, Y., Gimbline JM, Lande ES, Bueno-Orovio AG. Comparative epigenomic analysis of mouse and human adipogenesis. Cell 2010; 143:852-67; PMID:21037795; http://dx.doi.org/10.1016/j.cell.2010.12.017

Sugrue DJ, Grant GR, Schupbach T, Teuber JH. Propagation of adipogenic signals from stromal cells to adipocytes. Mol Cell 2011; 41:266-78; PMID:21992070; http://dx.doi.org/10.1016/j.molcel.2011.03.015

Mikkelsen TS, Xu, Z., Zhang, X., Wang, Y., Gimbline JM, Lande ES, Bueno-Orovio AG. Comparative epigenomic analysis of mouse and human adipogenesis. Cell 2010; 143:852-67; PMID:21037795; http://dx.doi.org/10.1016/j.cell.2010.12.017