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Mechanisms and dynamics of nuclear lamina–genome interactions

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The nuclear lamina (NL) interacts with the genomic DNA and is thought to influence chromosome organization and gene expression. Both DNA sequences and histone modifications are important for NL tethering of the genomic DNA. These interactions are dynamic in individual cells and can change during differentiation and development. Evidence is accumulating that the NL contributes to the repression of transcription. Advances in mapping, genome-editing and microscopy techniques are increasing our understanding of the molecular mechanisms involved in NL–genome interactions.

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Introduction of the nuclear lamina (NL) as a contact platform for the genome

The spatial organization of chromosomes in the interphase nucleus is determined by the laws of polymer physics, by inter-chromosomal and intra-chromosomal contacts, and by interactions with fixed nuclear scaffolds [1]. The NL is a prime candidate to act as an anchoring scaffold. The NL is a fibrous layer of proteins consisting mainly of lamins. Mammalian cells express four main lamins: A and C which are splice variants encoded by the LMNA gene, and B1 and B2, encoded by lamin LMNB1 and LMNB2 genes, respectively [2]. Every cell type expresses specific amounts of each lamin and, in some instances, lamin abundance affects cell differentiation [3,4]. Several transmembrane proteins are also part of the NL, such as lamin B receptor (LBR), lamin-associated protein 2β (LAP2β), and emerin (EMD) [2].

The DamID technique and Chromatin Immunoprecipitation (ChIP) have been used to map molecular contacts of the genome with the NL in human, mouse, fly, and worm cells [5–8] revealing domain-like patterns of interactions. Mammalian genomes harbor about 1100–1400 lamina-associated domains (LADs), which range in size from ~10 kb to ~10Mb and collectively cover nearly 40% of the genome. Here, we will discuss recent insights into the potential mechanisms responsible for LAD–NL interactions, the dynamics of these interactions, and the possible consequences for gene regulation.

Sequence-specific interactions

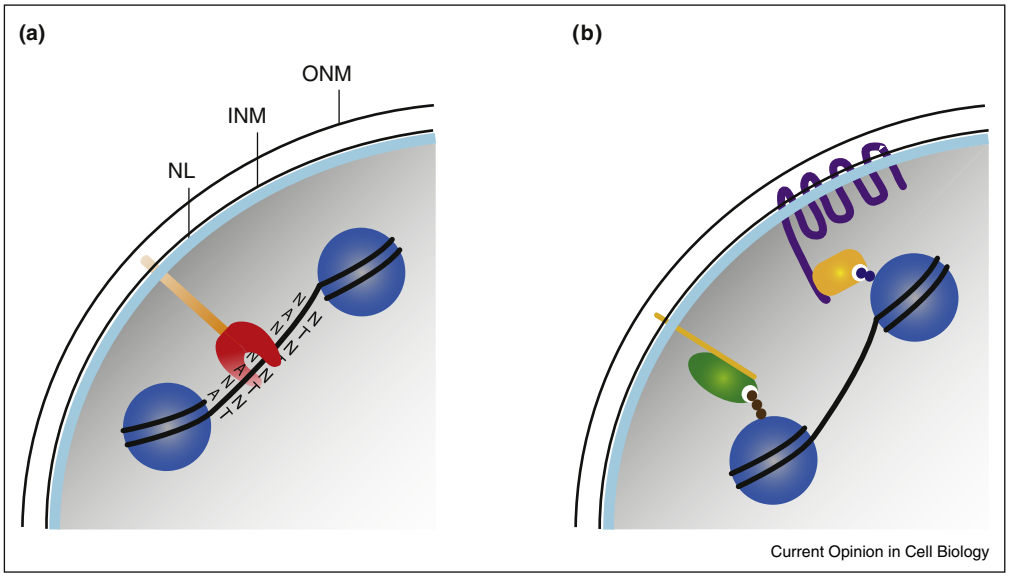
It is tempting to postulate that LADs are recruited to the NL by specific DNA-binding proteins embedded in the NL (Figure 1a). Indeed, analysis of two mouse LADs uncovered sequence elements that appear sufficient for NL targeting [9]. These LADs were enriched in $(GA)_n$ repeats, and artificial arrays of this motif could tether a locus to the NL. The transcriptional repressor cKrox may bind these elements and tether them to the NL by forming a complex with histone deacetylase 3 (HDAC3) and Lap2β [9]. Interestingly, a previous study found an 80 bp sequence, which also harbors a GA-rich motif, to be responsible for the NL targeting of the subtelomeric D4Z4 repeat in a Lamin A dependent manner [10]. $(GA)_n$ repeats are however not globally enriched in LADs [6], indicating that other NL-targeting sequences may exist. In this context, it is interesting to consider that mammalian constitutive LADs, that is, those associated with the NL in all cell types, coincide largely with A/T-rich isochores [11]; possibly the NL harbors a still unidentified DNA-binding protein with a broad A/T sequence specificity.

NL-targeting by chromatin

Not only DNA sequence, but also chromatin can drive NL interactions (Figure 1b). A screen in *C. elegans* revealed that MET-2, a histone methyltransferase responsible for H3K9 monomethylation and dimethylation, and SET-25 (Su(var)3-9, Enhancer-of-zeste, Trithorax), responsible for H3K9 trimethylation, act redundantly to tether a transgene repeat to the NL [12••]. A similar NL tethering mechanism may occur in human cells; G9a, a histone methyltransferase that produces H3K9me2, also promotes the NL contact frequencies of several human LADs [13••], and both H3K9me2 and H3K9me3 were found to be involved in the NL-targeting of the human beta-globin locus [14••]. Furthermore, H3K9me1 absence results in disintegration of heterochromatin foci and disruption of the nuclear lamina [15].

Other heterochromatin histone marks may also be involved in NL interactions. H3K27me3 and linker

Figure 1



Cartoon model depicting two possible non-exclusive mechanisms responsible for the tethering of LADs to the NL. **(a)** DNA-binding factors anchored to the NL may recognize specific sequence motifs enriched in LADs; (b) Certain proteins interacting with the NL may bind specific histone modifications, such as H3K9 methylation. INM = inner nuclear membrane; ONM = outer nuclear membrane.

histone H1 are enriched in LADs [6,8,16], but their roles in NL targeting have not been tested directly.

Lamins and chromosome organization

Integrity of the NL is important for the peripheral positioning of certain genomic loci. In *D. melanogaster*, depletion of the sole B-type lamin caused relocation and ectopic activation of a cluster of testis specific genes [17]. The same lamin was also found to be essential for proper peripheral positioning and silencing of the *hunchback* gene in differentiating fly neuroblasts [18••]. In *C. elegans*, artificial large heterochromatic repeats are positioned near the NL; depletion of the only worm lamin causes detachment of these repeats [19].

In mammals, evidence for a role of Lamin A in chromatin tethering comes from laminopathies, a variety of disorders characterized by mutation in Lamin A gene [20]. ChIP-seq of Lamin A/C in cells from Hutchinson Gilford Progeria Syndrome patients revealed that association of Lamin A/C with the genome is globally reduced [21]. However, in a second study, wild type and mutated Lamin A associated genomic regions were mapped in wild type cell lines, but virtually no difference was observed [22]. Follow-up experiments will need to clarify the role of this mutation for chromatin binding. Other Lamin A mutations can perturb the intranuclear positioning and compaction of chromosomes and alter their gene expression [23,24], again implying a role for Lamin A in chromatin tethering.

Alterations in chromatin organization and chromosome positioning are also observed upon depletion of Lamin B1 in cell lines [25,26]. Lamin B1 reduction is also associated with cell senescence, a cellular condition characterized by redistribution of heterochromatin foci inside the nucleus [27–29]. Direct comparison of Lamin B1 bound regions in proliferating and senescent cells shows that Lamin B1 binding in senescent cells is reduced in H3K9me3 regions, possibly facilitating the redistribution of this type of heterochromatin by decreasing its NL-tethering [30].

Perhaps surprisingly, double (B1 and B2) and triple (A, B1 and B2) lamin knockout murine embryonic stem cells (mESC) appear healthy, suggesting that lamins are dispensable in these cells [31•,32]. It will be interesting to study whether chromosome organization is affected in any way in these double and triple knockout mESCs.

Together, the available data generally indicate that lamins contribute to the spatial organization of the interphase genome. It is still unclear whether lamins bind DNA or chromatin directly, although earlier biochemical data support this notion [33]. It is also possible that lamins simply form the structural scaffold to which 'bridging' factors tether the chromatin fiber.

LBR and heterochromatin positioning

Two elegant genetic studies in mouse point to LBR as a likely candidate for chromatin tethering [34•,35••]. The nuclei of rod photoreceptor cells in the retina of nocturnal

animals (including mice) exhibit a peculiar chromatin architecture with active chromatin located at the nuclear periphery and pericentromeric heterochromatin being centrally located in a ''chromocenter'' [36,37]. This inverted chromatin architecture results in an improved light transmission through the nuclei to the photoreceptors, a potential crucial adaptation step for nocturnal vision. Solovei et al. discovered that in rod cells of nocturnal animals LBR and Lamin A/C are both absent, while in all other tissues – and in rod cells of diurnal mammals – at least one of the two proteins is present. Ectopic LBR expression in mouse rod cells was sufficient to revert the chromatin organization into the classical layer of peripheral heterochromatin. On the other hand, LBR removal caused chromatin inversion in non-rod cells that do not express Lamin A/C, and removal of both proteins caused inversion in all post-mitotic cells [35••]. These results point to redundant roles of Lamin A/C and LBR in the peripheral tethering of heterochromatin.

A similar mechanism appears to take place in mouse olfactory sensory neurons (OSNs). In OSNs, where LBR is absent, the inactive olfactory receptor genes (OR) are localized at a chromocenter in the nuclear interior, a chromatin organization that guarantees the expression of only one OR gene in each OSN. Ectopic expression of LBR in OSNs leads to disruption of the internal chromocenter and dispersal of the OR genes; conversely, knockout of LBR leads to the congregation of OR foci in a chromocenter in non-OSN cells of the olfactory epithelium [34•]. Thus, LBR prevents the clustering of heterochromatin into an internal chromocenter, presumably by keeping the heterochromatin anchored to the NL. On the basis of the study in rod cells, one might predict that OSNs also have low amounts of Lamin A/C, but this has not been tested.

Making the chromatin – NL connection

How is LAD chromatin connected to NL proteins? LBR has been reported to interact directly with heterochromatin proteins such as Heterochromatin Protein 1 (HP1) and methyl CpG binding protein 2 (MeCP2, Rett syndrome) [38,39]. It has also been suggested that LBR binds to the heterochromatin mark H4K20me2 [40], although this could not be confirmed in another study [41]. LBR may thus directly tether heterochromatic regions to the nuclear periphery.

Several other NL proteins are possible candidates for tethering in conjunction with certain chromatin proteins [42], such as EMD in complex with HDAC3 [43] or barrier to autointegration factor 1 (BANF1) [44,45], and LAP2α with the high mobility group nucleosome binding domain 5 (HMGN5) [46]. Table 1 provides a more extensive list of reported interactions between NL and chromatin components. Also noteworthy are the many uncharacterized Nuclear Envelope Transmembrane proteins (NETs) [47]. Some of these were found to control the positioning of individual chromosomes relative to the nuclear periphery [48], although the underlying mechanisms remain to be elucidated.

Table 1

NL-chromatin interacting proteins in mammalian cells

Nuclear lamina	Chromatin partner	Example reference
Lamin A/C	BANF1	[66,67]
	c-Fos	[68,69]
	EGF1	[70]
	hnRNP E1	[70]
	Lco1	[71]
	Mel-18	[70]
	MLIP	[72]
	MOK2	[73,74]
	NFAT5	[75]
	PRR14	[49]
	RB	[76,77]
	SLAP75	[78]
	SREBP1	[79,80]
Lamin B1	BANF1	[67]
	Oct1	[81]
Lamin B2	BANF1	[67]
Emerin	BANF1	[44,45,82]
	Btf	[83]
	HDAC3	[43]
	Lmo7	[84]
	GCL	[85]
	NCoR complex	[86]
Lamin B receptor	HA95	[87]
	HP1	[88–90]
	H4K20me2 (?)	[40]
	MeCP2	[39]
	Protamines	[91]
Lap2α	BANF1	[67,92]
	HMGN5	[46]
	RB	[93]
Lap2β	BANF1	[67,92]
	HA95	[87]
	HDAC3	[9,94]
	cKrox	[9]
	GCL	[95]
Man1/Lem3	BANF1	[67,92,96]
	BTF	[96]
	GCL	[96]
	SMAD	[97,98]

An interesting recent study points to a role for a protein named proline-rich protein 14 (PRR14). In interphase this protein is associated with the NL via Lamin A and it can also interact with HP1α Knockdown of PRR14 leads to partial loss of H3K9me3 from the nuclear periphery. PRR14 may thus help to tether heterochromatin to the NL in interphase [49].

Considering that mammalian genomes have more than a thousand LADs, it is probable that each of the proteins listed above contributes to the tethering of only a subset of LADs. It is also likely that additional tethering factors are still to be identified.

Stochastic behavior of genome – NL contacts

The term 'tethering' might suggest that LAD–NL interactions are tight and robust, yet the contrary may be true. Recently, a new method was developed to track LADs in live cells after they contact the NL. In interphase, these LADs are somewhat mobile, but they remain confined just underneath the NL. Surprisingly, after mitosis only a fraction of these LADs return to the NL in the daughter cells, while many others remain in the nuclear interior [13••]. Some of these internal LADs are associated with nucleoli, which is consistent with the observed overlap of LADs and nucleolus-associated domains (NADs) [50,51]. These data are in agreement with earlier evidence for substantial chromatin rearrangement from mother to daughter cells [52] and suggest that LAD–NL interactions are intrinsically stochastic, that is, in any cell only a subset of LADs contacts the NL (Figure 2).

A recent single-cell application of the Hi-C technology [53•] directly demonstrated the wide range of conformations that a chromosome can adopt. Despite this flexibility, LADs were generally modeled to be on one surface of the chromosome, suggesting that, when LADs on one chromosome contact the NL, they might do so as a collective.

NL and gene repression

Most genes in LADs are expressed at very low levels [5–7]. High-throughput analysis of thousands of random genomic integrations of two reporter genes showed that transcription in mouse LADs is reduced about 6-fold compared to inter-LADs [11]. Furthermore, artificial tethering of genomic loci to the NL can lead to reduced gene activity, but not in all instances [54].

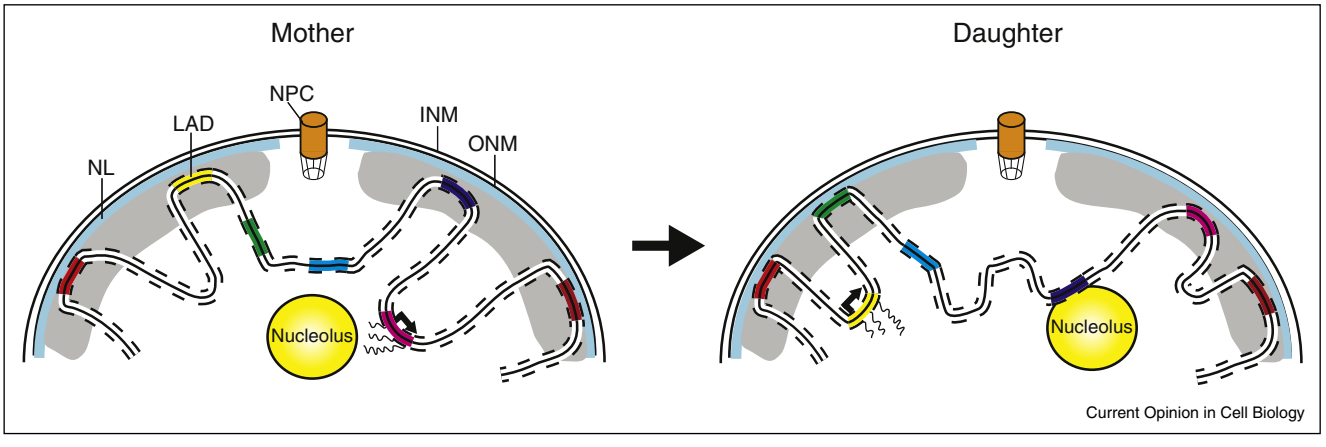
The repressive effect of gene positioning near the NL may help to coordinate gene activity during development. Hundreds of genes move towards or from the NL during differentiation of mESC, concomitant with reduced or increased expression, respectively [7]. Similar results were obtained in adipogenic cell differentiation model [55]. Interestingly, some genes that detach from the NL do not become immediately expressed, but rather become 'unlocked' to be active in a later differentiation step [7]. In *D. melanogaster*, failure to target the *hunchback* gene to the NL coincides with extended activity of this gene, leading to delayed neuronal differentiation [18••].

It seems counter-intuitive that the NL functions in gene repression while at the same time LAD–NL interactions vary stochastically from cell to cell, as this might lead to stochastic gene activity. Indeed, a sequential ChIP-DamID assay indicated that several genes in LADs randomly alternate between a transcriptionally active state in the nuclear interior and a repressed, H3K9me2-marked state when they are NL-associated [13••]. The majority of genes in LADs, however, appear to be robustly repressed.

Mechanisms of repression at the NL

How do contacts with the NL affect gene expression? Targeting of Lamin A to promoters can cause transcriptional repression [56], but the mechanism is unclear. The NL can bind transcriptional repressors and increase their local concentration at the nuclear periphery. In *D. melanogaster* HDAC1 and HDAC3 were reported to be enriched at the NL, and it was proposed that they accomplish different roles, with HDAC1 involved in transcriptional repression and HDAC3 in tethering the

Figure 2



Cartoon model of the genome–NL interactions in mammalian cells during interphase. Individual LADs are depicted as blocks with different colors. In each cell only a subset of LADs are associated with the NL. This subset is determined in a stochastic manner after each cell division. The dotted lines depict the constrained dynamics of chromatin during interphase. The nuclear pore complex (NPC) forms a distinct microenvironment from the NL. The gray areas represent the repressive environment present underneath the NL. Arrows indicate LADs with transcriptional activity and the wavy lines transcribed mRNAs. INM = inner nuclear membrane; NPC = nuclear pore complex; ONM = outer nuclear membrane.

genome to the NL [57]. In mammalian cells HDAC3 can bind NL proteins and, upon interaction, increase its enzymatic activity [9,43,58]. Moreover, inhibition of HDACs alleviates the repression caused by targeting of a reporter to the NL [59].

The chromatin structure of LADs may render DNA less accessible to protein binding and DNA-DNA contacts. The probability that transcription factors bind their recognition motifs is substantially reduced inside LADs [11]. Perhaps this effect is even factor-specific, as in myoblasts the myogenic differentiation gene (MyoD) interacts with the TATA box-binding protein-associated factor IID (TFIID) at the NL, while in differentiated myotubes this gene relocates to the nuclear interior to bind TAF3 [60]. In addition, Hi-C data show that LADs have fewer chromosomal contacts than other genomic regions [53•].

Future outlook

With the new experimental results highlighted above we are beginning to learn how the NL contributes to the spatial organization of chromosomes, and how it plays an active role in regulating gene expression. Considering that LADs collectively cover nearly 40% of the genome, it is likely that many other aspects of their interactions with the NL are to be discovered. Screens such as previously done in worms [12••] should identify additional molecular players, both at the NL and in chromatin. Furthermore, it will be important to find out to what extent laminopathies such as HGPS may be explained by defects in chromosome organization.

The stochastic nature of LAD–NL interactions pose some new conceptual challenges. How does this relate to 'noisy' gene regulation? What is the impact on other nuclear processes such as double-strand break repair and the timing of DNA replication [61]? How are LAD–NL interactions established after mitosis? To address these issues, 'bulk' assays on populations of cells may need to be replaced with new single-cell methods [53•] to monitor chromosome conformation and NL interactions. New imaging techniques, such as improved FISH [62,63] and *in vivo* visualization of specific genomic sequences [13••,64] and histone modifications [65], assure exciting times to come for the NL field.

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