Genome–nuclear lamina interactions: from cell populations to single cells

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Lamina-associated domains (LADs) are large genomic regions that interact with the nuclear lamina (NL) and help to guide the spatial folding of chromosomes in the interphase nucleus. LADs have been linked to gene repression and other functions. Recent studies have begun to uncover some of the molecular players that drive LAD–NL interactions. A picture emerges in which DNA sequence, chromatin components and nuclear lamina proteins play an important role. Complementary to this, imaging and single-cell genomics approaches have revealed that some LAD–NL interactions are variable from cell to cell, while others are very stable. Understanding LADs can provide a unique perspective into the general process of genome organization.

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Introduction: lamina associated domains

The spatial organization of chromosomes within the interphase nucleus has been linked to important biological processes such as gene regulation, DNA replication and DNA repair [1]. One key aspect of this spatial organization is the positioning of genomic loci relative to the nuclear lamina (NL). By means of the DamID (DNA adenine methyltransferase identification) [2,3] and ChIP (chromatin immunoprecipitation) [4] methods the genomic regions that interact with the nuclear lamina have been mapped in detail. These regions are generally referred to as lamina associated domains (LADs; Figure 1) [2–5].

There are >1000 LADs distributed throughout the mammalian genome. Their median size is in the range of 0.5 Mb, and they comprise ∼35% of the genome [3]. LADs are relatively gene-poor, and most of the genes located in LADs have very low expression levels. LADs are thus mostly transcriptionally silent. Studies of nuclear lamina interactions during differentiation have revealed two different types of LADs [5–7]. Constitutive LADs (cLADs) are shared among all tested cell types, while facultative LADs (fLADs) are only associated with the NL in some cell types but not in others (Figure 1). cLADs contain even fewer genes than fLADs and their positions throughout the genome are highly conserved between mouse and human [6]. These features suggest that cLADs may be stable NL anchoring points that form a structural backbone of interphase chromosomes [6,8••].
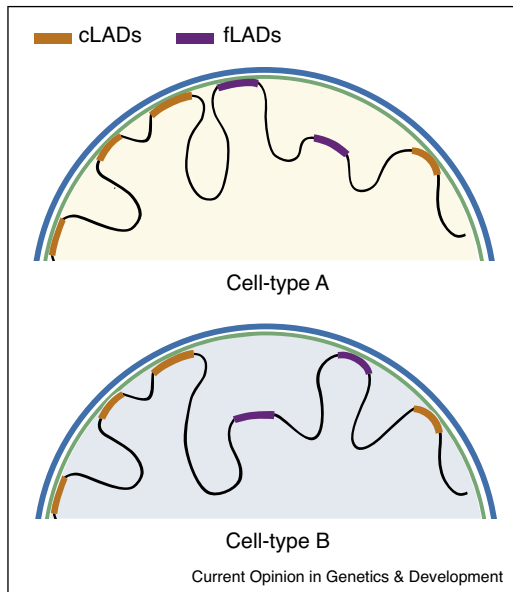
Here, we review new insights into the relationship between LADs, and gene regulation and other functions; the single-cell dynamics and cell-to-cell variability of LAD–NL interactions; and the molecular mechanisms that govern these interactions.

NL interactions, gene regulation and other nuclear functions

LADs have low levels of histone marks associated with gene activity, and are enriched in repressive histone marks such as H3K9me2 and H3K9me3 [3,5,8••,9,10] and in certain cell types also H3K27me2 [3]. During differentiation of mouse cells, several hundreds of genes (located in fLADs) move away or towards the NL, and this generally correlates with their activation or inactivation, respectively [2,5,11]. These data suggest that the nuclear periphery is a repressive compartment. Indeed, artificial tethering of an integrated reporter gene to the NL can result in the transcriptional down-regulation of the reporter gene and of some neighboring genes [12–15]. Conversely, forced activation of a gene located inside a LAD can induce relocation towards the nuclear interior, and this new position was observed to be maintained even after the transcriptional activator was lost [16•].

One possible interpretation of these data is that inactive genes are somehow preferentially positioned at the NL, and that the peripheral environment helps to reinforce their repressed state. Thus, once an inactive gene is located at the NL, it may be more difficult to activate it. A strong activator or chromatin remodelling factor may be able to overcome this repressive feedback loop and

Figure 1

Scheme showing the interaction between the nuclear lamina (NL) and two major classes of LADs: constitutive LADs (cLADs; orange), and facultative LADs (fLADs; purple) in two hypothetical distinct cell types.

cause relocation of the gene to the active chromatin compartment in the nuclear interior. There, the active environment of the nuclear interior might reinforce its active state.

However, observations in *Caenorhabditis elegans* that mutations in components of heterochromatin resulted in the loss of transcriptional repression of certain genes while still having perinuclear localization, or in the loss of perinuclear localization while still being repressed (see below), shows that transcription and localization can be uncoupled [17,18••]. Detachment of silent genes from the NL without their activation has also been observed in differentiating mouse embryonic stem cells [5]. Interactions with the NL may therefore not be essential for repression, but rather could add robustness to the repressed state of genes. In support of this, recent experiments have shown that mutations in genes required for sequestration at the NL hampered differentiation [18••,19].

In addition to reciprocal links with gene repression, evidence is accumulating that NL interactions are also linked to other nuclear functions. For example, the choice of DNA double-strand break repair pathway can be altered by relocalization of a locus to the NL [20]. Furthermore, LADs are known to overlap with domains that replicate late during S-phase [3,5]. The protein Rif-1 marks and controls the replication timing of these domains, as well as their spatial organization [21].

Single-cell dynamics of LAD–NL interactions

In vivo tagging and tracking of genomic regions that contact the NL in human cells revealed that the interactions are dynamic in interphase nuclei, because NL-interacting loci could be observed to move as much as ~1 µm from the NL within several hours [22]. However, this mobility is constrained because NL-contacting loci were never seen to move all the way to the nuclear interior, which is in agreement with earlier single-locus tagging results [23,24]. After mitosis the picture is very different: a substantial fraction of loci that contact the NL in the mother cell are relocated to the nuclear interior (often near nucleoli) in the daughter cells [22] (Figure 2a). This result has two implications: (i) some LADs associate with the NL in only a subset of cells in an otherwise homogeneous population – hence, not all of the ~35% of the genome classified as LADs interacts with the NL in each individual cell; (ii) after every mitosis LAD–NL interactions are reshuffled.
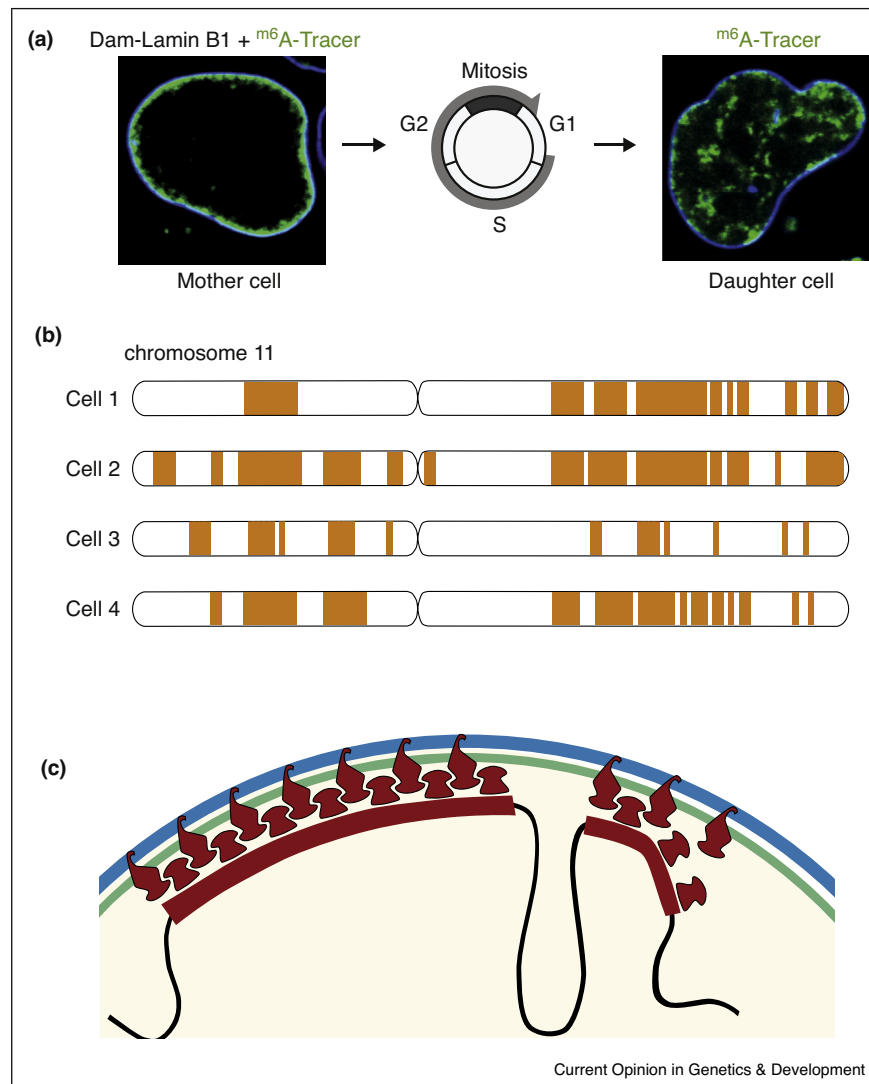
Recent genome-wide DamID mapping of NL contacts in single human cells [8••] underscored these findings. Analysis of hundreds of single-cell maps showed that each LAD has its own characteristic NL contact frequency (Figure 2b). The most consistent interactions involved the gene-poor cLADs, further supporting the notion that these may have a structural role. fLADs exhibit more cell-to-cell variation of their NL interactions, and the contact frequency correlates with the levels of H3K9me3 and inversely correlates with gene expression across the population of cells.

Interestingly, contacts of individual LADs in single cells typically involved long stretches of DNA, suggesting a zipper-like model of multivalent interactions (Figure 2c). At a bigger scale, LAD-dense chromosomes exhibited tighter contacts with the NL than LAD-poor chromosomes. Additionally, LADs on the same chromosome often showed coordinated interactions. Together, these results indicate that neighboring LADs interact with the NL in a cooperative manner [8••]. Single-cell maps thus reveal principles of NL interactions that cannot be easily inferred from population-averaged maps.

Sequence determinants of NL interactions

It is likely that the ability of a LAD to interact with the NL is at least in part encoded in the DNA sequence [7,25,26], but how is still unclear. One possible hint is the observation that cLADs have a relatively high AT content [6]. A wide range of proteins may exist that bind to AT-rich DNA without much additional sequence specificity [27], and it will be interesting to test their role in cLAD–NL interactions. Additionally, borders of LADs are often bound by the insulator protein CTCF [3]. Interestingly, CTCF seems to have an important role in defining NL interactions, as knockdown of CTCF affects the recruitment of specific loci to the nuclear periphery [28]. CTCF has been

Figure 2

LAD–NL interactions are dynamic. **(a)** Reshuffling of NL–LAD interactions from mother (left) to daughter cell (right). The mother cell expressed Dam-Lamin B1 that methylate adenines in close proximity to the lamina. A fluorescent protein that specifically binds to adenine-methylated DNA (^{m6}A-Tracer; green) visualizes genomic regions in contact with the NL in a mother cell (left) and a daughter cell after passing through mitosis (right). The NL is labeled in blue. Images kindly provided by Jop Kind [22]. **(b)** Mapping of cell-to-cell variation of NL interactions. Shown are NL contacts (orange) for chromosome 11 mapped by DamID in four individual human haploid cells (Cell1-4). Data is from Ref. [8••]. **(c)** Cartoon illustrating the zipper-like interaction model. Shown are two LADs (red thick lines) with several contact points with the NL (red zipper 'teeth'). The bigger LAD in the left contains more contact points and thus it is more strongly interacting with the NL than the smaller LAD in the right.

implicated in the formation and delineation of chromatin loops [29], raising the interesting possibility that such loops are involved in LAD organization.

Several labs have systematically fragmented LADs into smaller pieces in order to identify the minimal NL-targeting elements. Dissection of a LAD near the beta-globin locus identified several regions, one as small as 6 kb, that could promote NL interactions when ectopically integrated into the genome [26]. However, the NL-targeting activity was strongly dependent on the ectopic integration site and could not be detected when the 6 kb element was inserted into a free plasmid. Thus, these LAD fragments may only be targeted to the NL in particular chromosomal contexts. A similar approach applied to two other LADs identified many fragments, ranging from 900 bp to 30 kb, with NL-targeting ability [7]. The observation that several fragments within a LAD independently promote NL interaction is additional evidence supporting a zipper-like model.

Small sequence motifs, such as short GAGA repeats (suggested to act via the transcription factor cKROX) [25] and binding sites for the DNA-binding factor YY1 have been proposed to recruit genomic loci to the NL [7]. However, these sequence motifs occur by chance every few kb throughout the genome and are not particularly enriched in LADs [3], so it seems unlikely that they play a direct role. In fact, the tethering role of cKROX was not observed in another study [26]. In contrast, the artificial tethering of ∼100 molecules of YY1 could target a locus to the NL [7], showing that YY1 has the potential to trigger LAD formation if present in sufficient amounts.

Proteins that mediate LAD–NL interactions

As discussed above, certain sequence-specific DNA-binding proteins may help to tether certain LADs to the NL. The most compelling evidence for protein-mediated anchoring, however, points to the role of H3K9 methylation. Depletion of the enzymes that deposit H3K9me2 and H3K9me3 causes dissociation or weakening of LAD–NL interactions [7,17,22,26,30]. Both marks promote NL targeting in a redundant manner [17,26].
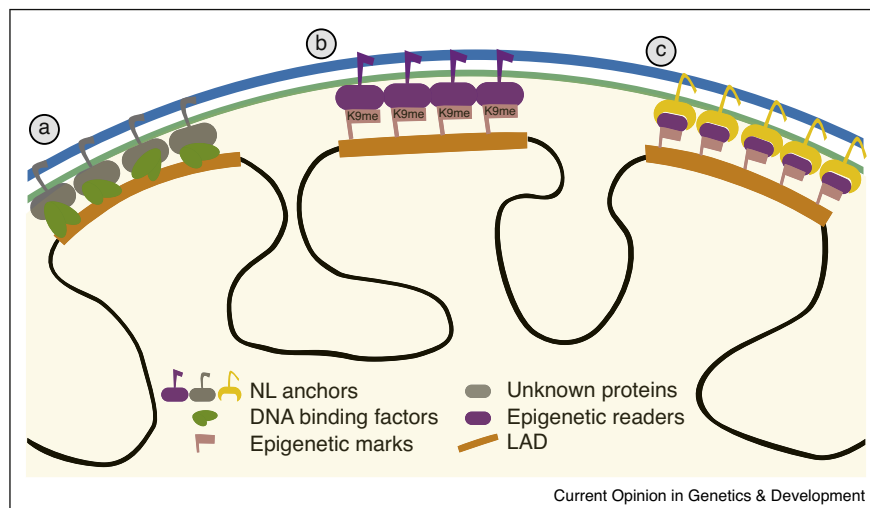
A recent study in *C. elegans* embryos has unraveled a mechanism through which these heterochromatic histone modifications can drive NL interactions. A small protein named Cec-4 binds H3K9me2/3 through its chromodomain, while another part of the protein associates tightly with an as yet unidentified component of the inner nuclear membrane. Deletion of Cec-4 causes detachment of a reporter construct from the NL, and leads to a genome-wide partial loss of NL interactions. Thus,

Cec-4 is a 'bridging factor' that anchors heterochromatin to the NL [18••]. There is no obvious Cec-4 homolog in mammals, but there is an interesting parallel with PRR14, a human protein that associates with the NL and can interact with the heterochromatin protein HP1α. The HP1α–PRR14 interaction was proposed to be involved in the re-assembly of the NL during anaphase [31].

NL proteins themselves have also been implicated in tethering of LADs. In mouse, the Lamin B receptor (LBR), an integral protein of the inner nuclear membrane, is needed for peripheral localization and proper silencing of olfactory receptor genes in olfactory neurons [32]. In other tissues LBR and Lamin A redundantly act to keep heterochromatin at the NL. An interesting exception are rod cells in the retina of nocturnal animals, which lack both proteins and show a striking 'inverted' nuclear organization, where heterochromatin is aggregated in the nuclear interior. Forced expression of LBR in these cells re-establishes peripheral localization of heterochromatin [33]. Interestingly, LBR also interacts with Xist, a non-coding RNA that coats the inactive X chromosome in female mammals. This interaction was reported to assist in recruiting the inactive X to the NL and enabling Xist to spread to actively transcribed genes along the chromosome [34•].

A largely unexplored set of proteins are the Nuclear Envelope Transmembrane (NET) proteins, many of which are cell-type specific. A recent study found three muscle-specific NETs, NET39, Tmem38A, and WFS1 to be involved in the positioning of specific genes at the nuclear periphery and to contribute to proper repression

Figure 3

Models of LAD–NL interactions. Cartoons illustrating alternative or complementary models for the interaction between LADs (orange) and the NL. **(a)** DNA-binding factors (green) recognize the certain sequence within the LADs. The combinatorial binding of DNA-binding factors and unidentified proteins might drive the LAD–NL interaction. **(b)** Epigenetic readers (purple) with affinity for methylated-H3K9 and attached to the NL, such as Cec-4, anchor LADs to the NL. **(c)** LAD are anchored to the NL by the interaction of NL proteins (yellow), such as PRR14, and epigenetic readers (purple), such as HP1α, with affinity to heterochromatin.

of these genes during myoblast to myotube differentiation [19].

It is likely that many more proteins regulate NL interactions, perhaps even at the level of individual LADs. Their identification may be challenging due to redundancies [26,33]. For example, depletion of all lamins in mouse embryonic stem cells did not detectably affect LAD–NL interactions genome-wide [35]. A high-throughput microscopy screen found ~50 human proteins that modulate the nuclear positioning of one or more genomic loci [36], but in most instances the magnitude of the relocations was modest.

Outlook

A diversity of experimental approaches has provided exciting insights into the dynamic organization of LADs. A key challenge for the near future is to thoroughly understand the molecular mechanisms that drive LAD––NL interactions. These interactions may be driven by sequence-specific DNA-binding factors (perhaps recognizing AT-rich DNA) that still need to be identified (Figure 3a). Alternatively, specific chromatin modifications, such as H3K9me2/3 might be bound by proteins that are part of the NL (such as the Cec-4 [18$^{\bullet\bullet}$]; Figure 3b) or by 'bridging' proteins (such as HP1α–PRR14 [31]; Figure 3c). These mechanisms are not mutually exclusive and may in fact act in a concerted manner. It is likely that fLADs and cLADs are anchored to the NL through distinct mechanisms. Further optimization of single-cell mapping and imaging approaches to study LAD–NL interactions with enhanced spatial and temporal resolutions may provide additional insights. Understanding of such architectural principles will undoubtedly offer new handles to dissect the functional relevance of LAD organization.

Conflict of interest

Nothing declared.

Acknowledgements

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Bickmore WA: **The spatial organization of the human genome**. *Annu Rev Genom Hum Genet* 2013, **14**:67-84.

2. Pickersgill H, Kalverda B, de Wit E, Talhout W, Fornerod M, Van Steensel B: **Characterization of the *Drosophila melanogaster* genome at the nuclear lamina**. *Nat Genet* 2006, **38**:1005-1014.

3. Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, Talhout W, Eussen BH, de Klein A, Wessels L, de Laat W *et al.*: **Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions**. *Nat Publishing Group* 2008, **453**:948-951.

4. Ikegami K, Egelhofer TA, Strome S, Lieb JD: ***Caenorhabditis elegans* chromosome arms are anchored to the nuclear membrane via discontinuous association with LEM-2**. *Genome Biol* 2010, **11**:R120.

5. Peric-Hupkes D, Meuleman W, Pagie L, Bruggeman SWM, Solovei I, Brugman W, Gräf S, Flicek P, Kerkhoven RM, van Lohuizen M *et al.*: **Molecular maps of the reorganization of genome–nuclear lamina interactions during differentiation**. *Mol Cell* 2010, **38**:603-613.

6. Meuleman W, Peric-Hupkes D, Kind J, Beaudry J-B, Pagie L, Kellis M, Reinders M, Wessels L, Van Steensel B: **Constitutive nuclear lamina–genome interactions are highly conserved and associated with A/T-rich sequence**. *Genome Res* 2013, **23**:270-280.

7. Harr JC, Luperchio TR, Wong X, Cohen E, Wheelan SJ, Reddy KL: **Directed targeting of chromatin to the nuclear lamina mediated by chromatin state and A-type lamins**. *J Cell Biol* 2015, **208**:33-52.

8. Kind J, Pagie L, de Vries SS, Nahidiazar L, Zhan Y, Lajoie B, de
•• Graaf CA, Amendola M, Fudenberg G, Imakaev M *et al.*: **Genome-wide maps of nuclear lamina interactions in single human cells**. *Cell* 2015, **163**:134-147.
The authors developed a single-cell DamID approach to characterize the variability of DNA–NL interactions. They observed clear differences between cells in almost one third of the LADs. Their results also suggest cooperativity between individual DNA–NL interactions and the coordination of DNA–NL interactions across the same chromosome.

9. Wen B, Wu H, Shinkai Y, Irizarry RA, Feinberg AP: **Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells**. *Nat Genet* 2009, **41**:246-250.

10. Towbin BD, Meister P, Pike BL, Gasser SM: **Repetitive transgenes in *C. elegans* accumulate heterochromatic marks and are sequestered at the nuclear envelope in a copy-number- and lamin-dependent manner**. *Cold Spring Harb Symp Quant Biol* 2010, **75**:555-565.

11. Lund E, Oldenburg AR, Delbarre E, Freberg CT, Duband-Goulet I, Eskeland R, Buendia B, Collas P: **Lamin A/C-promoter interactions specify chromatin state-dependent transcription outcomes**. *Genome Res* 2013, **23**:1580-1589.

12. Finlan LE, Sproul D, Thomson I, Boyle S, Kerr E, Perry P, Ylstra B, Chubb JR, Bickmore WA: **Recruitment to the nuclear periphery can alter expression of genes in human cells**. *PLoS Genet* 2008, **4**:e1000039.

13. Reddy KL: **Transcriptional repression mediated by repositioning of genes to the nuclear lamina**. *Nat Publishing Group* 2008, **452**:243-247.

14. Kumaran RI, Spector DL: **A genetic locus targeted to the nuclear periphery in living cells maintains its transcriptional competence**. *J Cell Biol* 2008, **180**:51-65.

15. Dialynas G, Speese S, Budnik V, Geyer PK, Wallrath LL: **The role of *Drosophila* Lamin C in muscle function and gene expression**. *Development* 2010, **137**:3067-3077.

16. Therizols P, Illingworth RS, Courilleau C, Boyle S, Wood AJ,
• Bickmore WA: **Chromatin decondensation is sufficient to alter nuclear organization in embryonic stem cells**. *Science* 2014, **346**:1238-1242.
The authors observed that forced activation of an inactive gene inside a LAD resulted in the relocalization of the gene towards the nuclear interior. This relocalization was independent of activation of transcription but associated with chromatin decondensation.

17. Towbin BD, González-Aguilera C, Sack R, Gaidatzis D, Kalck V, Meister P, Askjaer P, Gasser SM: **Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery**. *Cell* 2012, **150**:934-947.

18. Gonzalez-Sandoval A, Towbin BD, Kalck V, Cabianca DS,
•• Gaidatzis D, Hauer MH, Geng L, Wang L, Yang T, Wang X *et al.*:
**Perinuclear anchoring of H3K9-methylated chromatin
stabilizes induced cell fate in *C. elegans* embryos**. *Cell* 2015,
163:1333-1347.
By performing RNAi screens in *C. elegans* to find anchor proteins for
heterochromatin, the authors identified the protein Cec-4. Cec-4 is
localized at the NL and has affinity for H3K9me1, me2, and me3.

19. Robson MI, de Las Heras JI, Czapiewski R, Lê Thành P, Booth DG,
Kelly DA, Kerr ARW, Schirmer EC: **Tissue-specific gene
repositioning by muscle nuclear membrane proteins
enhances repression of critical developmental genes during
myogenesis**. *Mol Cell* 2016, **62**:834-847.

20. Lemaître C, Grabarz A, Tsouroula K, Andronov L, Furst A,
Pankotai T, Heyer V, Rogier M, Attwood KM, Kessler P *et al.*:
Nuclear position dictates DNA repair pathway choice. *Genes
Dev* 2014, **28**:2450-2463.

21. Foti R, Gnan S, Cornacchia D, Dileep V, Bulut-Karslioglu A, Diehl S,
Buness A, Huber W, Johnstone E, Loos R *et al.*: **Nuclear
architecture organized by rif1 underpins the replication-timing
program**. *Mol Cell* 2016, **61**:260-273.

22. Kind J, Pagie L, Ortabozkoyun H, Boyle S, de Vries SS, Janssen H,
Amendola M, Nolen LD, Bickmore WA, Van Steensel B:
Single-cell dynamics of genome–nuclear lamina interactions.
Cell 2013, **153**:178-192.

23. Chubb JR, Boyle S, Perry P, Bickmore WA: **Chromatin motion is
constrained by association with nuclear compartments in
human cells**. *Curr Biol* 2002, **12**:439-445.

24. Schermelleh L, Carlton PM, Haase S, Shao L, Winoto L, Kner P,
Burke B, Cardoso MC, Agard DA, Gustafsson MGL *et al.*:
**Subdiffraction multicolor imaging of the nuclear periphery
with 3D structured illumination microscopy**. *Science* 2008,
320:1332-1336.

25. Zullo JM, Demarco IA, Pique-Regi R, Gaffney DJ, Epstein CB,
Spooner CJ, Luperchio TR, Bernstein BE, Pritchard JK, Singh H:
**DNA sequence-dependent compartmentalization and
silencing of chromatin at the nuclear lamina**. *Cell* 2012,
149:1474-1487.

26. Bian Q, Khanna N, Alvikas J, Belmont AS: **β-Globin cis-elements
determine differential nuclear targeting through epigenetic
modifications**. *J Cell Biol* 2013, **203**:767-783.

27. Quante T, Bird A: **Do short, frequent DNA sequence motifs
mould the epigenome?** *Nat Rev Mol Cell Biol* 2016, **17**:257-262.

28. Zhao H, Sifakis EG, Sumida N, Millán-Ariño L, Scholz BA,
Svensson JP, Chen X, Ronnegren AL, Mallet de Lima CD,
Varnoosfaderani FS *et al.*: **PARP1- and CTCF-mediated
interactions between active and repressed chromatin at
the lamina promote oscillating transcription**. *Mol Cell* 2015,
59:984-997.

29. Merkenschlager M, Nora EP: **CTCF and cohesin in genome
folding and transcriptional gene regulation**. *Annu Rev Genom
Hum Genet* 2016, **17**:17-43.

30. Pinheiro I, Margueron R, Shukeir N, Eisold M, Fritzsch C,
Richter FM, Mittler G, Genoud C, Goyama S, Kurokawa M *et al.*:
**Prdm3 and Prdm16 are H3K9me1 methyltransferases required
for mammalian heterochromatin integrity**. *Cell* 2012,
150:948-960.

31. Poleshko A, Mansfield KM, Burlingame CC, Andrake MD,
Shah NR, Katz RA: **The human protein PRR14 tethers
heterochromatin to the nuclear lamina during interphase and
mitotic exit**. *Cell Rep* 2013, **5**:292-301.

32. Clowney EJ, LeGros MA, Mosley CP, Clowney FG, Markenskoff-
Papadimitriou EC, Myllys M, Barnea G, Larabell CA, Lomvardas S:
**Nuclear aggregation of olfactory receptor genes governs their
monogenic expression**. *Cell* 2012, **151**:724-737.

33. Solovei I, Wang AS, Thanisch K, Schmidt CS, Krebs S, Zwerger M,
Cohen TV, Devys D, Foisner R, Peichl L *et al.*: **LBR and lamin A/C
sequentially tether peripheral heterochromatin and inversely
regulate differentiation**. *Cell* 2013, **152**:584-598.

34. Chen C-K, Blanco M, Jackson C, Aznauryan E, Ollikainen N,
• Surka C, Chow A, Cerase A, McDonel P, Guttman M: **Xist recruits
the X chromosome to the nuclear lamina to enable
chromosome-wide silencing**. *Science* 2016, **354**:468-472.
The authors observed that LBR is important for the Xist-mediated silen-
cing of the inactive X chromosome through a direct LBR–Xist interaction.
This interaction is required for Xist to spread along the inactive X
chromosome. Mutation analysis suggests that an Arginine-Serine motif
in LBR is important for the Xist–LBR interaction.

35. Amendola M, Van Steensel B: **Nuclear lamins are not required
for lamina-associated domain organization in mouse
embryonic stem cells**. *EMBO Rep* 2015, **16**:610-617.

36. Shachar S, Voss TC, Pegoraro G, Sciascia N, Misteli T:
**Identification of gene positioning factors using high-
throughput imaging mapping**. *Cell* 2015, **162**:911-923.