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Stochastic genome-nuclear lamina interactions

Modulating roles of Lamin A and BAF

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The nuclear lamina (NL) is thought to aid in the spatial organization of interphase chromosomes by providing an anchoring platform for hundreds of large genomic regions named lamina associated domains (LADs). Recently, a new live-cell imaging approach demonstrated directly that LAD-NL interactions are dynamic and in part stochastic. Here we discuss implications of these new findings and introduce Lamin A and BAF as potential modulators of stochastic LAD positioning.

Keywords: Lamin B1, nuclear organization, LADs, single cell, Lamin A, chromatin domains, BAF

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Introduction

The NL is a thin filamentous meshwork that lines the inner nuclear membrane (INM) and forms a structural scaffold that is thought to support the spatial organization of the genome. The NL associates with very large genomic regions that range in size from ~10 kb to ~10 Mb and together cover approximately about 40% of the genome. The mammalian genome typically harbors about 1100–1400 of such lamina-associated domains (LADs).[1-4]

In mammalian somatic cells, the NL consists of two B-type lamins—B1 and B2—and two splice variants of A-type lamin named Lamin A and C. In most cell types, the B-type lamins are almost exclusively located at the NL, while A-type lamins additionally reside in the nucleoplasm.[5,6] The role of this nucleoplasmic pool of Lamin A/C remains largely enigmatic.

Besides lamins, the NL contains many other proteins. One of these is the barrier-to-autointegration factor (BAF, encoded by the BANF1 gene), a ~10 kDa evolutionary conserved protein that associates with the NL through interaction with LEM-domain containing proteins of the INM. BAF binds non-specifically to DNA[7,8] and chromatin.[9,10] One possibility is that BAF acts as a "bridging" factor that connects chromatin to the NL, but direct evidence for this has been lacking so far. Like Lamin A, BAF is partially nucleoplasmatic,[11] raising the question whether it binds to chromatin at the NL, in the nuclear interior, or both. In fact, it is not known whether BAF interacts with LADs at all.

Recently we reported that the interactions of LADs with the NL appear intrinsically stochastic, i.e., they are variable from cell to cell. Here, we discuss and build on these findings and present data in support of a role for Lamin A and BAF in controlling LAD positioning between the nucleoplasm and the NL in single cells.

Stochastic Positioning of LADs

Our laboratory developed the ^{m6}A-Tracer technique to specifically mark and trace chromatin that contacts the NL in single cells.[12] The method utilizes a GFP-tagged protein module that recognizes ^{m6}A on chromatin after deposition of this adenine modification by Dam-Lamin. Thus, any DNA that contacts the NL can be visualized and followed in live cells. Using Dam-Lamin B1 to label DNA in contact with the NL, this approach revealed that during interphase LADs are

somewhat mobile but confined to a narrow zone in the immediate vicinity of the NL. However, after mitosis these same LADs are seemingly randomly redistributed throughout the nucleus of the daughter cells, with only a subset returning to the NL. From these and other data we inferred that many LADs do not contact the NL in every single cell, but rather in a stochastic manner[12]; after every mitosis, a different subset of LADs is located at the NL. This model is concordant with various microscopy studies[13] and with recent single-cell HiC experiments,[14] which revealed that chromosomes adopt remarkably diverse configurations from cell to cell.

Interestingly, we noticed that some of the nucleoplasmic LADs accumulate around nucleoli.[12] This is in agreement with the partial overlap between LADs and nucleoli-associated-domains (NADs) as identified by genome-wide mapping.[15,16] It thus appears that some LADs can be located either at the NL or at a nucleolus, and that this choice may be random in a population of cells.

Stochastic Chromatin Modification State of LADs

According to genome-wide studies, LADs tend to be enriched in the heterochromatic histone modification H3K9me2.[3,17] However, by microscopy and combined ChIP-DamID experiments we observed that H3K9me2 is primarily present on those LADs that are actually located at the NL, while the same LADs carry less of this histone mark when they are located in the nuclear interior.[12] By inference, this means that the H3K9me2 status of LADs is also stochastic, and directly linked to their nuclear position.

Additional experiments indicated that H3K9me2 in part drives NL contacts, because inactivation of G9a, the principal enzyme responsible for H3K9 dimethylation, caused a reduction in LAD–NL contact frequencies.[12] In line with these findings, H3K9me2 and H3K9me3 were also shown to be involved in targeting the β-globin locus to the NL[18] and a similar mechanism was observed in *C. elegans*.[19]

Thus, LADs that are stochastically positioned in the nuclear interior do not detectably interact with Lamin B1, and they tend to have reduced H3K9me2 levels. Below we consider the possibility that the nucleoplasmic pools of Lamin A and BAF substitute for these associations, and even compete for the association of LADs with the NL.

Lamin B1, Lamin B2, Lamin A, and BAF Associate with the Same Genomic Regions

Previously we showed that, despite their partially different nuclear localization,[20] Lamin B1 and Lamin A interact with the same genomic loci in populations of mouse and human cells.[21] An example of the nearly identical DamID patterns is shown for human HT1080 cells in **Figure 1A and B**. For further comparison, we now also mapped the interaction pattern of Lamin B2. Lamin B2 again yielded a highly similar interaction profile (**Fig. 1A and B**).

We then performed DamID of BAF, also in HT1080 cells. Remarkably, this protein again showed a genome-wide interaction profile very similar to that of the three lamins (**Fig. 1A and B**). Thus, BAF also interacts preferentially with LADs.

Lamin A Preferentially Contacts Chromatin around Nucleoli

Due to the stochastic positioning of LADs, an interaction profile of a population of cells does not distinguish between peripheral vs. interior interactions of BAF and Lamin A in single cells. The ^{m6}A-Tracer approach indicated that Lamin B1 in interphase exclusively contacts chromatin in the vicinity of the NL, with virtually no signals in the nuclear interior, whereas Lamin A contacts chromatin both at the NL and in the nuclear interior.[12]

To study the nucleoplasmic ^{m6}A-Tracer signals obtained with Dam-Lamin A in more detail, we repeated these experiments with a cell line that stably expresses ^{m6}A-Tracer, which makes it easier to score distribution patterns. In this cell line, 20 h after transfection with Dam-Lamin A, ^{m6}A-Tracer signals are apparent both at the NL and throughout the nucleoplasm. Interestingly, in 35 ± 7% of the cells a clear accumulation of ^{m6}A-Tracer signal appeared around nucleoli (**Fig. 2A**). Interestingly, peri-nucleolar enrichment is not detected with antibody staining of Lamin A/C,[12] indicative that the enrichment of the ^{m6}A-Tracer around nucleoli likely reflects a accumulation of ^{m6}A over time either due to re-current transient and/or more specific chromatin interactions of Lamin A with peri-nucleolar chromatin. In parallel experiments, transfection with Dam-LaminB1 and Dam-LaminB2 did not yield any detectable peri-nucleolar ^{m6}A-Tracer staining (**Fig. 2B-D**), indicating that the cells had not progressed through mitosis since the time of transfection, which would lead to reshuffling of LADs.[12]

The peri-nucleolar ^{m6}A-Tracer enrichments indicate that Lamin A preferentially interacts with certain genomic regions that are positioned adjacent to nucleoli. Combined with the previous findings that (1) Lamin A and Lamins B1/B2 can interact with the same LADs, (2) some LADs are stochastically positioned near nucleoli, and (3) LADs partially overlap with nucleolus-associated-domains (NADs)[15,16], these results lead us to propose that nucleolus-associated LADs interact with Lamin A. We note that LADs at the NL also interact with Lamin A, as demonstrated by the clear labeling of the nuclear rim by ^{m6}A-Tracer in the presence of Dam-Lamin A.

Lamin A and BAF Compete for Genome-NL Interactions

Because the ^{m6}A-Tracer data suggest that Lamin A can interact with internally positioned LADs, we reasoned that Lamin A could sequester these LADs in the nuclear interior and prevent them from interacting with the NL. To test this “tug of war” model, we reduced the levels of Lamin A by siRNA-mediated knockdowns, and then determined the contact frequencies of LADs with the NL (using Dam-Lamin B1) as previously described.[12] Strikingly, reducing the levels of Lamin A results on average in a ~1.4-fold increase in contact

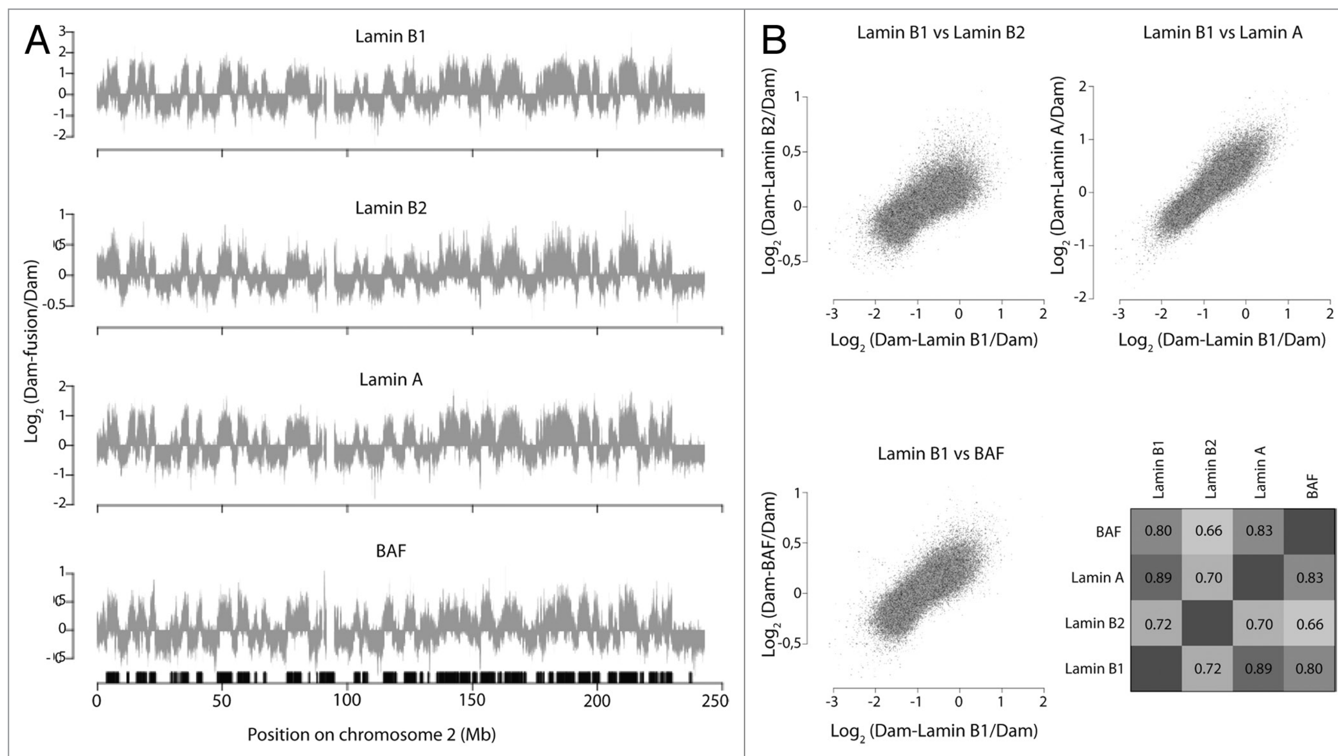

Figure 1. Lamin B1, Lamin B2, Lamin A, and BAF bind to the same genomic regions. (**A**) Interaction profiles of chromosome 2 in HT1080 cells for Lamin B1, Lamin B2, Lamin A, and BAF. Each profile represents the average of two independent experiments. Data for Lamin B1 and Lamin A are from references 12 and 21. Samples were smoothed with a running median window over 11 probes. The black boxes at the bottom of the graph depict LADs as defined in reference 2. (**B**) Scatterplots of Lamin B1 in relation to: Lamin B2 (top left), Lamin A (top right), and BAF (bottom left). Samples were smoothed with a running median window of 11 probes. Bottom right: genome wide Pearson correlation matrix for all smoothed (as above) samples.

frequencies between several LADs and Lamin B1 (**Fig. 3A and B**). This is in agreement with a model in which Lamin A and B1 compete for the same LADs.

We also determined the effect of BAF on LAD-Lamin B1 interactions. Again, knockdown of BAF substantially increased the contact frequency of several LADs with Lamin B1 (by ~1.8-fold on average). Albeit modestly, a double knockdown of both Lamin A and BAF results in an even further enhancement of the contact-frequencies (~2.2-fold compared with the control knockdown). In contrast, reducing the levels of H3K9me2 by knocking down G9a results into a reduction of genome-NL interactions (**Fig. 3A**), which is consistent with what we reported previously.[12] Hence, Lamin A/BAF and G9a are opposing forces in the regulation of genome-NL contact frequencies.

We do not know at which stage of the cell cycle Lamin A and BAF interfere with the positioning of LADs at the NL. This could happen right after mitosis, when the NL is reassembled onto chromosomes, or in early G1, when chromatin is still mobile[22] and LADs stochastically assume their positions in the nucleus[12] (**Fig. 4**). Some LADs could be localized to the nucleolar periphery by an interaction of BAF and Lamin A with nucleophosmin[23] and nucleolin,[24] respectively.

We note that our observation that Lamin A counteracts peripheral positioning of LADs seems contradictory to observations in mouse, where Lamin A and Lamin B Receptor (LBR) are thought to be redundantly involved in the anchoring of heterochromatin to the nuclear periphery.[25] Perhaps the modulatory role of Lamin A is different in the presence and absence of LBR, or is different between mouse and human cells.

Stochastic NL Interactions: Stochastic Gene Repression?

Most genes in LADs have very low expression levels, indicating that LADs constitute a repressive chromatin environment. In differentiating mouse ES cells, dissociation of genes from the NL often precedes the actual transcriptional activation at a next differentiation step.[3] Conversely, in *Drosophila* neuronal progenitor cells, tethering of the *hunchback* gene to the NL is necessary for its stable repression.[26] These observations support the notion that the NL is a repressive environment. Indeed, reporter genes integrated in LADs tend to be ~5-fold less active than the same reporter genes integrated in inter-LAD regions,[27] and tethering experiments have indicated that contact with NL itself can contribute to this transcriptional repression,[28-30] although not in all instances.[31]

Even though LADs exhibit detachment from the NL in a subset of cells, for the majority of genes in LADs no expression is detected by mRNA profiling of pools of cells.[12] Apparently, for these genes, the stochastic detachment from the NL does not lead to strong transcriptional activation. Possibly, association of Lamin A with these genes preserves their repressed

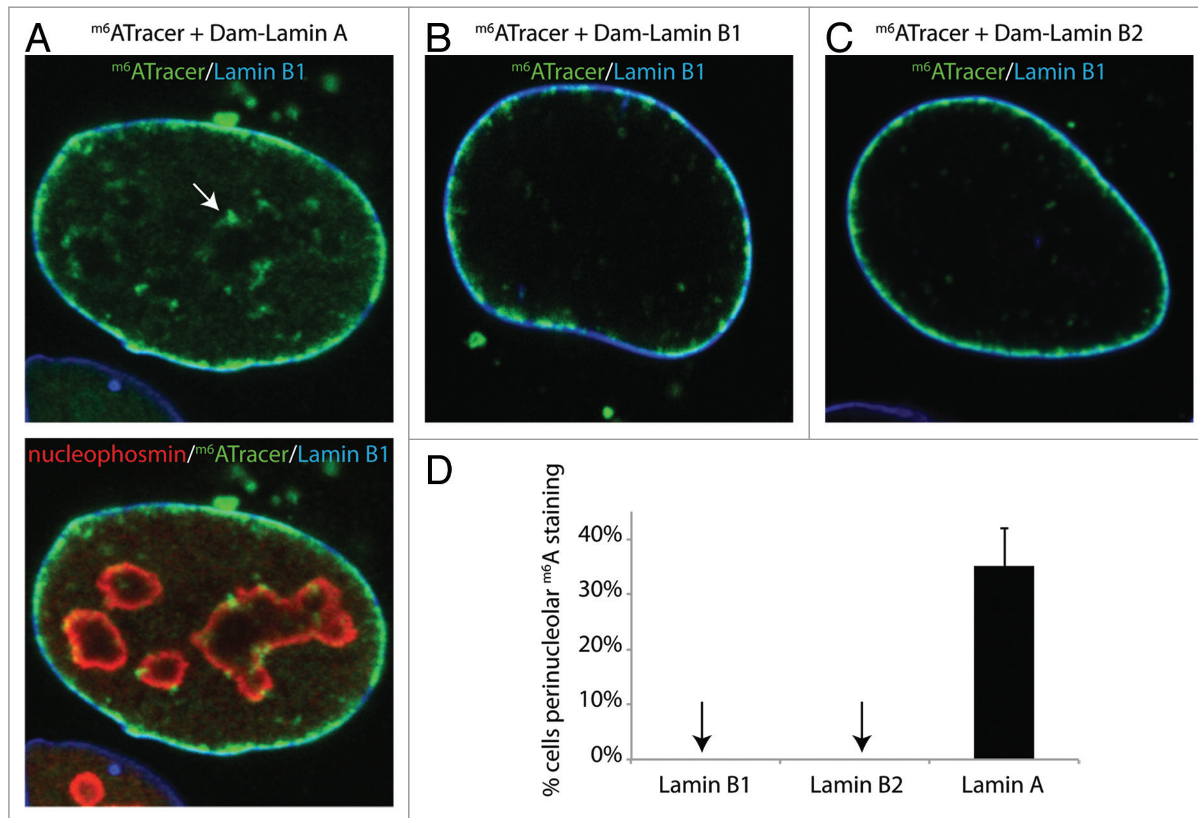

Figure 2. Lamin A interacts with peri-nucleolar chromatin. (**A-C**) Clonal HT1080 cell line expressing ^{m6}A-Tracer construct (in green) transfected with: Dam-Lamin A (left), Lamin B1 (top middle), or Dam-Lamin B2 (top right). The cells were harvested 20 h post transfection. Nucleoli are labeled with an antibody against nucleophosmin (red, bottom panel in A) and the NL is labeled with an antibody against Lamin B1 (blue). The extensive cytoplasmic labeling originates from transfected plasmid molecules that carry ^{m6}A and are therefore bound by ^{m6}A-Tracer. (**D**) Percentage of cells that display peri-nucleolar ^{m6}ATracer staining. Error bars indicate standard deviation (two independent experiments).

status in the nucleoplasm. Indeed, binding of Lamin A to promoters is generally incompatible with transcription,[32,33] and downregulation of Lamin A results in increased H3K4me3 levels at promoters, a mark that is thought to be permissive for transcription.[33] Other nucleoplasmic proteins such as BAF may further mark the detached LADs for repression.

It is also possible that transient NL contacts are sufficient to reinforce epigenetic silencing mechanisms for longer periods. For example, the histone deacetylase HDAC3 was found enriched at the NL[34-36]; perhaps an occasional visit to the NL is sufficient to remove histone acetylation from LADs and thereby preserve repression over multiple generations.

Nevertheless, some genes located in LADs are transcriptionally active according to mRNA profiling data of cell pools.[2,12] We observed for several of these genes that their stochastic location at the NL is inversely linked to levels of H3K36me3, which is a marker of transcriptional activity.[12] Stochastic activity of genes has been extensively studied,[37,38] but so far had not been linked to nuclear positioning to our knowledge. Thus, for some genes, the stochastic detachment from the NL coincides with transcriptional activation. This leads to intrinsic cell-to-cell variability in gene expression, a feature that may be exploited during cell-fate transitions.[39]

Future Directions

Nuclear architecture is much more dynamic than anticipated. Whether all cells at different stages of development exhibit similarly stochastic nuclear organization of LADs should be further investigated. It is tempting to speculate that, when cells become gradually committed to a particular lineage, the stochastic positioning of LADs decreases concomitantly. It will be interesting to compare the dynamics of LAD positioning in various cell types.

At present it is not clear whether NL contacts are equally stochastic for all LADs, or whether some LADs interact more robustly with the NL than others. This may be studied by fluorescence in situ hybridization of individual LADs, by tracking of selected LADs using new tagging methods,[40] or perhaps in the future by the construction of genome-wide maps of NL interactions in single cells.

So far, we have identified three proteins that modulate the NL contact frequency of LADs: G9a, Lamin A, and BAF. It is likely that other proteins are involved in this regulatory process. Identification of these proteins can provide us with tools to further investigate the links of dynamic LAD-NL interactions with stochastic gene expression, and perhaps with the single-cell dynamics of other nuclear processes such as DNA replication and DNA repair.

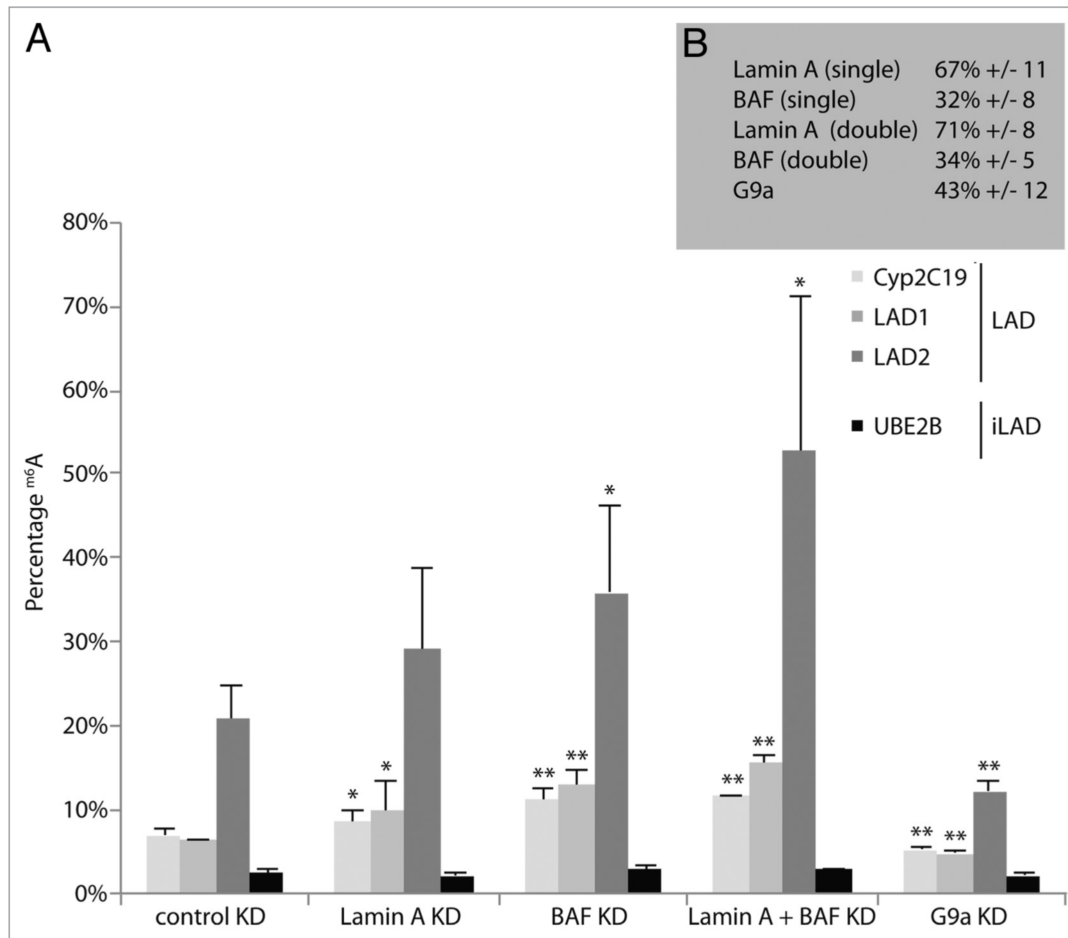

Figure 3. Lamin A and BAF compete with Lamin B1 for LAD binding. (**A**) ^{m6}A accumulation by Dam-LaminB1 at three LADs (Cyp2C19, LAD1, and LAD2) and one inter-LAD region (UBE2B) after siRNA knockdowns (KD) as indicated. Error bars indicate standard deviations (n = 4). *$P < 0.05$, **$P < 0.01$ according to a paired *t* test. (**B**) Estimated knockdown efficiencies, expressed as residual mRNA levels compared with control. Note the significant effect of Lamin A KD on Lamin B1 interactions with two LADs (**A**), despite the rather mild knockdown of Lamin A (mRNA level reduced to 67% of control).

Experimental Procedures

DamID

DamID of BAF and Lamin B2 was performed as described in reference 41 except that Dam-Lamin B2 was introduced by transfection of the pLgw-EcoDam-V5-Lamin B2 plasmid with lipofectamine (Clontech) 48 h prior to genomic DNA collection. DamID microarray data was normalized as described in reference 42.

Immunofluorescence labeling and microscopy

Immunofluorescence was performed as described in reference 12. The antibodies used in this study are from Abcam: Lamin B1 (ab16048) and NPM1 (ab10530). Images were acquired on a confocal laser scanning Leica TCS SP2.

qPCR quantification of ^{m6}A levels

Quantitative PCR measurements of ^{m6}A levels at selected GATC sequences were performed as described in reference 12; Dam-LaminB1 expression was induced three days post siRNA transfections for 20 h. RNAi efficiencies were determined by RT-qPCR of the respective mRNAs 72 h after siRNA transfection.

Cell line

The ^{m6}A-Tracer line was derived by transfection of HT1080 cells with TetO-puromycin-IRES-^{m6}A-Tracer and subsequent clonal selection with 2 μg/ml puromycin (Sigma).

^{m6}A-Tracer experiment

The ^{m6}A-Tracer line was grown in absence of doxycycline and transfected with pLgw-EcoDam-V5-Lamin B1, pLgw-EcoDam-V5-Lamin B2, or pLgw-EcoDam-V5-Lamin A. Twenty hours after transfections cells were fixed and prepared for Immunofluorescence. For **Figure 2D**, n = 40 for Lamin B1 and Lamin B2, and n = 40 and n = 33 for Lamin A. Cells were manually scored as having peri-nucleolar enrichment when a striking enrichment of the ^{m6}A-Tracer signal was apparent around nucleoli in respect to the overall signal throughout the nucleoplasm.

RNAi ^{m6}A-quantification assay and RT-qPCR

As performed in reference 12, Lamin A primer sequences for RT-qPCR: CCGAG TCTGA AGAGG TGGTC (forward), AACTC CTCAC GCACT TTGCT (reverse). BAF primer sequences for RT-qPCR: GAACC GTTAC GGGAACTGAA (forward), CCCAG GACTT CACCA ATCC (reverse).

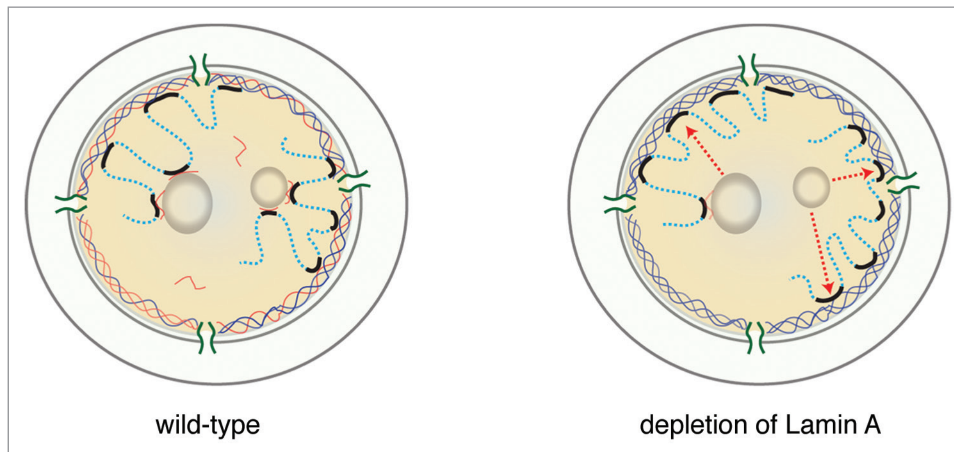

Figure 4. Cartoon model of a possible role of Lamin A in modulating NL interactions. In wild-type cells, LADs may be anchored at the NL by interacting with Lamin A (orange) or B (blue), or be kept in the nuclear interior by interacting with Lamin A, at the surface of nucleoli (left panel). In the absence of Lamin A, anchoring in the nuclear interior is lost and LADs are more likely to interact with Lamin B at the NL (right panel, arrows indicate relocated LADs).

Data availability

Genome-wide DamID data are available from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), accession number GSE55066.

Disclosure of Potential Conflicts of Interest

No conflict of interest was disclosed.

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