The nuclear lamina, a filamentous protein network that coats the inner nuclear membrane, has long been thought to interact with specific genomic loci and regulate their expression. Molecular mapping studies have now identified large genomic domains that are in contact with the lamina. Genes in these domains are typically repressed, and artificial tethering experiments indicate that the lamina can actively contribute to this repression. Furthermore, the lamina indirectly controls gene expression in the nuclear interior by sequestration of certain transcription factors. A variety of DNA-binding and chromatin proteins may anchor specific loci to the lamina, while histone-modifying enzymes partly mediate the local repressive effect of the lamina. Experimental tools are now available to begin to unravel the underlying molecular mechanisms.

Introduction
The cell nucleus is enclosed by a double lipid bi-layer with interspersed nuclear pore complexes (NPCs) that facilitate selective nuclear-cytoplasmic exchange of macromolecules. In metazoans, the nucleoplasmic surface of the inner nuclear membrane (INM) is structurally supported by the nuclear lamina (NL), a filamentous meshwork consisting of specialized intermediate filament proteins named lamins. There are two types of lamins: B-type lamins are found in all cell types, whereas the A-type lamins are found only in differentiated cells [1,2]. Lamins interact with many other proteins, some of which are integral components of the INM [1,3]. A wide spectrum of human disorders has been linked to mutations in lamins or lamin-interacting proteins [4], illustrating the importance of the NL.

For decades it has been thought that the NL may act as a surface for the anchoring of specific DNA sequences, thereby providing a scaffold for the folding of chromosomes inside the nucleus. In addition, the NL may play an active role in the regulation of gene expression. Recent microscopy studies, gene-tethering approaches and the mapping of genome–NL interactions at molecular resolution have yielded new insights into these processes. In this review we discuss the possible roles of the NL in chromosome organization and transcriptional regulation, with emphasis on new data reported over the past two years.

The genome in association with the NL and the NPC
Classic electron micrographs [5] and recent high-resolution light microscopy images of mammalian cell nuclei [6*] show that the NL tends to be in close contact with relatively compact chromatin, while NPCs are surrounded by much less, or decondensed, chromatin. Genome-wide mapping using the DamID technology has identified the regions of the genome that are in molecular contact with the NL in both human and fly cells [7,8*]. Human fibroblasts have more than 1300 of such genomic contact regions, which are named Lamina-associated domains (LADs). Human LADs are remarkably large genomic segments (0.1–10 Mb) and together harbor thousands of genes at a low gene-density. The vast majority of those genes have very low expression levels with only few active ‘escaper’ genes. Most LADs have sharp borders that are often marked by specific sequence elements, such as binding sites for the insulator protein CTCF [8*].

Recent data indicate that NPCs in metazoans also interact with specific genomic loci, as was previously found for budding yeast [9]. Molecular mapping studies have identified hundreds of sites in the *Drosophila* genome that are bound by NPC proteins [10–12]. In contrast to LADs, genes in association with NPCs are generally found active [9,10], or at least moderately transcribed [11]. Interestingly, a substantial pool of nuclear pore proteins freely diffuses and binds to genes located in the nuclear interior [11,12]. By a clever strategy with engineered NPC proteins, Kalverda *et al.* [11] managed to generate genome-wide maps that discriminate intranuclear interactions from NPC interactions at the nuclear periphery. This revealed that *bona fide* NPC-associated sequences are distinct from NL-associated regions, and primarily overlap with moderately active genes.

The molecular interaction maps for NL and NPC proteins offer an explanation for the sometimes confusing observations obtained by Fluorescence In Situ Hybridization (FISH) microscopy when correlating the nuclear
radial position of genes to their expression status [13,14]. The molecular mapping results emphasize that NL and NPCs represent different microenvironments (Figure 1), which are difficult to resolve by conventional light microscopy. The possibility of NL ‘microdomains’ composed of different lamin subtypes [15] further emphasizes that the nuclear periphery is a heterogeneous structure.

Whether interacting with the NL or NPCs, loci that associate with the nuclear periphery typically do so in a stochastic manner. Even in a homogeneous cell population, the frequency of association is never found to be 100% [7,8,11,14]. Partly, this may be attributed to intranuclear mobility of the chromatin fiber [16], and to some extent the folding of interphase chromosomes may have a stochastic component.

In addition, the organization of genes and chromosomes in the nucleus is dependent on the differentiation state of the cell. An extreme example of this are nuclei of rod cells in the retina of nocturnal animals. Here, the organization of chromatin is radially inverted, such that heterochromatin aggregates in the center of the nucleus and active euchromatin is located at the periphery. These inverted nuclei act as light-collecting lenses, thus enhancing the light sensitivity of rod cells [17**]. This fascinating anecdote illustrates the remarkable plasticity of nuclear architecture.

Mechanisms of genome–NL interactions

Do LADs adhere to the NL owing to specific biochemical interactions, or are they passively pushed towards the periphery because other chromosomal regions have a preference to be located in the nuclear interior? Interesting new computer simulations of chromosome polymer dynamics suggest that local differences in certain basic physical properties of the chromatin fiber, such as flexibility and thickness, may partly drive the peripheral location of heterochromatin by self-organization principles [18,19]. Yet, the NL does appear to play an active role in the nuclear organization of the genome by binding specific chromosomal regions. Indirect evidence comes from the observation that compacted chromatin is only touching the NL but not the neighboring NPCs [5,6*]. Passive brushing of chromatin against the NL is also difficult to reconcile with the sharply defined LAD structure and the presence of border elements that demarcate LADs [8*]. Furthermore, cells that lack Lamin B1 show abnormal positioning of some chromosomes [15,20], while cells from patients with a rare Lamin A mutation (causing Hutchinson Gilford Progeria Syndrome) have substantially reduced amounts of heterochromatin as judged from electron microscope images [21]. It is therefore likely that specific biochemical interactions between the NL and LADs help to organize chromosomes inside the nucleus.
A key question is how the NL interacts with specific genomic regions. Several DNA-binding factors physically interact with NL proteins [1,3,22] and are therefore good candidates to mediate specific genome–NL interactions. A notable candidate is the transcription factor Oct1, which binds to Lamin B1 and is present at the NL in a Lamin B1-dependent manner [23]. Oct1 recognition motifs are enriched within LAD sequences [8*], suggesting that Oct1 may help to tether LADs to the NL. Sequence-specific NL interactions are also illustrated by the recent identification of an 80 bp repeat sequence within a human subtelomeric region that is sufficient to localize an adjacent telomere to the NL in a CTCF and lamin A dependent fashion [24]. It is likely that additional sequence elements will be discovered that can be anchored to the NL via DNA-binding factors.

Besides ‘hard-coded’ sequence elements, local chromatin properties may also provide recognition sites for the NL. Lamins and INM proteins interact biochemically with histones and various chromatin proteins [1,3]. The histone modifications H3K9me2 and H3K27me3 in mammals are enriched at the nuclear periphery [14,25*] and are over-represented in LADs [8*,26]. However, microscopy studies of individual loci have so far not yielded direct evidence that these histone marks mediate NL interactions [25*,27], although more extensive investigation on this issue is needed. It is worth considering that the absence of activating histone marks (such as methylation of H3K4 and acetylation of several other histone lysines) may serve as a recognition cue for NL interactions. This notion is supported by the observation that treatment with trichostatin A, an inhibitor of histone deacetylases (HDACs), disrupts NL interactions genome-wide in Drosophila cells [7], and also dissociates some mammalian genes from the nuclear periphery [28].

**Rebuilding genome–NL interactions after mitosis**

During mitosis, phosphorylation of NPC-components and NL-components initiates the disassembly of the nucleus [29,30] and results into the dissociation of INM proteins and lamin from chromatin [31]. As cells need to progress through cell division for de novo NL-genome interaction to occur, the nuclear architecture is probably established during nuclear reassembly [32**,33**]. The molecular basis for the reassembly of the nuclear envelope at the end of mitosis is not understood in great detail, but a number of recent papers provide valuable insight in the order of events. At the onset of nuclear reassembly, BAF binds first to the telophase chromosomes at a distinct ‘core-structure’ followed by Lap2αa and many – but not all – NL-associated proteins [34,35*]. Lamins bind to telophase chromosomes only after the core region is assembled. Lamin A binds to the core region, while Lamin B first localizes to the spindle pole to gradually associate with chromatin in a rather diffuse pattern [34,35*]. The core region itself stays devoid of the nuclear membrane until all NL-associated components have been docked to this structure [35*]. Many NL-associated proteins collectively contribute to reassembly of the nuclear envelope, as reducing the levels of individual components significantly slows down nuclear reassembly [36].

The recognition basis for the distribution of these components to telophase chromosomes is unknown, although non-specific DNA binding by NL-associated proteins appears to play a role [37]. It is unlikely that BAF recognizes the core-structure through sequence-specific DNA binding, as BAF binds to dsDNA in a sequence-independent manner [38]. Instead, BAF and other NL-associated components could be directed to the telophase chromosomes via interactions with distinct chromatin proteins. Given the rough similarities of the core region and interphase LADs (i.e., presence of NL-associated proteins and absence of most NPC components), it is tempting to speculate that the chromosomal regions in the telophase core region correspond to some LADs in interphase.

**Gene regulation by the NL**

Does the NL play an active role in gene regulation, or is it merely an innocent bystander? Microarray and in situ expression analyses have shown that the depletion of lamins and other NL proteins causes misregulation of hundreds of genes [20,23,39–41], as does expression of a lamin A mutant that causes premature aging in humans [42]. In Drosophila, the knockdown of the only B-type lamin causes derepression of a testis-specific gene cluster, together with the relocalization of this cluster towards the nuclear interior [43], strongly suggesting that the genes in this cluster are repressed via direct contact with the NL.

To investigate the impact of gene–NL interactions on transcription more directly, a number of laboratories have artificially tethered reporter genes (integrated in the genome) to the NL. While in two studies this resulted into partial repression of the reporter and some of the surrounding genes [33**,44**] virtually no effect on reporter expression was found in a third study [32**]. However, a similar set of tethering experiments in Drosophila by the Wallrath laboratory showed strong repression of one reporter gene in three different genomic integration sites, while a second reporter positioned in tandem was only repressed in two of the three integration sites (George Dialynas, Sean Speese, Vivian Budnik, Pamela K. Geyer and Lori L. Wallrath, personal communication, paper of special interest). Together, these studies indicate that

---

1. This study reports, in analogy to mammalian laminopathies, that the expression of a N-terminal truncated form of Lamin A causes muscle defects in flies. Furthermore, the authors convincingly show, in an NL-targeting assay, that Lamin A is involved in gene-repression.
contact with the NL can contribute to gene silencing, yet the magnitude of the repressive effect depends on local chromatin context and regulatory sequences.

Interestingly, treatment with an HDAC inhibitor reverses the repression caused by NL-tethering \[44\]. Histone deacetylation is therefore a likely contributor to gene repression at the NL. Consistently, histones in chromatin near the NL are normally hypo-acetylated \[45\], indicative of high local HDAC activity. Indeed, several NL-components interact with HDACs, including Lap2β and Emerin \[46,47\]. An attractive model, therefore is that the NL helps to repress genes in LADs partly by deacetylation of histones (Figure 2a).

A second histone modification that appears to be involved in gene regulation at the NL is H3K9me2. In human cells this modification was found to be enriched at the nuclear periphery \[25\] possibly mediated by an interaction of BAF with G9a, the histone methyltransferase that is primarily responsible for dimethylation of H3K9 \[48\]. Chromatin immunoprecipitation data show that H3K9me2 occupies long stretches of the genome that substantially overlap with LADs \[8,26\]. Knockout of G9a causes derepression of many late-replicating genes that tend to be positioned at the NL \[25\]. Within the resolution of light microscopy, loss of G9a does not cause a significant relocation of the affected genes, suggesting that H3K9me2 is not required for genome–NL interactions, but

---

**Figure 2**

Models of regulatory interactions of the NL with chromatin and DNA-binding proteins. (a) Putative links between NL-associated chromatin and histone-modifying enzymes. G9a and HDACs interact with NL proteins \[46–48\] and may thereby locally dimethylate H3K9 and deacetylate histones in chromatin that is in close proximity, thus helping to repress gene activity near the NL. PRC2, Polycomb Repressive Complex 2, which methylates H3K27me3. (b) Hypothetical dual function of Oct1–NL interactions. Oct1 may anchor some LADs to the NL by binding simultaneously to Lamin B1 and to its binding motifs that are enriched in LAD sequences (left); at the same time, sequestering of Oct1 at the NL may prevent Oct1 from binding to its binding motifs at genes located in the nuclear interior (right) \[23\].
rather is needed for the repressive environment at the NL (Figure 2a).

The NL also contributes to gene regulation in indirect ways. For example, Oct1 can be sequestered away from its target genes by an interaction with Lamin B1. A number of genes involved in the oxidative stress response appear to be regulated through this mechanism. Both Oct1 and Lamin B1 null cells, show increased susceptibility to oxidative stress, which is probably due to the down-regulation of genes involved in this response [23]. Similar mechanisms involving sequestering by the NL have been reported for other transcription factors [22]. Note that Oct1 may have a dual partnership with the NL: on the one hand it may help to tether certain LADs to the NL, while at the same time the sequestration of Oct1 at the NL affects the expression of Oct1 target genes in the nuclear interior (Figure 2b).

Conclusions
The availability of new tools, such as molecular tethering methods, genome-wide mapping techniques, and subdiffraction light microscopy, has created exciting new opportunities to dissect the causal relationships among genome–NL interactions, interphase chromosome folding, and gene regulation in mammalian cells. A picture emerges in which the NL contributes to the spatial organization of the genome and helps to repress genes that are in close proximity. DNA-binding factors as well as chromatin components help to anchor LADs to the NL, and conversely the NL contributes to gene regulation by modulating chromatin and the activity of transcription factors.

Acknowledgements
We apologize to colleagues whose work we could not discuss owing to space constraints. We thank G. Filion, W. Meuleman, U. Braunschweig, and A. Pindyurin for helpful comments, and the M. Fornerod, M. Hetzer, A. Akhtar, and L. Wallrath labs for sharing unpublished results. Supported by an EMBO Long-term Fellowship to Jk and an NWO-VICI grant to BvS.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest •• of outstanding interest


Using three-dimensional structured illumination microscopy, the authors show, with unprecedented spatial resolution that the locale at the site of NPCs is devoid of lamins and heterochromatin.


The authors show, at molecular resolution, that the genome in association with the NL is organized in remarkably large domains with sharp borders. These domains represent regions of very low transcriptional activity.


17. Solovei I, Kreising M, Laoent C, Kosem S, Peichl L, Cremer T, Guck J, Jeffe B: Nuclear architecture of rod photoreceptor cells adapts to vision in mammalian evolution. Cell 2009, 137:356-368. This remarkable study shows that the nuclear architecture of nocturnal rod photoreceptor cells is inverted, such that heterochromatin aggregates in the center of the nucleus and euchromatin is located at the periphery. These inverted nuclei act as light-collecting lenses, thus enhancing the light sensitivity of rod cells.


44. 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. This study reports that the artificial tethering of two genomic regions to the INM results in the suppression of some, but not all genes. Inhibition of histone deacetylation reverses the repressive effect indicating a role for HDACs in gene regulation at the NL.


