

Nuclear Envelope: Connecting Structural Genome Organization to Regulation of Gene Expression

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Abstract For many years, the nuclear envelope was viewed as a passive barrier that separates the genetic material in the nucleus from the cytoplasm of the cell and permits regulated trafficking of various molecules through the nuclear pores. Research in the past two decades has shown that the nuclear envelope is a complex cellular compartment, which harbors tissue-specific resident proteins, extensively interacts with chromatin and contributes to spatial genome organization and regulation of gene expression. Chromatin at the nuclear periphery is organized into active and silenced domains punctuated by insulator elements. The nuclear envelope transmembrane proteins and the nuclear lamina serve as anchoring sites for heterochromatin. They recruit chromatin that has been modified with specific epigenetic marks, provide silencing factors that add new epigenetic modifications to genes located at the nuclear periphery, and sequester transcription factors away from the nuclear interior. On the other hand, proteins of the nuclear pores anchor as well as help generate active chromatin, promote transcription, and coordinate gene expression with mRNA export. The importance of these functions is underscored by aberrant distribution of peripheral chromatin and changes in gene expression that occur in cancer and heritable human diseases linked to mutations in nuclear envelope proteins. Although many mechanistic questions addressing the role of the nuclear envelope in genome organization and function have been answered in recent years, a great deal remains to be discovered in this exciting and rapidly moving field.

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Abbreviations

BAF	Barrier-to-autointegration factor
EDMD	Emery–Dreifuss muscular dystrophy
HP1	Heterochromatin protein 1
HDAC3	Histone deacetylase 3
HGPS	Hutchinson–Gilford progeria syndrome
INM	Inner nuclear membrane
IPTG	Isopropyl β -D-1-thiogalactopyranoside
lacO	Lac operator repeats
lacI	Bacterial lac repressor
NE	Nuclear envelope
NET	Nuclear envelope transmembrane protein
NPC	Nuclear pore complex

Introduction

The nuclear envelope (NE) forms extensive connections with the cytoplasm of the cell and chromatin in the nuclear space. Thus, the NE is uniquely positioned to integrate extracellular, cytoplasmic and nuclear signaling networks. There are multiple ways in which the NE contributes to the regulation of gene expression ranging from transmitting signals from the cytoplasm to the nucleus to spatial genome organization and fine-tuning of gene expression at the nuclear periphery. A wide range of cellular functions that are highly relevant to tissue homeostasis, human disease, and cancer biology are dependent on the composition and the organization of the NE. Therefore, there is an ever-growing interest in exploring the mechanisms of nuclear architecture and gene expression that are regulated by spatial cues at the nuclear periphery in conjunction with the NE and its resident proteins.

Structurally, the NE is a complex structure consisting of two separate concentric lipid bilayers, the outer (ONM) and the inner (INM) nuclear membranes, respectively (Fig. 1). The ONM is studded with ribosomes and continuous with the endoplasmic reticulum [1], but it has many unique proteins that connect to the cytoskeleton as well as to the proteins residing in the INM [2]. The connectivity of the NE to the endoplasmic reticulum also means that the NE lumen is continuous with the endoplasmic reticulum lumen. The ONM provides an impenetrable barrier for proteins and most small molecules except where the nuclear pore complexes (NPCs) are inserted. The NPCs regulate directional transport of soluble macromolecules in and

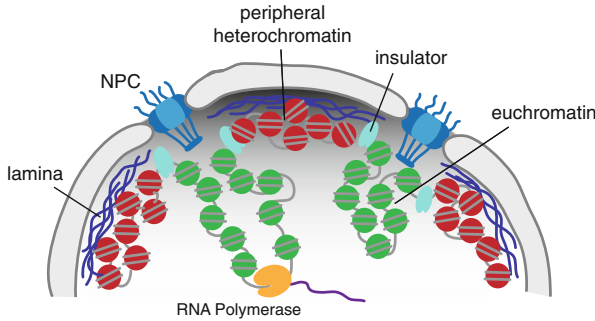


Fig. 1 Chromatin organization at the nuclear envelope. The nuclear envelope is a double membrane system perforated by nuclear pore complexes (NPCs) that regulate transport of molecules in and out of the nucleus. Between NPCs the inner nuclear membrane is lined by a filamentous polymer of the intermediate filament lamin proteins (lamina). At a simplistic level, peripheral heterochromatin tends to be associated with the lamina while peripheral euchromatin tends to be associated with the NPCs. Insulators on the DNA separate the peripheral euchromatin from peripheral heterochromatin

out of the nucleus and are extremely large (>40 MDa) structures made up of at least 30 distinct polypeptides present in multiple copies [3]. Where NPCs are inserted, the ONM curves around the outer face of the NPCs into the INM. The INM has its own unique set of transmembrane proteins, many of which interact with the intermediate filament lamins (in higher eukaryotes) that form a polymeric meshwork under the INM [4, 5]. The integral membrane proteins of both membranes are generally referred to as NETs for *nuclear envelope transmembrane proteins* [6].

Lamins, many NETs, and some NPC proteins bind directly to chromatin and/or transcriptional regulators and some have been reported to directly bind DNA (Fig. 1; reviewed in [7]). Moreover, some of the chromatin-binding partners of NETs specifically reflect transcriptionally silenced chromatin [8–10]. These interactions, accordingly, have been shown to direct the distribution of heterochromatin at the nuclear periphery [11, 12]. The affinity between lamins/NETs and chromatin provides a mechanism whereby binding interactions with lamina proteins can both sequester certain parts of the genome to the periphery and provide regulatory proteins to these genome regions.

Most of the NE proteins initially found to bind chromatin were widely expressed. However, subsequent proteomics studies found a much larger set of proteins at the NE than previously expected [13–16] and more recent studies further revealed that many of these NETs possess a high degree of tissue specificity [17–19]. Recent work indicates that tissue-specific NETs can influence spatial genome organization and gene expression [17, 20]. These findings suggest that the NE is a complex cellular compartment, which integrates both cytoplasmic and extracellular signals to regulate gene expression.

Spatial Genome Organization Directed from the Nuclear Periphery

The three-dimensional architecture of the genome is not random. For example, the heterochromatin, historically defined as denser chromatin observed by electron microscopy, tends to be concentrated at the nuclear periphery and around nucleoli and centromeres in most cell types [21]. Of these structures, the NE provides a large two-dimensional surface with a specific set of proteins to organize the genome. In general, if one were to consider the area spanning 50 nm inwards from the NE against the total volume of a typical mammalian nucleus (~5–10 μm in diameter), the NE could be considered to control roughly 1/30th of the nucleus. As each chromosome is one long folded strand of DNA, this NE tethering could physically propagate effects along the DNA polymer deep inside the nucleus. The idea that there appears to be a direct physical contact between chromatin and the NE has been supported by microscopy studies [22, 23] and biochemical experiments detecting retention of chromatin components after NE purification and extraction with high ionic strength buffers [24].

The notion that higher order chromatin structure might play regulatory roles was implied by observations that the distribution of heterochromatin is relatively uniform within a given cell type, yet it can vary widely between different cell types. For example, neurons tend to have very little peripheral heterochromatin while hepatocytes display a uniform and patchy heterochromatin distribution, epithelial cells have a less uniform patchy distribution, fibroblasts have a uniform distribution, and lymphoblasts tend to have an enormous amount of heterochromatin that extends several microns into the nucleus from the periphery [21]. In the case of lymphoblast cells, this dense chromatin largely dissipates upon immune activation, consistent with the idea that the strongly negatively stained material represents transcriptionally inert, but plastic facultative chromatin [25, 26].

Heterochromatin is now defined by specific chromatin epigenetic modifications such as histone H3 lysine 9 dimethylation and trimethylation (H3K9me2 and H3K9me3) and lack of histone acetylation and H3 K4 dimethylation and trimethylation (H3K4me2 and H3K4me3), which are normally present at genes that are actively transcribed or poised for transcription. Recent studies clearly demonstrate that antibodies against H3K9me2, a mark characteristic of facultative heterochromatin, detect chromatin positioned close to the NE, while antibodies against H3K9me3, a modification found at constitutive heterochromatin associated with telomeres and centromeres in higher eukaryotes, stains internal heterochromatic domains [27]. These data support earlier biochemical and microscopic observations indicating that the heterochromatin protein 1 alpha (HP1 α) seems to have a distinct subpopulation at the NE [28]. Recently, many specific interactions have been reported between the NETs, lamins and silent chromatin as well as cross talk between NPCs and transcriptional regulatory mechanisms. However, the precise relationship between gene activity and higher order chromatin organization at the nuclear periphery is not yet entirely clear.

Centromere and Telomere Patterns of Spatial Genome Organization

While early observations made by clinicians suggested that changes in nuclear morphology in tumor cells might be functionally important, it was not until the late 1800s that cell biologists began to notice that genome organization is not completely random [29]. The first basic description of such nonrandom nuclear organization came from Carl Rabl who found that the centromeres in nuclei from salamander larvae were located at the nuclear periphery, concentrating at the side of the nucleus where the centrosome was located (though on the outside) [29]. This “Rabl configuration” has since been observed in many plants and higher mammals, though it is more often transiently occurring just before or during mitosis and particularly meiosis [30, 31]. It also has been observed for telomeres, with associations at one pole of the nucleus occurring in both mitotic cells and in some interphase cells. One possible role for the Rabl configuration in meiosis is to orient the synaptonemal complex and align chromosomes for homologous recombination [32].

In interphase mammalian cells centromeres are not typically located at the periphery, but they do accumulate at the NE in certain cell types such as human neutrophils [33]. Though NE tethering of telomeres is also usually transient, this connection appears to be permanent in budding and fission yeast [34, 35] and is maintained throughout sperm development in mammals [36]. The NET SUN proteins have been linked to both telomere and centromere tethering to the NE in certain systems [36–40]. Most recently, the interaction between the fission yeast Sad1, a SUN family member, and a novel kinetochore protein Csi has been found to direct the tethering of centromeres to the NE [40]. Older studies suggested that lamins are also able to bind to specific chromatin structures such as centromeres and telomeres [41, 42]; however, as lamins also bind SUN proteins, it is possible that these reports reflect the SUN interactions.

Centromere function can be very important for cancer biology both by being essential for proper chromosome segregation in mitosis and, through these NE connections, for the formation of the synaptonemal complex. Improper formation of synaptonemal complex could contribute to chromosome translocations and aneuploidy. Telomeres also have been shown to play important roles in aging, cancer, and cell immortalization as maintenance of telomere length is essential for the immortalization process. Although it is not known what effect telomere length has on spatial positioning, the NE tethering of telomeres and centromeres provides potential links between the NE, genomic instability and cancer, which merit further studies.

Large-Scale Patterns of Tissue-Specific Chromosome and Chromatin Organization

While the positioning of structurally important chromosome regions, such as centromeres and telomeres that are rich in repetitive sequences and present on all chromosomes, tends to be transient in higher organisms, the nonrandom distribution

of individual chromosomes tends to be tissue-specific. Theodor Boveri first suggested roughly 100 years ago that chromosomes tend to occupy particular domains in the interphase nucleus while studying eggs of the worm *Ascaris* [43]. However, only recently has work indicated that specific chromosomes have higher than random probability to occupy characteristic positions within the three-dimensional framework of the nucleus with respect to the NE. Whole chromosome fluorescence in situ hybridization (FISH) revealed that in human fibroblasts chromosome 18 tends to be located at the nuclear periphery while chromosome 19 tends to be positioned internally [44]. Much of this positioning appears to correlate with gene density of individual chromosomes [45, 46]; however, gene density cannot fully account for differences in chromosome positioning. For example in hybrid nuclei containing mouse and human chromosomes, the human chromosomes adopt a spatial position in the mouse cell nucleus based on the synteny with the mouse chromosomes rather than their gene density [47]. Moreover, the positioning of some chromosomes can vary between cell types in a tissue-specific manner although the molecular mechanisms that determine this phenomenon are as yet very poorly understood. For example mouse chromosome 5 tends to be peripheral in lung cells while being internal in blood and liver cells; also chromosome 6 is peripheral in CD8+ T-cells but internal in CD4+ T-cells [48, 49].

The spatial organization of chromosomes is often altered in tumors. Determining the position of chromosomes 18 and 19 in both normal and tumor cell lines revealed that their respective radial positioning with respect to the NE was highly conserved in normal fibroblasts, but in seven of eight tumor cell lines this particular positioning was much less pronounced [46]. It is not clear whether these observations reflect tissue-specific differences in nuclear organization between fibroblasts and epithelial tumors or altered chromosome numbers that are characteristic of tumor cells: further studies using matched cancer and normal tissues should elucidate this question. Importantly, the maintenance of tissue-specific chromosome positioning patterns may explain why some chromosomal translocations occur with higher frequency in certain tumor types. Recently, a study aiming to test whether chromosomes most commonly involved in tissue-specific tumor translocations were positioned adjacent to one another during interphase has found a statistically significant correlation between adjacent positioning of chromosomes in normal tissues and translocation frequency observed in tumors [50].

This was not only one of the first indications that different cell types favor certain chromosome groupings [48, 49], but it also highlights that chromosome positioning may explain the tissue-specific patterns of genomic instability observed in cancer and other human disease conditions.

Large-scale differences in chromosome positioning may also reflect the above-mentioned tissue-specific patterns of heterochromatin distribution in different cell types or, as an extreme example, the evolutionary adaptation of similar cell types to function under diverse conditions. For example, a large comparative survey of heterochromatin organization in a variety of tissues and species detected profound tissue-specific differences in heterochromatin patterning as well as differences between similar cell types, such as the eye rod cell nuclei, in diurnal, nocturnal and aquatic mammals [11, 51]. Further experiments have suggested that heterochromatin

positioning in some cases could be explained by tissue- and cell type-specific expression of lamin B receptor (LBR) and lamins A/C, which contribute to tethering of heterochromatin to the nuclear periphery [11]. Whether this is the most prominent mechanism to achieve tissue-specific genome architecture is yet to be determined. Notably, many tissue-specific NETs could also fulfill this function.

Specific Gene Positioning with Respect to the NE

The finding that centromeres, telomeres, and gene-poor chromosomes associate with the NE together with the knowledge that most of the genome is noncoding might suggest that this spatial positioning is directed by general repetitive elements in the genome. The first indication that specific coding gene loci could be preferentially positioned at the NE came from *Drosophila* where several individual gene loci were observed to be reproducibly proximal to the NE [52]. This conserved positioning would be expected to have functional consequences.

We now know that many individual genes have nonrandom positions in the nucleus and many genes change position under certain conditions, particularly those under strong regulation during development. For example, the immunoglobulin H (*IgH*) locus moves from the nuclear periphery to the nuclear interior during B lymphocyte development at a critical time when the locus undergoes *V(D)J* recombination. Specifically, the *IgH* locus is at the NE in early lymphocyte lineages such as Pro-B cells and T-cells but is in the nuclear interior in later stages such as Pre-B cells [53]. Also during neurogenesis the *Mash1* (*Ascl1*) locus moves away from the periphery [54] and during adipogenesis several genes involved in lipid biogenesis that are upregulated during differentiation have been observed to move from the nuclear periphery to the interior [55]. Finally, the cystic fibrosis transmembrane conductance receptor (*CFTR*) gene is positioned at the NE in some cell types and in the interior in other cell types in a reproducible fashion [56]. Notably, in most cases described genes associate with the nuclear periphery in their inactive state and reposition towards the nuclear interior when active. Some genes, however, have been observed to exhibit a spatial preference for the nuclear periphery that is maintained rather than changing with differentiation and activation. These include the proteolipid protein (*PLP*) gene locus [57], the interferon- γ locus [58], the breast cancer *ERBB2* locus [59], and the osteogenesis collagen type 1 alpha 1 (*COL1A1*) locus [60].

In most studies, when the repositioning of individual genes between the nuclear periphery and interior was observed the rest of the chromosome was not tested. In cases when this was tested, many genes were found to move between the periphery and interior without a corresponding change in the position of the whole chromosome territory [55, 61]. This is consistent with numerous observations that chromosome territories often exhibit “looping out” of small regions [62–64]. However, the *FABP4* gene and its host chromosome both strongly shift from the nuclear periphery to the nuclear interior during adipocyte differentiation [55]. Thus genes and chromosomes may not always exhibit synergistic behavior and it remains unclear if the spatial gene positioning regulates or simply reflects gene activity.

Tethering of Chromosomes and Loci to the NE

In attempts to investigate the consequences of gene and/or chromosome positioning within the nuclear space several laboratories developed artificial tethering systems allowing recruitment of a single locus to the NE [65–67]. Three independent studies used mammalian cell lines with bacterial lac operator repeats (lacO) inserted into a genomic region that tended to be in the nuclear interior. Separately, the bacterial lac repressor (lacI) that specifically binds these repeats was fused to either GFP or to a NE protein (lamin B1 or the NETs LAP2 β and emerin) and expressed in the cells carrying the array. Expression of the lacI-reporter fusion to GFP had no effect on the position of the lacO locus within the three-dimensional organization of the nucleus, but when the lacI fused to a NE protein was expressed the lacO array repositioned from the nuclear interior to the NE [65–67]. Once at the NE, the tethering could be reversed because the binding of lacI to lacO sequences can be disrupted by addition of isopropyl β -D-1-thiogalactopyranoside (IPTG). Addition of IPTG to disrupt this interaction caused the locus to be released from the periphery. Several important conclusions stemmed from these studies. In all cases, tethering of the lacO array to the nuclear envelope led to repositioning of the entire chromosome carrying the lacO array from the nuclear interior to the nuclear periphery. However, the effect of this repositioning on gene expression was not consistent between individual studies (see below). It was also observed that when chromosome 11 containing the lacO repeats moved to the periphery, another chromosome, chromosome 4, moved away from the periphery suggesting that genes/chromosomes may compete for space based on the strength of affinity interactions. As the NE represents only $\sim 1/30$ th of the nuclear volume it would not be likely to be able to accommodate all chromosomes in a typical nucleus. Lastly, the positioning of lacO repeats and their recipient chromosome was not heritable - the loss of lacO-lacI affinity interaction upon treatment of the cells with IPTG resulted in loss of peripheral localization of the lacO array in daughter cells. This argues that in the case of endogenous loci/chromosomes a particular pattern of affinity interactions must be restored at the end of each mitosis for a specific cell type to achieve a particular chromosome configuration. Such reestablishment would not be expected to be completely accurate. Thus, not surprisingly, a particular organizational pattern using the directed lac array system was never achieved in more than 89 % of cells and endogenous patterns tend to be achieved at frequencies of 60–80 %. A recent study using an elegant experimental set up to follow specifically the localization of peripheral chromatin through several rounds of mitosis also clearly demonstrates that sequences labeled as peripheral in mother cells may (with some degree of probability) end up localizing internally in daughter cells [27]. This is likely to reflect the randomness in chromosome movements when aligning at the metaphase plate that may result in some chromosomes not being accessible when NETs bind to reform the NE in telophase. Such lack of accurate heritable propagation places the spatial genome organization outside the classical definition of epigenetics.

The lacO-lacI system provides an extremely strong tether not just because of the high binding affinities between lacO and lacI, but also because the lacO sequence usually is amplified 128–256 times in the array. Though this is certain to be stronger than any individual chromatin–NE interactions in mammalian cells, interaction sites on human chromosomes responsible for spatial genome organization would likely be both abundant and widely distributed, thus providing many tether points that would in the end have the same effect as the amplified lacO array. Alternatively, large gene clusters such as at the *IgH* locus, *Hox* loci, and olfactory receptor gene clusters might provide unique binding sites that would create distinct microenvironments at the NE. Indeed, a recent study found that olfactory receptor gene clustering is associated with the NE [68].

So how are loci and/or chromosomes tethered to the NE? The NE tethers for chromatin should be strongly embedded core components of the NE, e.g., lamins and NETs. Lamins form an intermediate filament polymer resistant to most standard cell extraction conditions such as 1 M NaCl and 2 % detergent and some lamin-lamin interactions can withstand 6 M urea extraction [69]. Thus, the nuclear lamina provides a strong scaffold to tether chromosomes. Lamins have been shown to bind core histones, particularly the H2A/H2B subtypes [70–72]. These interactions would not be expected to discriminate any particular areas of the genome, however, these studies were performed before the identification of many histone modifications and it is possible that if revisited some specificity might be observed. Some specificity was also observed in lamin binding to DNA, in particular to repetitive AT-rich sequences and the minor groove of single-stranded DNA in matrix- and scaffold-attachment regions (MARs and SARs; [41, 73–75]).

Just as the NET SUN proteins are responsible for the tethering of centromeres and telomeres to the NE, other NETs are likely to contribute to specific chromosome attachments. As the NETs are embedded in the membrane, they provide a strong anchor to chromatin, but this strength is further increased when considering that most NETs tested thus far have been shown to bind the lamin polymer [6]. That the NETs could contribute specificity to genome organization was first supported by observations that LBR and emerin bind to distinct positions on chromosomes at the earliest stages in NE assembly in telophase [76]. LBR has, like lamins, been found to bind histones, though in this case histones H3/H4 [77], and also to heterochromatin protein 1 (HP1) [10], which binds with high affinity to methylated histone H3 tails (H3K9me2 and H3K9me3) [78, 79]. These interactions provide a mechanistic explanation for the enrichment of H3K9me2 modified heterochromatin at the nuclear periphery. Notably, either pharmacological inhibition or knockdown by RNA interference of the enzymes responsible for depositing H3K9me2 (G9a and GLP, also known as EHMT2 and EHMT1, respectively) led to dissociation of peripheral chromatin from its proximity to the nuclear lamina [27].

The NET LAP2 β has also been shown to bind to core histones [80], but also binds directly to DNA and to the barrier-to-autointegration factor (BAF) [81, 82]. BAF is a soluble protein that binds both to histones and DNA and so can contribute to higher order chromatin structure [83]. BAF binding could bring some specificity

to NE–chromatin interactions because it has particular affinity for selected linker histones including H1.1 [84]. The NET MAN1 can also bind BAF, but separately binds directly to DNA through a winged helix fold domain in its carboxyl-terminal domain [85].

The NETs mentioned above are all widely expressed and so it is relatively easy to imagine how they could contribute to general spatial genome organization patterns such as those related to gene density—and indeed LAP2 and LBR have been linked to such general organization [11, 12, 68]. However, it is not easy to explain how these ubiquitously expressed proteins, except perhaps LBR, could contribute to the tissue-specific patterns of genome organization observed for certain chromosomes and gene loci. It would seem more likely that tissue-specific NETs recently discovered in proteomic analyses of NEs isolated from several different tissues [17–19] might perform this function. Indeed, screening of novel blood-specific NETs identified one that promoted chromatin condensation and two others that repositioned a gene locus [17]. Moreover, a recent study indicates that several NETs expressed preferentially in either liver, fat, or muscle cells can reposition chromosomes to the NE when exogenously expressed in fibroblasts and, in the case of the liver-specific NETs 45 and 47, their knockdown resulted in release of certain chromosomes from the NE in liver cells [20]. The same study showed that different NETs are able to affect the positioning of different subsets of chromosomes, indicating that these are indeed the likely endogenous players that provide tissue specificity to spatial genome organization [20].

Relationships Between Nuclear Positioning and Gene Expression State

The idea that changing the position of a gene with respect to the nuclear periphery could lead to a change in gene expression received its first strong support in *Drosophila* when an insulator sequence called *gypsy* was found to be preferentially located at the nuclear periphery. When *gypsy* and a reporter gene were inserted into a more internal area of the genome, the locus was translocated to the periphery and the expression from the reporter was correspondingly reduced [86]. This came to be known as one of the many examples of position effect variegation in gene expression that have been observed in *Drosophila*, yeast and mammalian cells. The example above is somewhat anecdotal and may reflect caveats with experimental design as insulator sequences, such as *gypsy*, serve as boundary elements protecting active genes from spreading of nearby heterochromatin [87, 88]. Insulator elements bind specific proteins and are involved in long-range chromatin interactions (chromatin looping), blocking of enhancer activity, and notably, delineation of subnuclear localization of chromosomes [87, 89].

In mammalian cells, insulator sequences are bound by zinc-finger proteins CTCF (CCCTC-binding factor) and its relative BORIS (*Brother Of the Regulator of Imprinted Sites*) [90, 91]. In recent years, the mapping of CTCF binding sites in the

genome [92] and the lamina-associated chromatin domains [93, 94] in conjunction with maps of chromatin modifications and gene expression profiles have provided important insights into the relationship between nuclear positioning, chromatin architecture, chromatin modifications and gene expression state.

Gene Activity at the Nuclear Periphery

Apart from the *gypsy* insulator experiment mentioned above, multiple examples can be found in literature suggesting that genes located at the nuclear periphery tend to be transcriptionally inactive, late replicating and marked by chromatin modifications indicative of silenced chromatin [95]. When genes were found to migrate to the nuclear interior upon differentiation or under the influence of external stimuli, this in some cases was accompanied by upregulation of transcriptional activity, increase of histone acetylation and the presence of RNA polymerase II at gene promoters [53, 54]. However, as alluded to earlier, examples exist demonstrating that not all genes located at the nuclear periphery are transcriptionally silenced. Moreover, the cause and consequence relationship between gene location and gene activity has always been difficult to infer from studies on individual gene loci.

The experiments using lacO arrays to tether genomic loci to the NE [65–67] were partly designed to test the effect of nuclear positioning on gene expression in a more controlled fashion. Two of these studies had a selectable marker inserted in the array and both found that transcription of this particular marker was reduced when the locus was at the periphery [65, 67]. Correspondingly they found that release of the locus from the periphery with IPTG restored the lost activity to the marker genes [65, 67]. One of the studies also tested endogenous genes close to the area where the lacO array was inserted, finding that repression was not general with only some genes being repressed when the locus was at the periphery [65]. In the third study no repressive effects or deficiencies in the induction dynamics of a reporter gene inserted by the array were observed between its internal and peripheral positioning [66]. However, in this study the reporter was strongly and actively induced from a promoter that could potentially overcome any repressive effects of the periphery. Therefore, the question of whether and how tethering a locus to the periphery directly results in its repression remained unresolved by these studies, but it is clear that changes in gene regulation can occur concomitantly with changes in gene positioning.

The general features of gene activity at loci in close proximity to the NE became apparent when the nuclear lamina associated chromatin domains were mapped on a genome-wide scale in *Drosophila* and mammalian cells by a technique known as DamID [93, 94]. The DamID method used in these studies employed a fusion of lamin B with a GATC sequence-specific bacterial DNA adenine methylase (Dam). As adenine methylation in GATC context does not exist in higher eukaryotes, any DNA carrying this mark in cells expressing the Dam-laminB fusion signifies proximity to the nuclear lamina. This approach defined the *lamina-associated domains*

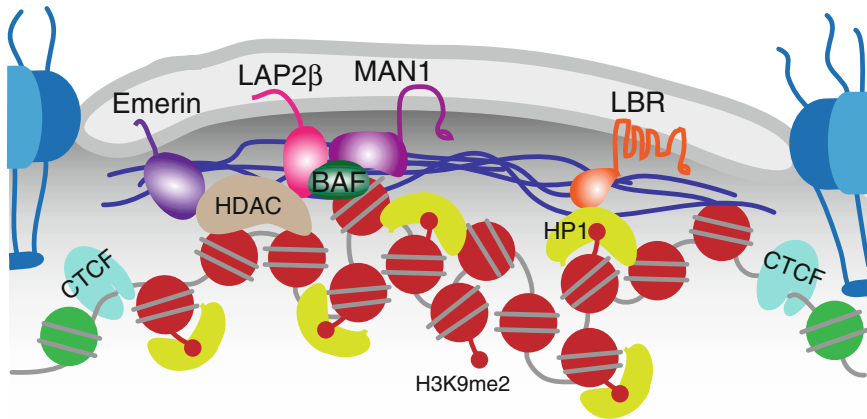


Fig. 2 Anchoring and silencing of chromatin at the nuclear envelope. Silenced chromatin is both recruited to and maintained at the NE through interactions of lamins and NETs with chromatin and chromatin modifying enzymes. Lamina-associated domains (LADs) are bounded by CTCF on chromatin. Silent marks such as histone H3 lysine 9 di and tri methylation (H3K9Me2 and Me3) recruit the additional silencing factor heterochromatin protein 1 (HP1), which in turn can bind to the NET lamin B receptor (LBR, so named because it in turn binds the lamin polymer). One part of the barrier-to-autointegration factor (BAF) can bind histones while another part binds to the NETs emerin, LAP2 β and MAN1. LAP2 β and emerin have also been found to bind histone deacetylases, which further promote and maintain silencing marks at the periphery

(LADs) as stretches of DNA 0.1–1 mega bases (Mb) in length. In human fibroblasts, there are more than 1,300 of such genomic regions in close contact with the nuclear lamina. LADs are present on all chromosomes, associated with repressive histone modifications (H3K9me2 and H3K27me3) and are relatively gene-poor (Fig. 2). Genes embedded in LADs have low levels of expression with very few active genes that escape silencing. Most LADs have sharp boundaries that are marked by insulator elements bound by CTCF [94] (Fig. 2). This organization of chromatin into LADs and inter-LAD domains is also conserved in *Drosophila* [93] with a different set of insulator elements marking the LAD boundaries [87].

Given the vast changes in gene expression that occur during differentiation and the observations that the radial positioning of chromosomes varies in a tissue-specific manner, one would expect LADs and CTCF binding sites also to change substantially during differentiation. Surprisingly, the LADs and H3K9me2-rich domains, which largely overlap with LADs, remain largely invariable during differentiation of embryonic stem cells into neuronal progenitors and further into mature neurons [96–98]. Instead of a global rearrangement of genome-nuclear lamina interactions, small local changes affecting individual genes were observed in these studies. Thus, LAD-embedded genes, which were upregulated during neuronal differentiation, were seen to dissociate from the lamina and lose silencing histone modifications [96, 97]. This may seem surprising, however, such global maps represent population average and cell-to-cell variations are lost in such studies. Thus, LADs can be viewed as a probabilistic map of genome-nuclear lamina interactions. Techniques that permit global genomic studies on a single-cell level are starting to emerge and promise

to be instrumental in determining cell-to-cell variation in cultured cells and, excitingly, cells derived from specific tissues. As mentioned earlier, tracking individual cells through mitosis detected substantial rearrangement and dissociation of LADs from the nuclear lamina in daughter cells [27]. This, perhaps, allows rearrangement of LADs and activation of LAD-embedded genes. It also suggests that rearrangement of chromatin–lamina interactions may be proportional to the number of cell divisions undertaken by cells before they acquire the differentiated state. In the case of in vitro differentiation models using ES cells, the differentiated state is achieved within very few mitotic divisions, which may not allow sufficient time for significant LAD rearrangements to take place.

Is the dissociation of genes from the nuclear periphery sufficient to induce gene expression? In the lacO tethering experiments to the NE, it was found that the release of the locus from the periphery either by IPTG (disrupting lacO–lacI interactions) or treatment with histone deacetylase (HDAC) inhibitors could restore the lost activity to marker genes and few, but not all, endogenous genes in close proximity to the lacO array [65–67]. However, recruitment to peripheral chromatin of the strong transcriptional activator VP16, which interacts with histone acetyltransferases (HATs) and disrupts nuclear lamina–chromatin contacts, induces relatively few changes in gene expression on a global scale [27]. Taken together, these experiments suggest that movement of loci away from the nuclear lamina is perhaps permissive, but not sufficient for gene activation.

Recruitment and Silencing of Chromatin at the NE

The question of whether or not genes are silenced before they are anchored to the NE is not yet comprehensively answered. Notably, many interactions between chromatin, the nuclear lamina and the NETs may require preexisting histone modifications. Thus, chromatin anchoring to the INM embedded lamin B receptor LBR via HP1 α and HP1 γ [10] requires H3K9me2 to allow HP1 binding (Fig. 2). Although there is some evidence that BAF (barrier-to-autointegration factor), which binds the NETs LAP2 β , emerin, and MAN1 through a shared sequence motif called the LEM domain [81, 99, 100] interacts with histone H3 methylase G9a (EHMT2) [101], in most mammalian cell types G9a is distributed throughout the nucleoplasm and not exclusively anchored to the NE. However, additional evidence suggests that NETs emerin and LAP2 β interact with histone deacetylases, HDAC3 being one of them [102, 103] (Fig. 2). Therefore, it is possible that localized HDAC activity at the NE provides G9a with a suitable substrate for subsequent methylation of H3 tails generating H3K9me2 required for anchoring to LBR-interacting HP1 proteins.

The interactions of BAF with NETs raise another interesting issue regarding anchoring to the NE of silenced chromatin. The NET LAP2 β has several soluble splice variants that also bind BAF [104–106]. One of these, LAP2 ζ , principally resides in the cytoplasm and its upregulation compared to LAP2 β causes BAF to be captured in the cytoplasm before nuclear import, thus reducing intranuclear pools and their corresponding functions in cross-linking chromatin and recruiting it to the

NE [106]. As many NETs have multiple splice variants, this type of competitive inhibition will likely prove to be used commonly as a regulatory mechanism for chromatin recruitment to the NE.

In addition to chromatin-mediated interactions, several DNA-binding factors have been found to interact with NETs and the nuclear lamina. One of these is the transcription factor Oct1, which interacts with lamin B and localizes to the nuclear periphery in a lamin B-dependent manner [107]. As Oct1 motifs are enriched within LADs, this suggests that Oct1 may contribute to tethering to the NE of genomic loci in a sequence-specific manner.

Activation of Chromatin at the Nuclear Periphery

Among the proteins identified in a proteomic study of rat liver NEs were several that can modify histones for not only repression, but also activation. One of these, NET43/hALP, is a histone acetyltransferase [16]. Interestingly, the membrane prediction for NET43/hALP only occurs in some organisms and was absent in the human homologue. Nonetheless, the human version localizes to mitotic chromosomes in mammalian cells by binding to another NET, SUN1 [108]. Depletion of SUN1 in human tissue culture cells resulted in delayed chromosome decondensation and a reduction in histone H2B and H4 acetylation in a manner dependent on hALP [108]. Interestingly NET43/hALP appears to be upregulated during lymphocyte activation when the large amount of dense peripheral chromatin of resting lymphocytes becomes decondensed [17].

Functions of the NPC in Spatial Genome Organization and Gene Regulation

Each NPC is a large protein assembly of >40 MDa in yeast and >60 MDa in mammalian cells made up of at least 30 core proteins [13, 15]. The assembly has an eightfold symmetry so that each protein is represented in a minimum of eight copies and in some cases in as many as 64 copies in a single NPC. Among these proteins is Tpr, a 270 kDa coiled-coil protein that extends roughly 100 nm into the nucleoplasm in a structure generally referred to as the nuclear basket [109], and Nup50, which also associates with the nuclear basket extending roughly 50 nm from the central plane of the NPC into the nucleoplasm [110]. These extensions allow interactions with chromatin of a different character to those at the level of the membrane. Electron micrographs and recent high-resolution imaging of mammalian nuclei [111] show that while heterochromatin contacts the areas of the NE which are lined with nuclear lamina, the NPCs are surrounded by less dense chromatin. Studies in yeast and higher eukaryotes demonstrate that NPCs contribute in several different ways to the spatial genome organization and regulation of gene expression.

The NPC in Spatial Genome Organization

Although in mammalian cells telomeres are tethered to the NE through interactions with SUN proteins, in yeast they are tethered by the NPC. In fact, the first specific interactions between telomeres and the NPC were determined in yeast with the yeast Tpr homologue, Mlp, as the anchoring site [34, 35]. The difference in telomere tethering between yeast and mammals together with the lack of yeast homologues for many NETs, particularly the more tissue-specific ones [18], suggests that many regulatory functions in yeast carried out by the NPC have been subsequently taken over by other NE proteins during evolution.

The recruitment of telomeres to the periphery in budding yeast is essential for silencing of subtelomeric genes. In addition to Mlp (Tpr), peripheral localization of telomeres also involves the soluble non-NPC protein Ku [112]. Mutation in Mlp and Ku proteins results in derepression of subtelomeric silenced genes [34, 35, 113, 114]. As a potential epigenetic mechanism, the heritability of this silencing was also addressed. Derepression of reporter genes integrated close to telomeres upon deletion of Mlp and Ku implied that silencing requires NE/NPC association [34, 114]. However, deletion of the NPC proteins also results in a redistribution of Sir3p fused to GFP [114]. The latter result argues that the observed derepression of genes could be a secondary consequence of NPC disruption, rather than due to relocation of the telomeres away from the nuclear periphery. Another study, using an elegant experimental setup to break the connection between the NPC and a silenced reporter after the silencing was established, found that the release from the periphery did not derepress the silent reporter [115]. This suggests that once established at the nuclear periphery the silenced chromatin state can be stably maintained without NPC association.

Active Chromatin at the NPC

In contrast to chromatin at the nuclear lamina, the NPCs in many species including yeast, flies and mammals associate with active chromatin [116–119]. Adaptor proteins that mediate these connections have been identified in a variety of systems. In yeast, the nuclear basket protein Mlp1 interacts with the chromatin-bound co-activator complex SAGA (Spt-Ada-Gcn5-Acetyltransferase), which is known to promote active transcription [120]. SAGA is a large protein assembly, containing two chromatin-modifying enzymes, the Gcn5 histone acetyltransferase and Ubp8 histone deubiquitinase [121, 122]. SAGA, via its Sus1 component, was also shown to interact with the NPC-bound TERX-2 complex, which plays important roles in transcript elongation and mRNA transport [123–125] (Fig. 3). Budding yeast TREX-2 consists of four proteins (Sac3-Thp1, Cdc31, and Sus1) [126]. Thus, tethering of active acetylated chromatin to the NPC via Sus1, a shared component of SAGA and TREX-2 complexes, may help to coordinate transcription-coupled mRNA export [127]. This arrangement seems to be conserved in other species. In *Drosophila*, the Sus1 ortholog E(y)2 via the TREX-2 complex protein Xmas-2, the

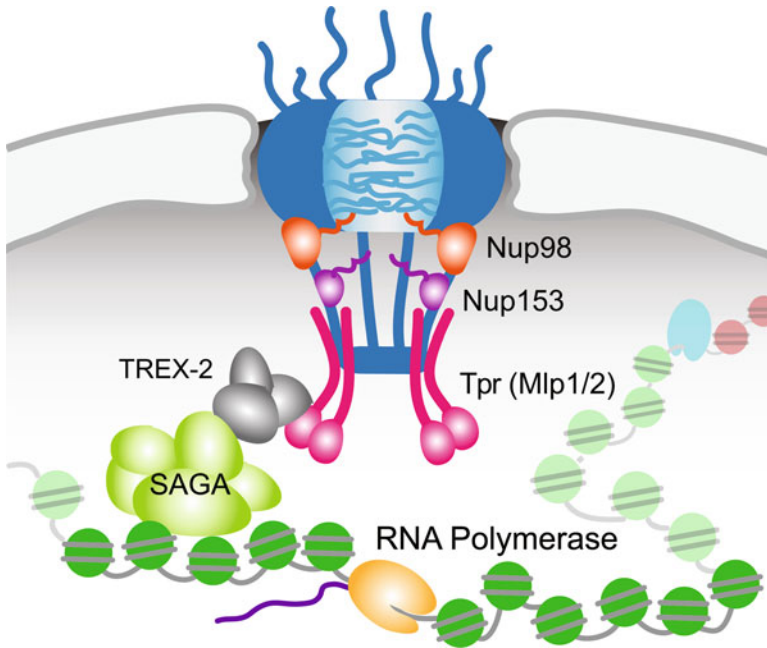


Fig. 3 Active chromatin at the nuclear pore. NPC proteins Nup98, Nup153, and Tpr form the nuclear basket, which tethers active chromatin through binding a complex of TREX-2 and SAGA. The SAGA complex promotes active transcription while the TREX complex is important for RNA export. Thus, recruitment of active genes to the periphery by this mechanism also facilitates rapid translocation of the mRNAs out of the nucleus so they can be translated on ribosomes in the cytoplasm. It is noteworthy that the average mammalian nucleus is estimated to have 2,000–3,000 NPCs and as they are 125 MDa complexes of greater than 100 nm diameter this translates to up to 40 % of the nuclear surface

equivalent of yeast Sac3, promotes transcription, mRNA export and positioning close to the NE of the *hsp70* gene cluster [128, 129]. Whether tethering of active chromatin follows a similar arrangement in mammalian cells is yet to be determined. Notably, the SAGA complex is highly conserved [122] and a Sac3-related protein GANP involved in mRNA export is present in human cells [130]. Therefore, it is likely that the positioning of active SAGA-bound chromatin at the NPC may operate in a similar manner across most eukaryotes.

Studies in yeast and *Drosophila* have also shown that several nucleoporins located at the nuclear side of the NPC are required for tethering of active chromatin to the nuclear periphery. Nup1, Nup2, Nup60, and Mlp1 (Tpr in metazoan species) have been implicated in mediating connections between active genes and the NPC [120, 131, 132]. One of the most interesting examples signifying the functional importance of such tethering is dosage compensation in *Drosophila*. Male flies, having one X chromosome, achieve balanced expression of X-linked genes relative to female flies with two X chromosomes by upregulating gene expression on the single male X. This upregulation requires *Drosophila* dosage compensation complex, which includes noncoding RNAs, histone acetyltransferase MOF and

additional proteins, as well as the nuclear basket proteins Nup153 and Tpr [117, 133]. Taken together, these examples implicate nucleoporins located at the nuclear side of the NPC in spatial genome organization and regulation of gene activity.

It has been suggested that, topologically, positioning of active genes at the NPC may occur via formation of chromatin loops mediated by promoter–terminator interactions as well as the boundary elements in yeast and their equivalent, the insulator elements, in other metazoan species [92, 134, 135] (Fig. 1). Such elements are often found at the boundaries between active and repressed chromatin and serve to antagonize heterochromatin spreading. Consistent with such a function, studies in yeast and human cells have shown that chromatin in close proximity to the NPC carries modifications indicative of both, active and repressed, chromatin states [92, 136]. A screen for proteins involved in boundary activity in budding yeast identified Mlp, Nup2 (the yeast homologue of Nup153 in humans), the NPC-associated proteins Nup60p, and the Ran-GTP exchange factor Prp20p [134, 137]. The typically mobile Prp20 is bound to the core structure of the NPC through Nup2 on one side and on the other it binds H2A.Z (also called Htz1), a variant of histone H2A that is loaded by the SWR-C chromatin-remodeling complex [138]. H2A.Z marks relatively immobile nucleosomes in the yeast genome and can be found at most gene promoters and some intergenic regions of the budding yeast genome [139]. Interestingly, the SAGA complex may also contribute to boundary/insulator element function. It has been reported that the Ada2 component of SAGA is recruited to yeast telomeres and required to suppress the spreading of telomeric heterochromatin into subtelomeric regions [140]. Moreover, Sus1, a shared component of SAGA and TREX-2 complexes, functions together with Su(Hw) at insulator elements in *Drosophila* [129]. The overall similarity between the function of boundary elements in yeast and the insulators in *Drosophila* and mammalian cells indicates that the compartmentalization of chromatin into active and inactive domains at the NE is widely used in evolution.

Transcription Factors at the Nuclear Envelope

The nuclear surface provides a large scaffold on which the genome can be spatially organized and regulated; however, in relative terms it represents only ~1/30th of the total nuclear volume. If a spherical nucleus has a radius of ~5 μm , then the volume of the nucleus would be 523 μm^3 and the surface area would be 314 μm^2 . However, the thickness of the NE from ONM to INM is only ~50 nm, and the penetration of NETs and lamins from the inner surface into the nucleoplasm is likely much less than this, while the NPC nuclear baskets have been measured to project roughly 100 nm into the nucleoplasm. Thus, if one considered an average penetration of 50 nm for the “volume” of the NE, the volume of the nuclear surface would be less than 16 μm^3 or roughly 30-fold smaller than the volume of the nucleoplasm. Thus, if, in addition to the genes tethered by the NE (discussed above), the NE also tethered transcriptional regulators for those genes, it would have the equivalent effect of increasing the local concentration of the transcriptional regulator by 30-fold.

The Function of NE in Direct and Indirect Repression of Transcription

Both LAP2 β and emerin bind the transcriptional repressor germ cell-less (*gcl*) that is known to affect E2F/DP transcription factor heterodimers [141, 142]. Moreover, overexpression of LAP2 β in tissue culture cells inhibited E2F-dependent transcription from a reporter construct [142]. Emerin also binds Btf, another transcriptional repressor with a different target specificity [143]. Of the lacO-lacI studies that used NE affinity tethering to recruit a lacO array to the periphery one fused the lacI to LAP2 β and the other fused lacI to emerin [65, 67]. The overlap and discrepancies in results between these studies might be in part because of the partly shared and partly different specific transcriptional repressors binding to these two NETs. In the emerin study the amino-terminus was deleted to minimize this potential criticism [67]; however, the deleted region only partly overlaps with the binding site on emerin for germ cell-less [141], so this may not have been sufficient.

Whereas it has been demonstrated that multiple different NPC proteins can recruit transcription factors and their target genes to the same location to effectively increase the relative local concentration of the transcription factor, NETs and lamins that have been found to bind to transcription factors appear to function in the opposite fashion. They sequester the transcription factor at the periphery away from the gene target in the nuclear interior. Thus, NE binding of transcription factors appears to function to prevent gene activation.

The first demonstration of transcription factor binding to NE proteins was binding of lamin A to the retinoblastoma protein (Rb) [144]. However, the effect of lamins on Rb may be due mostly to the nucleoplasmic pool of lamins. This is supported by findings that a soluble splice variant of the NET LAP2 β , LAP2 α , forms a complex with lamin A and Rb in the nuclear interior [145]. Interestingly, this can have both positive and negative effects on gene regulation depending on cell type. Although this complex on the one hand sequesters Rb away from gene targets, on the other hand the complex stabilizes Rb, which, without this binding, turns over rapidly. As a consequence of this, in cells stimulated by phosphorylation of Rb to initiate progression into S-phase, the higher levels of stabilized available Rb enable much stronger activation. Thus, cells with more or less LAP2 α and lamin would have different propensities to engage cell cycle progression upon the same activation stimulus. Consistent with this, LAP2 α knockout mice exhibit hyperproliferation of both erythroid and epidermal lineage cells [146]. Lamins are ideal for sequestering transcriptional regulators as their abundance (~3,000,000 copies per mammalian nucleus; [147]) could easily saturate any transcription factor. Lamin A has also been shown to bind cFos [148].

In addition to lamins, NETs also repress gene function by sequestering transcription factors. Emerin interacts with the transcription factor Lmo7 [149]. Intriguingly, this interaction has been found to function in a tightly regulated feedback loop to regulate the emerin gene itself. Lmo7 activates the emerin gene (*EMD*) so that emerin protein binding of Lmo7 sequesters Lmo7 at the periphery away from the emerin gene; thus, the more emerin is produced, the more it can sequester Lmo7 to

repress its own expression. Similarly, the NET MAN1 interacts with Smads and sequesters them away from target genes located in the nuclear interior [150, 151]. Very few of the many tissue-specific NETs recently identified have been characterized in detail, but it seems likely that some of these may bind to transcription factors for sequestration. The tissue specificity of these NETs would add a much greater complexity to NE regulation of gene expression.

Transcriptional Activation at the NE

In addition to binding Smad transcription factors, MAN1 directly binds DNA through a winged helix fold in its carboxyl-terminal domain [85]. Thus, though this has not been specifically demonstrated yet, it could potentially also tether both Smads and Smad-regulated genes to the NE to additionally activate transcription as shown for the NPC proteins.

While older studies showing the dissipation of dense chromatin at the nuclear periphery concomitant with activation of lymphocytes suggested a role for the NE in gene activation [26], several more recent studies show with certainty that transcriptional activation occurs from the NE. The *PLP* gene, involved in myelin production, becomes activated when already at the periphery [57]. It is interesting that this gene is active in glial cells, as it was noted earlier that brain cells tend to have minimal peripheral heterochromatin. It is thus reasonable to postulate that the ability to activate a gene at the NE may depend on cell type and the amount of peripheral heterochromatin. Other differentiation/cell state-associated genes observed to be active at the NE are the breast cancer *ERBB2* gene, the osteogenesis *COL1A1* gene and the interferon gamma *IFN- γ* locus [58–60].

Stabilization of the Genome by the Nuclear Envelope

Though only theoretical at this stage, the physical tethering of chromatin to the NE could play a major role in stabilizing the genome to protect against translocations and other factors that can lead to cancer. Proteins in the nuclear interior, even those associated with nucleoplasmic structures, tend to be relatively dynamic in FRAP studies [152]. In contrast, the movements of proteins at the NE are much less dynamic and indicate local constraints [153, 154]. One potential consequence of NE tethering could be to physically stabilize the genome, minimizing movement. Indeed, transgenes located near the nuclear periphery in mammalian cells have been shown to be less mobile than those residing in more internal positions [155]. In theory, tethering of chromatin to the NE could help maintain chromosome territories and prevent entanglement of chromosomes and potentially associated chromosome translocations that could lead to tumors. This could be particularly important during replication and even the reason why late-replicating DNA tends to be at the periphery [93, 156], i.e., because a peripheral tether combined with silent chromatin helps to stabilize chromosome territories so that chromosomes do not get entangled during replication.

The NE is a good tethering point for chromatin because it is a relatively stable structure due to the intermediate filament lamin polymer lining the inner surface of the NE [4]. It is striking that while the cytoplasm has actin filaments, microtubules and intermediate filaments, the NE has just the intermediate filament lamins. Unlike other cytoskeletal systems, intermediate filaments are highly elastic. Under compression or tension forces that would break actin filaments and microtubules the intermediate filaments are unaffected [157]. It is not surprising thus that spider's webs are made of intermediate filaments, tough yet elastic.

These properties are important because live cell microscopy indicates that nuclei move and exhibit frequent morphological aberrations while chromatin also moves dynamically. Thus, the NE needs to have a structural support that can bend, but not break connections. In this light it is not surprising that both lamins and INM NETs bind chromatin and each other, thus providing multiple contacts in an overlapping network embedded with a wide range of proteins into the membrane to create a very strong tether. Other INM NETs make connections across the lumen of the NE to outer membrane NETs [158] and these in turn connect the NE to the cytoskeleton providing an anchor for the nucleus in the cell [159]. Together these properties enable the NE to keep chromatin tethered while still being able to stretch in response to forces placed on the polymer by genome movements or the cytoskeleton. If the peripheral lamina nucleoskeleton were rigid like microtubules, it would likely break in response to such forces and genes and chromosomes would lose their tethering. Similarly, if tethered merely by transmembrane proteins, strong forces from chromosome movements might rip the tethering NETs out of the lipid bilayer. Thus, the use of both lamins and NETs is a sensible strategy to support the many dynamic movements of chromatin within the interphase nucleus.

Although much of the above is merely a hypothesis, it is clear that lamins contribute to the mechanical stability of the nucleus. Lamin depletion or mutant expression resulted in nuclear lobulation and increased deformation under mechanical stress [69, 160–163]. In theory the importance of this is manifold. Increased lobulation would also increase the ratio of NE to nucleoplasmic volume, thus enabling greater silencing and gene regulation from the NE. Due to the DNA of chromosomes being single rope-like molecules, connections to the NE could influence the ability of internal sections to engage in transcription factories.

Peripheral Chromatin Organization and Nuclear Envelope Disease

Though lamins, NETs, and NPC proteins have all been linked to cancer in a variety of ways, most of these are indirect, e.g., having functions in processes that are critical to cancer progression as opposed to mutations in a particular NE protein causing cancer. In contrast, mutations in NE proteins have been found to be causative of a

wide variety of inherited disorders ranging from muscular dystrophies to the premature aging progeroid syndromes [164–166]. In fact lamin A is now the most mutated gene in the human genome. Lamin diseases include muscular dystrophies [167–169], lipodystrophy [170–172], neuropathy [173, 174], cardiomyopathy [175], dermatopathy [176], and the aging disease progeria [177, 178]. Several NETs and associated proteins also cause diseases or syndromes affecting muscle [179–182], bone [183, 184], brain [185–189], skin [176] and immune cells [190]. Because most NETs tested bind to lamins, lamin mutations could also affect NET distribution and function. Thus, the pathology of lamin-based diseases could be as much due to secondary effects on NET function as to the loss of lamin function.

Chromatin Organization in Heritable Diseases Linked to the NE

The overall distribution of heterochromatin as defined by electron dense material in electron microscopy is altered in several NE-linked diseases. Normal fibroblasts typically have a reasonable amount of this dense peripheral chromatin generally distributed throughout the periphery. In fibroblasts from patients with Emery–Dreifuss muscular dystrophy (EDMD), the dense chromatin often seems to have broken away from the NE and resides about 500 nm in from the NE. This altered pattern is observed for two different NE-linked muscular dystrophies, EDMD and Limb-girdle muscular dystrophy, and for both NET-linked and lamin-linked disease [191–193]. It is also observed in cardiomyopathy linked to the NE [194]. In contrast, fibroblasts from patients with NE-linked progeroid diseases tended to lose all peripheral dense chromatin [195], and fibroblasts from NE-linked lipodystrophy patients exhibited an intermediate phenotype with partial loss of peripheral dense chromatin in some areas at the periphery and partial clumping in other areas [193]. The fact that the patterns are not just disrupted, but disrupted in reproducible ways for each disorder suggests that these spatial genome organizational patterns are functionally relevant.

These NE protein mutations had specific and reproducible effects on spatial genome organization also in controlled experimental systems. For example, a mutation in lamin A that causes Hutchinson–Gilford progeria syndrome (HGPS) yielded an abnormal distribution of telomeres and clustering of centromeres [196] while other mutations that cause variously a neuropathy, lipodystrophy and muscular dystrophy reposition chromosomes 13 and 18 away from the nuclear periphery [197]. However, the relevance of these changes to specific disease pathology is uncertain as different mutations that cause the same disease can yield different effects on chromosome positioning. For example, both E161K and D596N lamin A mutations cause cardiomyopathy, but only E161K causes chromosome 13 to lose its normal peripheral localization [198].

Epigenetic Changes in Heritable NE Diseases and Cancer

Some of the reorganization of chromosomes, observed by FISH, and altered distribution of peripheral dense chromatin, observed by electron microscopy, could be explained by changes to epigenetic heterochromatin marks. This appears to be the case for HGPS where primary fibroblasts from patients have a significant loss of silenced chromatin marks associated with facultative heterochromatin, such as H3K9me2 and H3K27me3, while marks of constitutive heterochromatin such as H4K20me3 were increased [199]. Similar changes could be induced in normal cells upon overexpression of lamin A carrying a progeria-causing mutation [199]. Fibroblasts from a female patient also lost silencing marks on the inactive X chromosome and this chromosome lost its tight association with the periphery. Similar changes in silent chromatin marks have also been reported in normal aging cells [200]. Most cases of HGPS are due to a splice site mutation that causes loss of an exon that contains a cleavage site close to the C-terminus of lamin A. Similar to the small G proteins, Lamin A acquires a farnesyl group at its C-terminal CaaX box. This modification is transient as the last 18 amino acids are cleaved at this cleavage site in mature lamin A. Blocking lamin A farnesylation had a positive effect on HP1 α accumulation [201] and some of the heterochromatin defects observed in progeroid cells in culture could be reversed by treatment of the cells with farnesyltransferase inhibitors [202].

A number of studies have detected altered expression of NE components in cancer cells. For example, reduced expression of Emerin in ovarian cancer [203] and Lamin A/C overexpression in colorectal, ovarian, and prostate cancer correlates with poor prognosis and advanced tumor stage [204, 205]. Although such examples are numerous [206], a direct causal relationship between either mutations or altered expression of NE proteins has been established in very few cases. Nevertheless, indications that NE–chromatin interactions play an important role in genome organization and gene expression in cancer have started to emerge. It is well known that tumors exhibit altered patterns of DNA methylation in comparison to normal tissues [207]. These alterations include localized gain of DNA methylation at normally methylation-free CpG-rich gene promoters (CpG islands), leading to stable silencing of tumor suppressor genes, and widespread loss of DNA methylation from extensive chromosomal regions potentially promoting genomic instability. Interestingly, recent reports have shown that loss of DNA methylation in colorectal and other types of cancer occurs in regions of the genome that coincide with LADs and the silencing H3K9me2 [98, 208]. Moreover, gain of DNA methylation at promoters also occurs at genes located in these domains [208]. Taken together, these findings suggest that genome organization mediated by chromatin–NE interactions is not only profoundly different in cancer cells, but that these rearrangements may have direct functional implications in cancer development.

Another striking connection between NE components and cancer has emerged when translocations involving the nucleoporin Nup98 were found in patients with acute myeloid leukemia (AML) [209]. The most commonly detected translocations generate a fusion between Nup98 and homeobox transcription factor HoxA9 genes [207, 210]. The resulting Nup98-HoxA9 chimeric protein is nucleoplasmic and

contains the GLFG repeats of Nup98, normally involved in shuttling of nuclear transport receptors through the NPC, fused to the DNA binding domain of HoxA9. The mechanism by which the fusion protein induces AML is as yet unclear. It has been suggested that GLFG repeats bind transcriptional co-activator CBP/p300 histone acetyltransferase [211], thus maintaining expression of HoxA9 target genes, which are normally downregulated during differentiation of hematopoietic stem cells following downregulation of HoxA9 itself. Fusions of Nup98, all containing the Nup98 GLGF repeats, with a variety of other partners have also been identified in AML. These fusion partners include a variety of co-activator proteins, histone methyltransferases such as NSD1, DNA topoisomerases, and RNA helicases [209]. Similar to Nup98-HoxA9, the Nup98-NSD1 fusion protein was found to bind regulatory elements of polycomb target genes, normally silenced during myeloid differentiation, and induce high levels of H3K36me3 (a mark associated with active transcription elongation) and histone acetylation, thus maintaining the expression of several homeotic genes, which contribute to malignancy [212]. Mutations abolishing H3K36me3 activity of NSD1 as well as deletion of GLGF repeats of Nup98 within the Nup98-NSD1 fusion protein context abolish activation of homeotic genes and inhibit leukemogenesis [212]. By a broadly similar mechanism, fusion proteins between Nup98 GLGF repeat region and PHD (plant homeobox domain) zinc fingers, which bind to histone H3 tails methylated at K4, function as potent oncogenes and induce AML in mouse models [213]. These observations suggest that most chimeric Nup98 proteins function to promote AML by generating aberrant active chromatin, which counteracts polycomb-mediated gene repression and allows self-renewal and maintenance of the undifferentiated state of myeloid progenitors [214]. Thus, epigenetic changes caused by a mutant NE protein are an essential component of AML and, perhaps, other malignancies.

Conclusions

Research in the past few decades has led us to conclude that the NE is a complex compartment of the cell, with functions that extend beyond the separation of the genetic material from the cytoplasm of the cell and the control of nuclear trafficking. The NE contributes to tissue-specific genome organization and regulation of gene expression through many different mechanisms, the details of which remain to be fully elucidated. Lamins provide a structural framework for the nuclear periphery and interact with silenced chromatin. NETs bind both the lamin polymer and the membrane in addition to both general and specific chromatin proteins. Moreover, NETs and NPC proteins recruit transcriptional regulators, epigenetically modified chromatin, and separately recruit enzymes that add epigenetic modifications to chromatin. As only a fraction of the many novel NETs identified by proteomics have been characterized in detail, there are a great many possibilities for diverse mechanisms in the regulation of spatial genome architecture and chromatin modifications from the NE. The finding that many NETs are tissue specific further adds to this complexity.

There are many questions that remain to be elucidated. To what degree does the NE silence genes by bringing them into an environment that is rich with silencing factors that directly modify the chromatin versus bringing already silenced chromatin to the periphery by affinity interactions? Does NE tethering also contribute a steric effect to this regulation? To what degree are the NE influences on chromatin organization heritable? To what degree does NE tethering stabilize the genome to protect it from inappropriate recombination events? And how much do these various functions contribute to the initiation of tumorigenesis? The NE provides many layers for regulating genome function, and many important discoveries are certainly yet to be made in this exciting and rapidly moving field.

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