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Abstract

In the past 15 years our perception of nuclear envelope function has evolved perhaps nearly as much as the nuclear envelope itself evolved in the last 3 billion years. Historically viewed as little more than a diffusion barrier between the cytoplasm and the nucleoplasm, the nuclear envelope is now known to have roles in the cell cycle, cytoskeletal stability and cell migration, genome architecture, epigenetics, regulation of transcription, splicing, and DNA replication. Here we will review both what is known and what is speculated about the role of the nuclear envelope in genome organization, particularly with respect to the positioning and repositioning of genes and chromosomes within the nucleus during differentiation.

Introduction

The nuclear envelope (NE) is double-membrane system with the outer nuclear membrane (ONM) continuous with the endoplasmic reticulum (ER) and facing the cytoplasm while the inner nuclear membrane (INM) faces the nucleoplasm (**Fig. 1**).[1] Both membranes are joined in points where the nuclear pore complexes (NPCs) are inserted. These are >40 MDa gateways built from multiple copies of roughly 30 proteins called nucleoporins that mediate transport of proteins and RNA between the nucleus and cytoplasm.[2] Both the INM and ONM harbor specific sets of proteins including several integral membrane proteins generally referred to as NETs for Nuclear Envelope Transmembrane protein.[3] Cytoplasmic filament systems are tethered to the nucleus through several NETs of the ONM. These in turn form connections across the lumen of the NE to INM proteins that are grounded in the lamin type V intermediate filament polymer that lines the INM.[4] Lamin mutations reduce nuclear resistance to mechanical stress, indicating the polymer confers structural support; however, the baseline levels of this structural support vary among different cell types.[5-9] This is because ratios of the different lamin isoforms also vary among cell types[10] and each isoform has a different binding strength.[7] NPC proteins, lamins, and several NETs of the INM interact directly with DNA, chromatin and/or chromatin-associated proteins.[11]

Within the interphase nucleus the 3-dimensional architecture of the genome is not random. For example, denser chromatin observed by electron microscopy tends to be concentrated at the nuclear periphery and around nucleoli and centromeres.[12] Individual chromosomes also have a non-random distribution.[13] Each chromosome occupies a discrete non-overlapping territory in the interphase nucleus, yet certain specific chromosomes tend to be at the nuclear periphery while others tend be in the interior.[14] Such preferred positioning is observed in most cell types for some chromosomes, while other chromosomes favor peripheral or internal positioning only in certain cell types. Most of the non-random genome positioning

that has been observed is in relation to the nuclear periphery, suggesting that the NE is a tethering point for chromatin. The NE is perhaps the most logical choice for a structure from which to establish 3-dimensional genome architecture because the lamin polymer gives it structural stability to serve as an anchor point and also the dynamic stability to serve as a reference point. The interaction of NPC proteins, lamins and NETs with chromatin[11] is moreover consistent with this idea; however at this point the interactions observed tend to be very general and cannot adequately explain the tissue specific aspects of spatial genome architecture.

Likewise our understanding of the functional relevance of such 3-dimensional genome organization remains obscured. The central idea embraced is that it adds an additional layer to genome regulation.[15,16] Many theories have been proposed for the detailed mechanism of gene regulation from sterically blocking access to genes to propagating silencing epigenetic marks to bringing trans regulatory elements together. Yet a greater number of contradictory results have been reported than there are theories and results have contradicted even on the basic question of whether the spatial organization of the genome contributes at all to regulation of gene expression. Thus the questions of how particular genome organizational patterns are established and what the consequences are for genome regulation remain the central questions in this area. We will focus on the former.

Patterns of Genome Organization

In the late 1800s Carl Rabl made the earliest scientific observation indicating that genome organization is not random, noting that the centromeres in nuclei from salamander larvae were located at the nuclear periphery.[17] In mammals centromeres are not typically located at the periphery, but they do accumulate at the NE in certain cell types such as human neutrophils.[18] It is also more common to find telomeres at the periphery than centromeres in mammalian cells; however, this non-random positioning of telomeres tends to be transient, occurring just prior to or during meiotic

recombination. The structure known as the meiotic bouquet, where telomeres are tethered to one side of the NE, could orient chromosomes so as to facilitate synaptonemal complex formation and homologous recombination.[19] Though NE tethering of telomeres is usually transient, this connection appears to be permanent in yeast[20,21] and is maintained throughout sperm development in mammals.[22]

While the non-random positioning of general repetitive sequences such as centromeres and telomeres that occur on all chromosomes tends to be transient in mammals, the non-random distribution of individual chromosomes is maintained and heritable. Studies using whole chromosome painting techniques revealed that specific chromosomes tend to have characteristic positions within the 3-dimensional framework of the interphase nucleus (**Fig. 2**). For example, in human fibroblasts chromosome 18 tends to be located at the nuclear periphery while chromosome 19 tends to be internal.[14] As chromosome 18 has a lower density of genes compared to chromosome 19 it was proposed that this non-random distribution might reflect the tendency to observe more electron dense/ silent chromatin at the periphery and indeed this is partially supported by other chromosomes that tend to be peripheral. However, differences were observed for the favored positioning of the same individual chromosome between cell types. For example mouse chromosome 5 tends to be at the periphery in lung cells while being internal in blood and liver cells.[23] Even among different blood cell lineages certain chromosomes favor peripheral or internal positioning: for example, chromosome 6 was peripheral in CD8+ T-cells but internal in CD4+ T-cells.[24] Where many chromosomes have been assayed, typically just a subset are observed to change in position. In addition to this distribution with respect to the periphery some internal chromosomes also tended to be located next to one another in particular tissues. In fact the tendency for two chromosomes to be adjacent in interphase in a particular tissue was found to correlate with types of translocations common to tumors of those tissues.[25]

In addition to this potential link to cancer the positioning of chromosomes in the nucleus may play a role in the pathology of several diseases linked to the NE. There are now over twenty diseases linked to mutations in NE proteins, both lamins and NETs.[26-28] Just as patterns of chromosome distribution are tissue-specific, so typically is the focus of pathology in NE diseases that range from muscular dystrophy to lipodystrophy, restrictive dermopathy, neuropathy and premature aging progeroid syndromes. A mutation in the *LMNA* gene (that encodes Lamin A) causing Hutchison-Gilford progeria syndrome, E145K, yielded an abnormal distribution of telomeres and clustering of centromeres,[29] while several other mutations in *LMNA* that cause variously a neuropathy, lipodystrophy and multiple muscular dystrophies reposition chromosomes 13 and 18 away from the nuclear periphery.[30] Mutations in the NET emerin that also cause muscular dystrophy had a similar effect on these chromosomes.[30] Nonetheless, the link between chromosome repositioning and disease pathology is not clear because different mutations that cause the same disease can yield different effects on chromosome positioning: a *LMNA* mutation causing cardiomyopathy, E161K, results in chromosome 13 losing its normal peripheral localization, while another mutation, D596N, causing the same disease maintains chromosome 13 at the periphery.[31] It is important to note that the positioning of only a small subset of chromosomes are affected in these disorders and that just as the diseases focus pathology in particular tissues the effects may be cell-type specific as changes in chromosome positioning were not observed in all studies.[32,33]

Individual genes on chromosomes also reposition during differentiation. For example, the immunoglobulin H (*IgH*) locus moves from the nuclear periphery to the nuclear interior during B lymphocyte development.[34] In such studies the repositioning of the chromosome containing the gene was often not tested to determine if gene repositioning correlated with chromosome repositioning. However this was done in a few recent studies where the gene locus of interest moved within and sometimes

beyond the general chromosome territory (as defined by whole chromosome painting) while the chromosome itself typically did not correspondingly change position with respect to the NE.[35,36] Nonetheless, in one of these studies that focused on repositioning in adipocyte differentiation, the *FABP4* gene and its host chromosome both strongly shifted from the nuclear periphery to the nuclear interior.[36] There is too little data available correlating gene and chromosome repositioning to determine whether gene repositioning can drive chromosome repositioning or *vice versa*, but the several instances where gene positioning changed without corresponding changes in chromosome positioning suggest that chromosome repositioning might be a fortuitous consequence of global developmental restructuring of gene positions. During neurogenesis the *Mash1* (*Ascl1*) locus also moves away from the periphery.[37] In this case the state of epigenetic marks on the locus was also followed during the differentiation process. Interestingly, when at the periphery the locus had accumulated silencing marks and these had largely disappeared when the locus was in the nuclear interior.[37]

This observation introduces yet another aspect of non-random positioning in the nucleus: the distribution of heterochromatin. The original definition of heterochromatin was the presumed denser material observed in negative stain electron microscopy. Long before capabilities existed for identifying individual chromosome territories or gene positions it was noted that a majority of this electron-dense chromatin tended to be at the nuclear periphery in most interphase cells. Focused studies moreover suggested that this chromatin is in direct contact with the mammalian NE.[38] These direct interactions, presumed to be with the lamin polymer, were moreover supported by NE retention of chromatin after extraction at high ionic strengths.[39]

While the tendency for this dense chromatin to accumulate at the periphery is common to nearly all cell types, there are particular patterns and degrees characteristic of distinct cell types. For example, fibroblasts tend to have a more

uniform distribution of dense chromatin throughout the periphery while epithelial cells tend to have a more patchy distribution. Neurons tend to have little observable dense chromatin while lymphoblasts tend to have an enormous amount that extends several microns into the nucleus from the periphery.[12] Thus the distribution of chromatin within the nucleus, particularly with respect to the nuclear periphery, is both non-random and tissue-specific.

The importance of these patterns of electron-dense peripheral chromatin distribution is underscored by the fact that, just like chromosome positioning, they are perturbed in cells from patients with NE diseases. Normal fibroblasts tend to have a reasonable amount of this dense peripheral chromatin generally distributed throughout the periphery. In contrast, in patients with NE mutations linked to muscular dystrophy much of this dense chromatin appears to have broken away from the nuclear periphery,[40-44] while in patients with progeroid syndromes and mandibuloacral dysplasia it appears to have completely dissipated.[45,46] Patients with familial partial lipodystrophy, Dunnigan type still have dense chromatin at the periphery, but it is no longer uniformly distributed.[46] 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.

One suggested function can be found in observations that the lymphoblast dense peripheral chromatin diminished upon activation, consistent with the idea that the strongly negatively stained material was silent chromatin.[47,48] However, immunostaining with antibodies to epigenetic markers of silent chromatin have not revealed a similarly strong enrichment at the periphery with the exception that heterochromatin protein 1 alpha (HP1α) seems to have a distinct subpopulation at the NE.[49] Nonetheless, several specific interactions have been reported between NE proteins and epigenetically silent marks on chromatin (see below) and the majority of chromatin found in contact with the NE by high-throughput studies is in a silent configuration.[50,51]

How these patterns are established is an important question in cell biology. As many specific chromosome regions appear to be in direct contact with the periphery by electron microscopy it logically follows that they are driven at least in part by physical tethering to NE proteins. Consistent with this idea several NE proteins have been found to interact with DNA and specific chromatin proteins.

Specific NE Interactions With Chromatin and DNA

NPCs, lamins and NETs all can interact with a variety of nuclear components (**Fig. 1**). These include DNA, chromatin proteins such as histones, epigenetic marks on chromatin, and transcriptional regulators. The first NPC interaction with chromatin described was the tethering of yeast telomeres that required the function of Mlp, which is a homolog of the mammalian nucleoporin Tpr.[20,21] Yeast NPCs also bind transcription factors[52,53] and it is thought that such interactions promote activation of genes where an epigenetic regulation referred to as boundary activity segregates active from inactive chromatin.[54] NPCs are likely to be the main tethering point for chromatin in yeast and other fungi because these organisms lack a nuclear intermediate filament lamin polymer (see below). Mammalian NPCs also interact with chromatin, but whereas yeast NPCs were strongly activating a preponderance of silencing epigenetic marks associated with mammalian nucleoporins.[55] Interpretation of NPC results was confounded by there being separate nucleoporin pools assembled into the megadalton NPC transport channels or distributed throughout the nucleoplasm. Fusing nucleoporins to a membrane span to keep them at the NPC/ NE clarified that the nucleoplasmic pools interact with active chromatin while the NPC/ NE pools tend to interact with silenced chromatin.[56,57]

Biochemical efforts to identify chromatin proteins interacting with lamins similarly suffer from the inability to properly distinguish nucleoplasmic pools from those assembled into the polymer at the NE. Because the vast majority is thought to be in the polymeric form based on the intensity of signals for the different pools by

immunofluoresence microscopy, the interactions observed are considered to reflect the NE pool; however, the abundance may be countered by the greater facility in isolating the unpolymerized population. It is also noteworthy that studies have generally NOT tested whether the different lamin subtypes bind to or have different affinities for the same chromatin proteins. There are three different lamin genes, *LMNA*, *LMNB1*, and *LMNB2*, each of which has multiple splice variants and the ratios of these proteins vary in different cell types.[10] Accessibility is also an issue as studies expressing different lamin subtypes in *Xenopus* oocytes revealed that the subtypes assembled different layered polymers such that Lamin A would be most proximal to chromatin;[58] however, lamin mutants of one subtype can disrupt the distribution of other subtypes in vivo, different subtypes are capable of heterotypic interactions in vitro, and polymer structure is less clear in somatic cells so that it remains possible that different subtype interactions might occur in some cell types.[7,8,59]

Bearing in mind the above-mentioned caveats, lamins have been shown to interact with DNA at matrix or scaffold attachment regions (MARs and SARs[60,61]), specific chromatin structures such as centromeres and telomeres,[62,63] and core histones, specifically H2A and H2B.[64-66] On the one hand the interaction of lamins with multiple types of general chromatin suggests that they would not be involved in tissue-specific aspects of spatial genome organization, but if each lamin isoform has a different affinity for different types of chromatin, it could potentially contribute to cell-type differences in genome architecture.

Unlike nucleoporins and lamins, NETs are restricted to the NE because they are integral to the membrane. NETs, like lamins, can interact directly with DNA. LAP2β binds DNA using a domain on its amino-terminus[67] while MAN1 binds *via* a winged Helix fold on its carboxyl-terminus.[68] However, the majority of NET interactions identified have been with chromatin proteins or chromatin-associated proteins.

In mammals the Lamin B Receptor (LBR) binds core histones H3/H4 directly.[69] LBR can also bind heterochromatin through direct interaction with HP1α and HP1γ.[70] Chromatin that coimmunoprecipitated with LBR was highly enriched in silent epigenetic marks.[71] LEM-domain NETs that include LAP2, emerin, and MAN1 all interact with chromatin through the DNA crosslinking protein BAF (barrier-to-autointegration factor.[72-74] In addition to bridging chromatin-NE interactions, BAF also compacts chromatin through promoting DNA looping.[75,76] LAP2 has multiple splice variants:[77,78] LAP2β is the predominant membrane bound form, but there are other both soluble and transmembrane variants that also bind to BAF.[79-81] One of these, LAP2ζ, occurs in the cytoplasm and its overexpression causes BAF to be captured in the cytoplasm thus reducing intranuclear pools.[81] As many NETs have multiple splice variants, it is likely that this type of competitive inhibition is common.

Finally, similar to NPC proteins, some NETs interact with transcription factors such as Lmo7 and Smads.[82-84] However, whereas the NPC interactions are thought to positively promote transcription, the NET interactions are generally thought to sequester transcription factors away from their targets.

After several proteomic analyses of the NE[85-88] the number of NETs has grown from roughly a dozen to many hundreds with many being tissue-specific and only a small number tested for interactions with chromatin. Running just ten novel NETs in two screens for effects on genome architecture yielded one that promoted chromatin condensation and two others that repositioned a gene locus.[86] Thus there is a strong likelihood that the complexity of NE interactions with the genome is exponentially greater than our current understanding permits. Nonetheless, the fact of these interactions argues that specific tethering of particular genes or chromatin underlies the many distinctive patterns of spatial genome organization observed in cells.

Mechanism for Establishment of Different Organizational Patterns

If the hypothesis that specific tethering interactions at the NE direct spatial genome organization is correct, then it follows that changing NE interactions with the genome should correspondingly change genome spatial organization. Replacing the players or adding post-translational modifications that alter affinities could change NE-genome interactions. It also follows that the interactions driving specificity in the system must themselves exhibit both specificity and dominance. To test this, one could introduce a new extremely high affinity interaction into the system.

Three recent studies did just this, introducing into mammalian cells an artificial high-affinity interaction between a specific genome region and a specific NE protein and finding that it could indeed dominantly alter genome spatial organization.[89-91] In all three studies an array of bacterial lac operator repeats (LacO) was first inserted into a mammalian genome in a region that tended to be in the nuclear interior. Separately the bacterial lac repressor (LacI) that specifically binds these repeats was fused to a NE protein and expressed in the cells carrying the array. Expression of the LacI-NE protein fusions in all cases resulted in the repositioning of the LacO array from the nuclear interior to the nuclear periphery. The binding of LacI to LacO sequences can be disrupted by addition of IPTG (isopropyl β-D-1-thiogalactopyranoside) and this correspondingly released the locus from the periphery.

Both recruiting the locus to the periphery and releasing it from the periphery required passage of the cells through mitosis. The density of proteins and established chromosome territories might be expected to block such major repositioning in interphase cells; however, in mitosis the mammalian NE breaks down early in prophase as the chromosomes condense, allowing the chromosomes to move freely. Moreover, the condensed chromosome state together with unrestricted space makes most chromosome surfaces available for interactions that would be established when the NE begins to reform in telophase. The form the

broken down NE takes during mitosis is still not entirely clear: some studies are consistent with its streaming into the ER[92-95] while others suggest it breaks down into vesicles.[88,96-102] It is also possible that both occur in the same or different cell types. For the purposes of drawing the following model (**Fig. 3**) we will assume that it forms vesicles, though the critical interaction with chromatin would be essentially the same whether it has flowed into the ER or vesiculated. Thus we postulate that in telophase vesicles containing NETs from the disassembled NE would bind to partner proteins on the accessible surfaces of mitotic chromosomes (**Fig. 3A**). Then as vesicles fuse to reform the NE the chromosomes with high affinity interactions would remain at the nuclear periphery while those that lack such high affinity interactions might slip into the interior (**Fig. 3B**). As the chromosomes with high affinity interactions decondense taking up more of the reforming NE surface area, those lacking strong tethering interactions would likely be sterically pushed into the interior (**Fig. 3C-D**). Indeed, in the affinity studies using the LacO-LacI system, whole chromosome painting revealed that the entire chromosome moved to or from the periphery along with the LacO array.[89]

The LacO-LacI system has an extremely high affinity as the LacO sequence was amplified 128-256 times in the array. Though this is certain to be higher than any individual interactions in mammalian cells, interaction sites on human chromosomes would likely be both abundant and widely distributed thus providing many tethering points that would in the end have the same effect as the concentrated array. Alternatively, large gene clusters such as at the *IgH* locus might provide unique binding sites that would create distinct microenvironments at the NE. Viewing the current set of NETs known to bind chromatin by microscopy gives no indication of microenvironments in the interphase nucleus, but, interestingly, LBR and emerin bind to distinct positions on chromosomes in telophase.[103] This would be consistent with some kind of microenvironment at least on mitotic chromosomes.

Once the high-affinity interactions have tethered genes/chromosomes to the NE the many general interactions such as those between lamins and histones might further cement a particular pattern of spatial genome organization. With roughly 3 million copies of lamins per mammalian nucleus and many more histones, this would provide thousands of binding sites at the NE for each chromosome at the periphery. This additional tethering could help to maintain the distinctive chromosome territories observed in interphase cells. Furthermore, some NETs can also bind to chromatin-modifying enzymes and so could function to propagate silencing through chromatin already at the NE or promote release through adding active marks. LBR and LAP2β also associate with HA95, a kinase involved in the regulation of NE and chromatin interactions.[104] For LAP2 this interaction is splice-variant specific and thus does not apply to the soluble splice forms. NETs can also interact with enzymes that add epigenetic marks to chromatin. The histone deacetylase HDAC3 binds to LAP2β[105] while the histone acetyltransferase hALP1 binds the NET SUN1.[106] An interaction has also been reported for LBR with the DNA methylating enzyme MeCP2.[107]

As the overall spatial patterns differ among cell types and tissues, the establishment of particular patterns of spatial genome organization requires some tissue-specific components. General interactions such as those described between lamins and histones could not drive the tissue-specific organizational patterns as both protein sets are ubiquitously expressed in all tissues. This specificity could come in the form of epigenetic modifications of chromatin that alter affinities for NE proteins, tissue-specific chromatin binding proteins or transcriptional regulators sitting on DNA that interact with NE proteins, or tissue-specificity in the complement of NE proteins that interact with particular types of chromatin. The first possibility is supported by the changes in the distribution of epigenetic marks that occur in different cell types and during differentiation[108] such as changes in epigenetic marks on *Mash1* between when it was at the periphery or interior.[37] The second possibility

is consistent with the finding that several NETs can bind transcriptional regulators, which would enable genes with bound tissue-specific transcriptional regulators to be recruited to the periphery.[82-84,109] Finally, the possibility that tissue-specific NETs drive the specific chromatin interactions is supported by the identification of many tissue-specific NETs in two recent proteomic studies.[86,88]

The dynamic scaffold

The ability of the NE to anchor chromosomes depends on the structural stability of the lamin polymer. However, the dynamic of this interaction is very different from that of an anchor sitting on the ocean floor. Though historically both the genome and the lamin polymer were viewed as being quite rigid, live cell microscopy has revealed that nuclei move and exhibit frequent morphological aberrations while chromatin also moves dynamically. Rather than being rigid, the polymer that lines the NE is made entirely of intermediate filaments that are highly elastic and have properties like a spider's web, tough yet elastic. In contrast intermediate filaments in the cytoplasm combine with the more brick-like qualities of microtubules and actin microfilaments to form the cytoskeleton. Moreover, the lamin polymer associates with many NETs to connect it strongly to the lipid bilayer. This enables the lamina to keep chromatin tethered while still being able to stretch in response to other forces placed on the polymer by genome movements (**Fig. 4**). If the peripheral lamina nucleoskeleton were stiff and rigid it would likely break in response to such strong forces and the chromosomes would lose their tethering (**Fig. 4C,E**). Similarly, if tethered merely by transmembrane proteins strong forces from chromosomes might rip interacting 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.

Though whole chromosome territories are generally maintained during interphase,[110] within these territories individual loci can move rapidly over large distances.[111] Often upon transcriptional activation a locus will decondense and move

until it associates with PML bodies to maximize transcriptional output.[112] Moreover, recent chromatin conformation capture studies[113,114] indicate that some loci on chromosomes move large distances to engage with other regions as far as 10 MB away and there are thousands of such interactions, some even occurring between two different chromosomes.[115-117] Such interactions might bring enhancers together to function in trans and so additionally contribute to gene regulation as has been proposed to occur in what are termed transcription factories (**Fig. 5A**). Additionally such higher order collections of active genes would increase the likelihood that processive transcriptional proteins would rapidly find new substrates after concluding one transcriptional round, effectively increasing the localized concentration of the transcriptional proteins. While large-scale movements have been observed for some loci, those at the nuclear periphery are much less mobile than those residing in the nuclear interior.[118] Chromatin is often considered as a rope. To carry this analogy further, one might consider the NE connections as the cowboy throwing a lasso who can then partially restrict the movements of an animal far away that is caught in the lasso. Thus NE tethering at various points along the chromosome rope could both restrict and facilitate these long-range movements with the result of enabling or preventing chromosome regions from participating in higher order structures such as the transcription factories (**Fig. 5B**).

In addition to the dynamics of motion, much of the chromatin in association with the NE may dynamically exchange their tethers. A method that uses bacterial Dam methylase to label DNA in contact with particular proteins[114] revealed that roughly 40% of the genome interacts with lamins.[119] This is obviously impossible if the same tethering points were always used as the NE has only ~1/40th the volume of the nucleoplasm. This discrepancy likely reflects to some degree differences in the genes tethered at the NE within a population of cells and the interactions of the more minor intranuclear lamin pools, but also dynamic exchanges in tethering points within individual cells. It makes sense that some NE-chromatin interactions would exchange

during the cell cycle because interactions likely must be broken when DNA is replicated. In this regard it is interesting that nearly all DNA at the nuclear periphery replicates late.[120]

Would the NET, lamin and NPC proteins and corresponding chromatin proteins involved in tethering also exhibit dynamic properties? This cannot be addressed until specific proteins are identified, but FRAP on NPC proteins suggested that they are highly dynamic despite their assembly into >60 MDa structures.[121] Additionally, a recent systems modeling analysis of several NETs found that their extremely varied dynamics could be attributed to differences in the half-time of tethering in the INM.[122] Interestingly, the longest half-time estimated from this study for a known chromatin-binding NET was on the order of 12 minutes, much shorter than a mammalian cell cycle. Such dynamic exchange may be driven by post-translational modifications on NETs or chromatin as the CFTR gene locus is peripheral in some cells and can be repositioned into the nuclear interior upon treatment with trichostatin A, which promotes histone hyperacetylation.[123] Thus, the many chromatin-modifying proteins discussed above could rapidly direct changes in the specific chromosome loci tethered during interphase without notably changing the spatial positioning of the chromosome territory.

Concluding observations

There is much left to be worked out about the molecular mechanisms supporting spatial genome organization. Not the least of these is identifying the endogenous NE and chromatin proteins that direct specific spatial genome organization patterns. The only published protein thus far linked to chromosome positioning is Lamin B1,[124] but it is hard to conceptualize how any of the interactions known for this protein could support a tissue-specific genome organization pattern because both lamin B1 and its known binding partners are widely expressed. The best candidates on the chromatin side are transcriptional regulators because they have been shown to bind to all NE

components (NPC, lamin and NET proteins) and could provide the needed tissue-specificity to the system. This idea is consistent with observations from chromatin conformation capture studies that long-range genome interactions reported have been found to be bridged by several transcriptional regulators and the insulator protein CTCF.[125,126]

The other outstanding questions relate to the functional consequences of a particular spatial genome organization. When the *gypsy* insulator DNA sequence was inserted into a locus in the nuclear interior it relocated this locus to the periphery with a concomitant reduction in gene expression.[127] Since this finding, many studies have attempted to correlate changes in gene expression with spatial changes in gene or chromosome position. The most common mechanisms proposed for how this could work are: 1) NE tethering of a gene and/ or a transcriptional regulator could be controlled to either bring the two together or keep them apart (**Fig. 6A**); 2) NE recruitment of a gene could bring it to the generally silencing environment of the periphery (**Fig. 6B**); or 3) NE recruitment of both a gene and a histone/ DNA modifying enzyme could add to the transcriptional regulation of the gene (**Fig. 6C**). However, many studies over many years have tendered many contradictory results. Even among the three studies mentioned earlier that all repositioned a LacO array to the NE, one found general gene repression as a consequence,[91] one found a mixture of repressed and unchanged genes,[89] and one found no repression at all.[90] Many possible explanations can be proposed to explain these discrepancies, but it is likely that such controversies will persist until the endogenous players have been identified as absent this information all these studies must use highly artificial reporter systems. Determining which proteins normally tether a particular gene or chromosome to the periphery should provide the necessary tools to answer this question.

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Figure Legends

Figure 1.
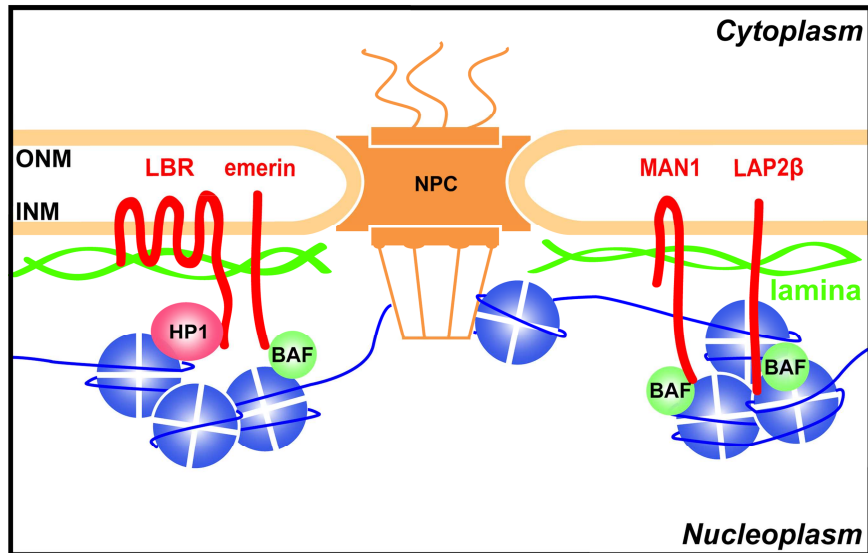
NE proteins interact with chromatin proteins.

The NE consists of outer (ONM) and inner (INM) nuclear membranes that fuse where the nuclear pore complexes (NPCs) are inserted. Both ONM and INM contain membrane spanning proteins that are generally referred to as NETs for Nuclear Envelope Transmembrane protein. Underlying the INM is the lamin intermediate filament polymer (green). The INM harbors a specific set of NETs (red), which together with the lamins are referred to as the lamina. Lamins, NETs and NPCs can all interact with chromatin components like Barrier-to-Autointegration Factor (BAF), Heterochromatin Protein 1 (HP1) and histones.

Figure 2.
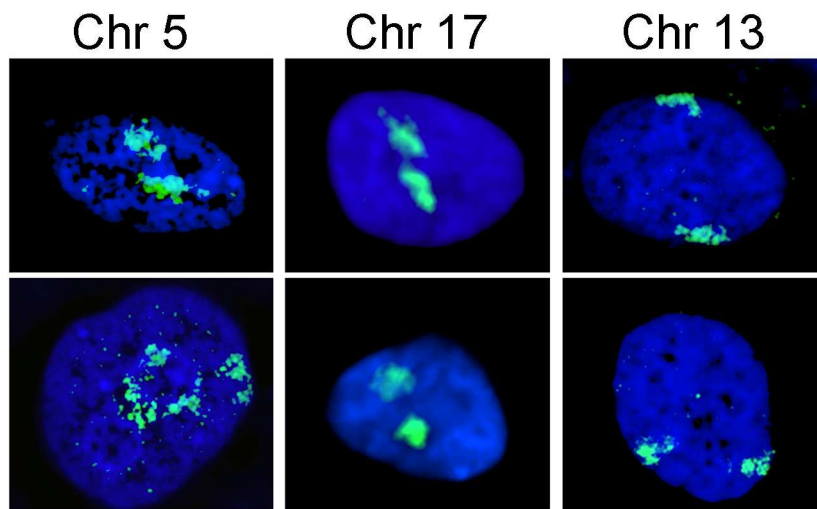
Each chromosome has a distinctive positioning in the nucleus with respect to the nuclear periphery.

In human HT1080 fibroblast cells chromosomes 5 and 17 tend to be in the nuclear interior while chromosome 13 tends to be at the periphery. The chromosome is shown in green and the DNA from DAPI staining in blue delineates the nuclear boundary. Cells were fixed with formaldehyde prior to processing for 2D FISH so that much of the 3D structure is maintained. Deconvolved sections from z-series through the nucleus are shown.
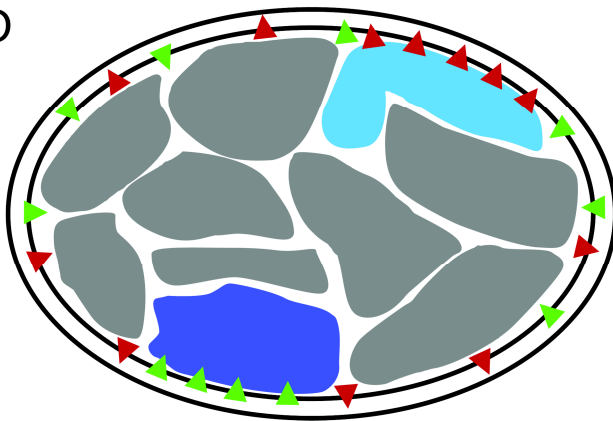
Figure 3.

A
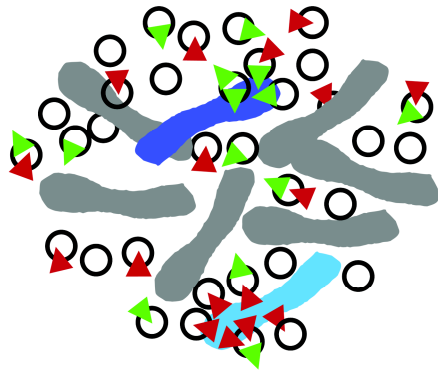
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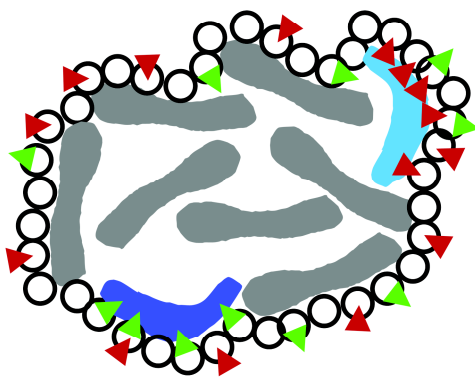
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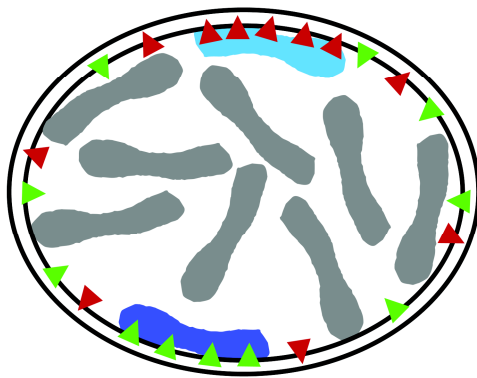
D

Affinity mechanism for establishment of spatial chromosome organizational patterns.

(A) During mitosis the NE either breaks down into ER/ NE vesicles or diffuses into the ER. The vesicle model is shown here. Distinct vesicles contain specific components (red and green triangles representing different NETs). Components of some vesicles interact with regions of particular condensed chromosomes. (B) At the end of mitosis the NE starts to reform from vesicles with some specific chromosomes still being attached to particular vesicles. (C) The NE has reformed with some chromosomes being trapped at the NE due to specific NE components that have a high affinity for these chromosomes. The chromosomes are still largely condensed. (D) During interphase the chromosomes decondense and occupy distinct territories within the interphase nucleus. At this point a multitude of less specific lower affinity interactions from the lamina would be expected to help maintain the positioning established in mitosis.

Figure 4.
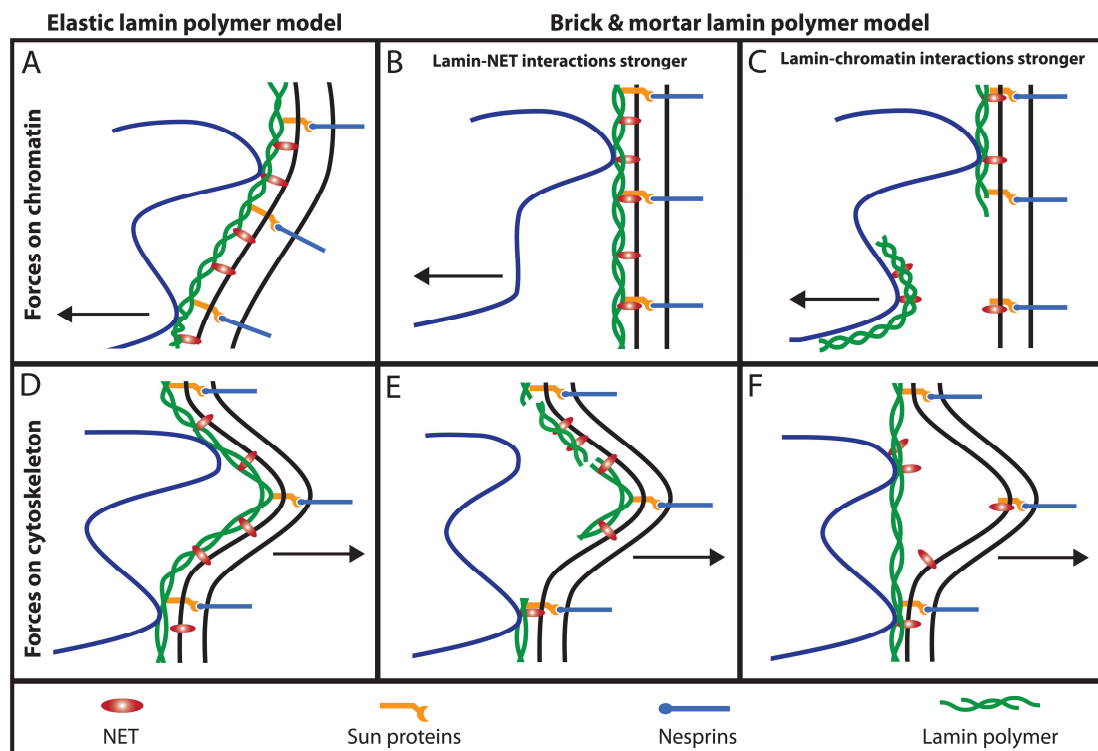
Elastic behavior of the NE.

If the intermediate filament lamin polymer supporting the NE were very stiff like other filament systems in the cytoplasm, it would be likely to rupture under the stresses exerted on it by the genome (e.g. growth during replication or rapid movements of regions within chromosome territories; A-C) or the cytoskeleton (D-F). When all components are bound and working together with an elastic nucleoskeleton the whole system can move slightly together while providing a counterforce to that exerted (A,D). In contrast, if the nucleoskeleton functioned like a brick and mortar scaffolding, then components of the system might pull apart from one another or the lamin polymer might physically rupture as do microtubules when subjected to strong bending forces (B,C,E,F). In this case NETs that have strong interactions with chromatin and/ or the lamin polymer might even be pulled out of the membrane.

Figure 5.
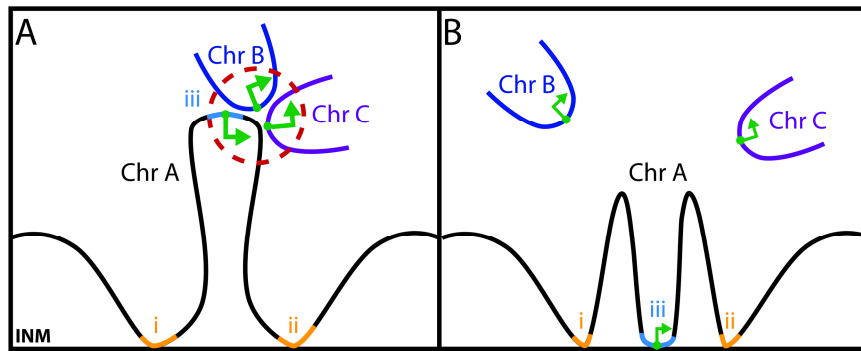
Transcription factories in the nuclear interior can be affected by NE affinity for distinct regions on chromosomes.

Because each chromosome is essentially a continuous strand of DNA folded over on itself for compaction into the 10 or 30 nm fibers observed by electron microscopy, it can be unraveled or compacted based on connections to the NE. (A) Recent 3C and 4C chromatin capture studies have revealed interactions within chromosomes and between adjacent chromosomes (e.g. chromosomes A, B and C in the diagram). Some of these are thought to act as transcription factories where greater local concentrations of transcriptional proteins can optimize transcription (larger green arrows). The availability of chromosome regions to participate in such transcription factories may depend on connections with chromatin and the INM (regions marked i and ii). (B) Changing the pattern of the connections to the INM (e.g. by recruiting also locus iii to the NE) will also affect the transcription factory structure and transcription levels.

Figure 6.
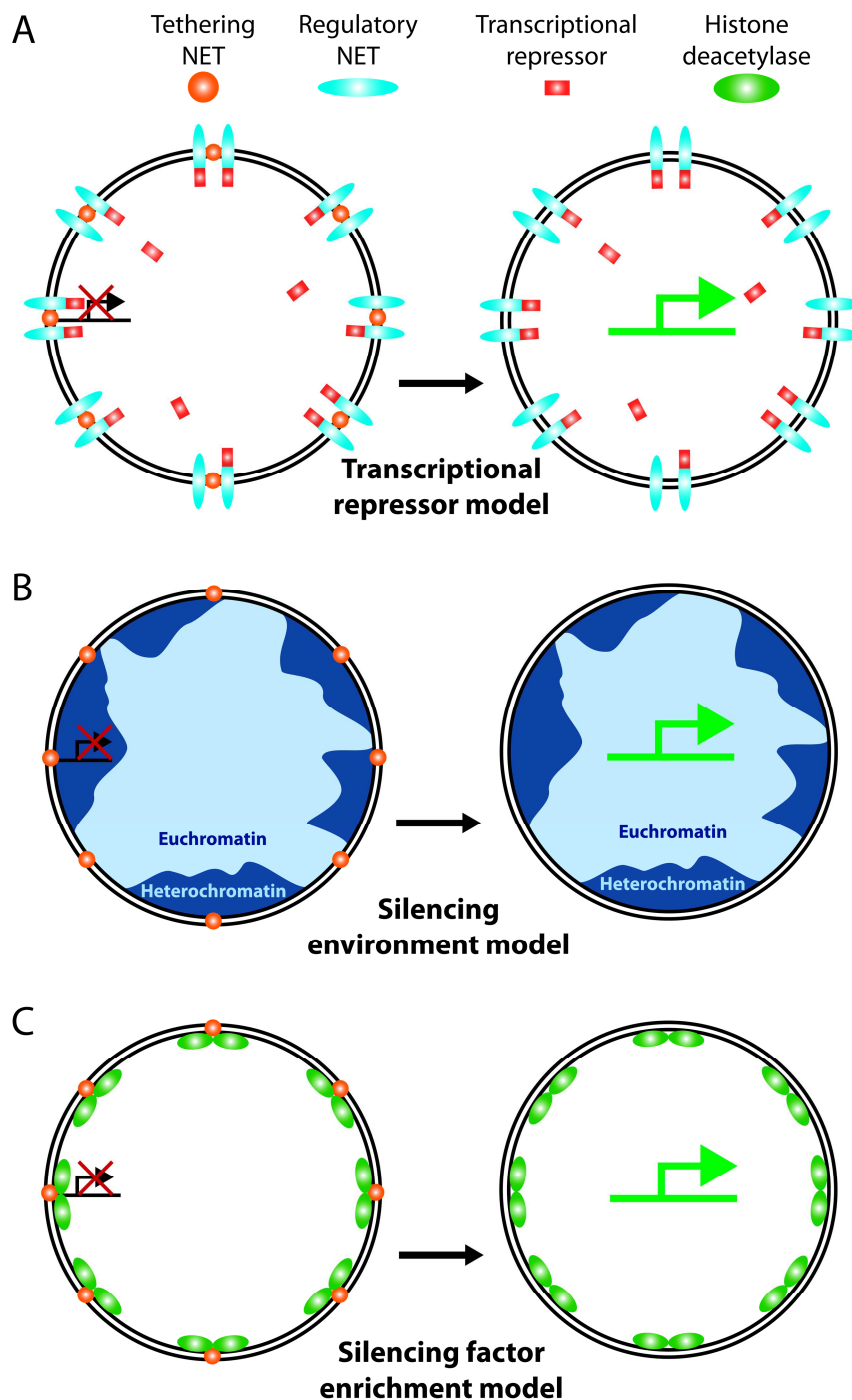
Possible mechanisms for NE interactions to regulate gene expression.

Various models have been proposed for how NE tethering of a chromosome or gene could affect gene regulation. (A) Transcriptional regulator sequestration. One NET (orange ball) recruits a gene to the periphery while another NET (blue oval) recruits a transcriptional regulator, in this case a transcriptional repressor (red rectangle).

Because the environment of the NE is only ~1/40 the volume of the nucleus this would effectively increase the local concentration of the transcriptional regulator to keep the gene more tightly repressed. During differentiation expression would shut down for the NET tethering the gene (or the transcriptional repressor) enabling the gene to move away from the high local concentration of the repressor and become more strongly activated. There are obviously many versions of this model. (B) Recruitment to a generally silencing environment. The majority of NE interactions with chromatin identified to date appear to involve heterochromatin by both the original definition of electron dense chromatin observed by electron microscopy and the more modern definition of histones carrying silencing modifications. Thus recruitment of a gene to the periphery could result in its silencing by the general environment. One flaw with this model is that NETs tend to be generally distributed throughout the INM and there are also patches of euchromatin at the periphery so the gene could conceivably move to an active region and not be repressed e.g. tethering NETs (gold balls) in lighter blue regions of euchromatin. (C) Silencing enzyme recruitment model. In addition to binding directly to silenced chromatin, some NETs have been found to recruit factors that modify chromatin to a silent configuration (e.g. the LAP2 interaction with HDAC3 and the LBR interaction with MeCP2). Thus merging aspects of the first two models, co-recruitment of a gene and a chromatin-silencing enzyme to the periphery would effectively shut expression from the gene.