The Epigenetics of Nuclear Envelope Organization and Disease

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Abstract

Mammalian chromosomes and some specific genes have non-random positions within the nucleus that are tissue-specific and heritable. Work in many organisms has shown that genes at the nuclear periphery tend to be inactive and altering their partitioning to the interior results in their activation. Proteins of the nuclear envelope can recruit chromatin with specific epigenetic marks and can also recruit silencing factors that add new epigenetic modifications to chromatin sequestered at the periphery. Together these findings indicate that the nuclear envelope is a significant epigenetic regulator. The importance of this function is emphasized by observations of aberrant distribution of peripheral heterochromatin in several human diseases linked to mutations in NE proteins. These debilitating inherited diseases range from muscular dystrophies to the premature aging progeroid syndromes and the heterochromatin changes are just one early clue for understanding the molecular details of how they work. The architecture of the nuclear envelope provides a unique environment for epigenetic regulation and as such a great deal of research will be required before we can ascertain the full range of its contributions to epigenetics.

Keywords: nuclear envelope; lamin; inner nuclear membrane protein; epigenetics; position effect; heterochromatin.
1. Introduction

Structurally, the nuclear envelope (NE) is a double membrane system continuous with the endoplasmic reticulum (ER) [1]. The outer nuclear membrane, like the ER, is studded with ribosomes yet also appears to contain some unique proteins (Fig. 1). The membrane curves around the outside of the nuclear pore complexes (NPCs), which regulate directional transport of soluble macromolecules in and out of the nucleus [2]. The inner nuclear membrane (INM) contains a set of unique proteins and also some proteins common to other organellar membranes [3]. Directly underlying the membrane in higher eukaryotes (but not yeast) is a polymer of intermediate filament lamins proteins [4], which is connected to the membrane by several of the unique INM proteins (Fig. 1).

Functionally the NE first appeared over a billion years ago as the defining characteristic of eukaryotes. The obvious importance of its barrier function, protecting the genetic contents of the cell from the wide-ranging enzymatic activities in the cytoplasm, precluded thoughts of additional functions for many years. However, as for epigenetics, our view of the NE and its functions has evolved considerably in the past few years. A mechanical stability role for lamins in nuclear shape was recently given strong experimental support in vivo [5-8]. Lamins and INM proteins can also influence replication [9-11], transcription [12-14] and signaling cascades [15-18].

The many links between the NE and gene regulation complicate the task of discerning its role in epigenetic regulation. For example, in mammals, one characterized method of NE transcriptional regulation is sequestration of the retinoblastoma protein (pRb) by lamins: this prevents release of E2F-transcription factors from pRb, thus blocking activation of their targets. This does not qualify as an epigenetic mechanism, but such NE functions could be the true mechanism behind an effect that is misinterpreted as gene silencing at the periphery. Despite the many studies that will be discussed here, it remains unclear whether the nuclear envelope silences genes merely by bringing them into an environment rich with other silenced genes (position effect), by actively modifying peripheral chromatin to a silent configuration or by preferentially recruiting already silenced chromatin.
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It is also unclear in most cases whether genes and/or modifying factors are directly tethered to the NE or simply in the environment of the periphery. Finally, it is important to keep in mind when thinking of these issues that while no data clearly demonstrate silencing through steric effects from tethering to the NE, no studies clearly show that this does not play a role in silencing.

2. Gene Silencing at the Nuclear Periphery

Early studies of transcriptional activation in many organisms inferred a connection between inactive genes and the darker staining areas of dense chromatin observed under the electron microscope that was referred to as heterochromatin [19]. In particular the heterochromatin at the periphery of resting lymphocytes was noted to diminish upon activation [20]. Heterochromatin has now been re-defined in the context of epigenetics as inactive or silent chromatin based on histone and DNA modifications [21,22]. How well the denser chromatin observed by electron microscopy correlates with these epigenetic marks has not been investigated, but the amount of nucleic acid in the darker-staining chromatin is surprisingly variable [23], suggesting that in some cases the density is achieved with proteins. Dense chromatin is observed at centromeres and nucleoli in relatively standard amounts, while that at the NE has distinctive patterns and amounts in different cell types, suggesting that it may play a role in gene regulation throughout differentiation.

That chromatin is generally transcriptionally silent at the periphery was subsequently confirmed in a study that identified genes located at the periphery. Genes in contact with lamins in *Drosophila melanogaster* tissue culture cells were isolated using a trick where lamins were fused to the *Escherichia coli* DNA adenine methyltransferase (DAM) and the uniquely methylated DNA in contact with lamins was isolated, labeled and used to probe whole-genome microarrays. This revealed an enrichment of late replicating DNA lacking active histone modifications at the periphery [24]. This is consistent with previous observations that late-replicating DNA tends to be at the periphery from bromodeoxyuridine (BrdU) pulse chase experiments [25]. A subsequent study fusing DAM to Suppressor of
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Under-Replication (SuUR), a Drosophila protein associated with heterochromatin, found considerable overlap with the genes identified as associating with lamins [26].

Co-immunoprecipitation of proteins associated with other abundant NE proteins further confirms the bias at the periphery for heterochromatin. For example mass spectrometric analysis of chromatin associated with NPC protein nucleoporin 93 (Nup93) in yeast [27] and the INM protein lamin B-receptor (LBR) in mammalian cells [28] revealed principally heterochromatic/silencing modifications.

3. Translocation of Genes To and From the Nuclear Periphery

3.1. Original studies in yeast

Although yeast do not have visible heterochromatin by electron microscopy, they provided the first molecular observations supporting NE silencing in that the Sir silencing proteins exhibited partial concentration in telomeric foci at the nuclear periphery [29,30]. This followed on work showing that inserting genes close to yeast telomeres results in their silencing in a process involving Sir proteins [31,32]. Soon afterwards it was shown that tethering a reporter gene to a nuclear membrane protein resulted in silencing of the reporter [33]. Together these findings suggested a position effect model whereby targeting genes to the silencing factor-rich environment of the nuclear periphery results in their silencing.

Several focused studies in both yeast and mammalian cells on telomere tethering to the periphery appeared to support this model. Ku and Mlp proteins in yeast were found to be required for peripheral telomere localization [34-36] and mutations in these proteins resulted in derepression of silenced genes [35,37,38]. The Mlp proteins are homologs of the mammalian Tpr, which is the outermost nucleoplasmic component of the NPC and gave the same effect when knocked down [36]. This resulted in speculation that silencing at the periphery might be specifically mediated by NPCs; however in yeast Sir4 interacts with a nuclear membrane protein Esc1 in areas that are distinct from telomeres [39], indicating that silencing is a general property of the periphery.
These studies also attempted to address the question of whether recruitment to the periphery silences chromatin in a heritable manner. Derepression of reporters in yeast upon deletion of NPC proteins involved in tethering telomeres argued that silencing required NE/NPC association [35,38]. However, deletion of the NPC proteins also resulted in a redistribution of Sir3p fused to green fluorescent protein [38]; thus derepression could be due to secondary consequences of NPC disruption, rather than simply relocation away from the periphery. Another report found that breaking the connection to the NE did not derepress the silent reporter [40], arguing that silencing results from changes to the reporter chromatin itself rather than being a consequence of the repressive environment of the periphery. As each study used different artificial experimental systems, this question remains unanswered in yeast and it may differ in mammalian cells.

3.2. Genes of higher eukaryotes undergo spatial regulation

The yeast studies relied heavily on genetics and artificial reporters as the small size of the yeast nucleus does not allow high-resolution visual analysis. The larger nucleus of higher eukaryotes enables high-resolution analysis of nuclear positioning/re-positioning. The relevance of nuclear positioning in gene regulation is underscored by observations of non-random localization of endogenous genes in Drosophila and mammalian nuclei and the influence of localization on their activity. In Drosophila several individual gene loci were observed to have conserved proximity to the NE [41]. Another interesting observation in Drosophila was that the suppressor of Hairy-wing [su(Hw)] protein that binds to the gypsy insulator and also the gypsy sequences themselves accumulate primarily at the nuclear periphery [42]. Insulators are DNA sequences that can protect a transgene from repression due to position effects if the transgene is inserted into a generally repressive environment. At the same time they can also block activity of an enhancer when inserted between it and the gene it activates [43]. Thus they can insulate a gene from propagation of surrounding epigenetic marks. The finding that gypsy and su(Hw) accumulate at the periphery was a major step in determining that NE positioning has a role in chromatin regulation.
In mammalian cells chromosomes achieve defined positions within the 3D-framework of the nucleus: the relatively inactive gene-poor chromosome 18 tends to be located at the periphery while the much more active and gene-rich chromosome 19 tends to be internal [44]. Centromeres [45], and telomeres [46,47] have all been found at the NE in specific cell types and/or cell cycle stages.

The number of individual genes whose intranuclear position has been determined by FISH is quite limited, but some of those tested appear to be regulated in part by positioning at the periphery. The immunoglobulin heavy chain IgH locus remains tethered to the nuclear periphery in early lymphocyte lineages but moves to the nuclear interior preceding the initiation of V(D)J recombination [48]. Among other genes regulated by peripheral association are the Mash1 (Ascl1) gene that moves away from the periphery when it needs to be activated in neural development [49], a transcription factor locus in T helper cells that needs to be repressed during stages of differentiation [50] and the cystic fibrosis transmembrane conductance regulator (CFTR) gene [51]. In all cases what proteins tether the locus to the NE and how these connections are broken remains unresolved.

3.3. Directed localization of reporters to the NE in higher eukaryotes

It was expected that revisiting the artificial tethering of genes to the NE in mammalian cells would further elaborate on the mechanisms uncovered in yeast. However, the mechanisms may be distinct as higher eukaryotes differ from yeast in having a dense and highly stable filamentous lamin polymer connected to the INM that could in theory amplify a silencing position effect due to steric constraints. Indeed transgenes located near the nuclear periphery in mammalian cells have been shown to be less mobile than those residing in more internal positions [52], despite the fact that soluble molecules can travel quickly through such environments [53]. Furthermore, some of the chromatin is directly apposed to the lamina in both insect and mammalian cells [54,55] and associations are retained after extraction [56]. A role for specific lamins in tethering chromosome 18 to the periphery is supported by observations that the whole chromosome moves away from the periphery in lamin B1 knockout cells with resultant gene derepression [57].
To get further at the molecular mechanism behind NE silencing, three elegant studies this year in mammalian cells used different systems for inducible tethering of genes directly to the NE [58-60]. All three studies used cell lines in which bacterial lac operator (LacO) repeat sequences were inserted into the mammalian genome in a locus that was not typically close to the NE and these cells were then transfected with lac repressor (LacI) fused to a reporter alone or to a reporter and different NE proteins (Fig. 2A): lamin B1 [59] and the INM proteins emerin [60] and the lamina-associated polypeptide LAP2β [58]. Two of the studies had a selectable marker inserted in the transgene array and both found that transcription of this reporter was reduced when the locus was at the periphery [58,60]. These systems allowed testing for the heritability of silencing as treatment of the cells with IPTG (isopropyl β-D-1-thiogalactopyranoside) disrupts the LacO-LacI binding and releases the locus from the periphery. The result of disrupting peripheral tethering was restoration of activity to the genes that had been silenced at the periphery in both studies [58,60]. One of the studies further found that many surrounding genes were repressed when the locus was at the periphery; however the pattern of gene regulation effected by the change in position was complex with some genes being downregulated and other genes unaffected [58]. If the environment of the periphery was generally repressive it could be due to a high concentration of general silencing factors or steric effects. For the environment of the periphery to repress only certain genes argues that a group of transcriptional repressors that have restricted targets are concentrated in this environment. The transcriptional repressors germ cell-less, Btf and Lmo7, each of which has target gene specificity, have been shown to bind variously to emerin and LAP2β [12,61-63]. Moreover germ cell-less was further shown to mediate specific repression of E2F-regulated genes when LAP2β was overexpressed [12,61]. Thus the use of emerin and LAP2β to mediate tethering to the periphery may have artificially repressed certain genes through recruitment of these transcriptional repressors. In one study the amino-terminus of emerin was deleted to minimize this criticism [60]; however the deleted region only partly overlaps with the binding site on emerin for germ cell-less [61], so this was probably not sufficient.
Gene repression was not observed in the third study when the locus was at the periphery; however the reporter system was quite different. In this case the reporter gene was under control of a strongly inducible promoter that could potentially overcome repressive effects of the periphery and a lamin B1-LacI fusion was used instead of the emerin and LAP2β fusions that, as noted above, could recruit transcriptional repressors [59]. Furthermore, the reporter gene contained MS2-binding sequences that allowed live visualization of its transcription with a fluorescent protein fusion to MS2 that bound to the mRNA [59]. Whether the locus was at the nuclear interior or the periphery the decondensation of the LacO spot and appearance of reporter-bound mRNA was the same, suggesting that gene repression did not occur. Therefore, the question of whether/how tethering a locus to the periphery directly results in its repression remains unresolved.

3.4. Affinity tethering of chromatin to the NE

In all three studies movement to the periphery was never observed during interphase, but required the cells to go through mitosis. During NE reassembly at the end of mitosis many INM proteins bind early to mitotic chromosomes [64,65]. Thus in the relatively mobile mitotic phase the chromosomes/ LacO array must sample their environment for higher affinity binding sites (LacI-NE protein fusions).

The use of higher affinity binding likely also functions to direct particular endogenous genes/ chromosomes to the periphery, but the proteins that tether them to the periphery have not been determined with the exception of Ku, Mlp/Tpr and SUN protein involvement in telomeric localization (shown in both yeast and mammalian cells) [34-36,46,47] and lamin B1 in chromosome 18 localization in human cells [57]. It is possible that both general and specific interactions are involved for different types of chromatin and for individual genes. Several nuclear envelope proteins interact with chromatin proteins in both yeast (see above) and mammalian cells (reviewed in [66]). In mammalian cells lamins bind histones H2A/H2B [67] while the INM protein lamin B receptor (LBR) can bind histones H3/H4 [68]. LBR can also bind heterochromatin, directly binding heterochromatin protein 1 (HP1) alpha and gamma [69] and preferentially binding histone H3 with the silent lysine 9 tri-
methylation (H3K9Me3) mark (Fig. 1) [28,68]. A higher affinity of HP1 for INM proteins compared to partners in other nuclear locations has not been directly tested, but might be inferred from observations that microinjected HP1α accumulated at the periphery before eventually being distributed to other nuclear locations [70]. Barrier-to-autointegration factor (BAF), which binds several mammalian INM proteins [71,72], in turn binds to histone H3 and linker histone H1 [73]; however, it does not appear to show any preference for association with modified histones such as H3K9Me3 [74]. Nonetheless BAF might be considered an epigenetic regulator because it can crosslink DNA/histones [75] (Fig. 1).

The identification of specific histones as binding to lamins raises the question of whether other histone variants might have higher affinity for NE proteins. In yeast INO1 and GAL1 genes were found to move to the periphery upon transcriptional inactivation. Concomitant with the relocalization was the replacement of histones with the histone variant H2A.Z, that is also found at promoters but has additionally been reported as a silencing factor [76]. This served as a mechanism for retaining memory of previous transcriptional activation as GAL1 at the periphery was activated faster than longer repressed GAL1 genes that were located in the interior of the nucleus. The tethering of INO1 to the nuclear periphery required both H2A.Z [77] and the integral membrane protein Scs2p [78], suggesting that H2A.Z may have a higher affinity for Scs2p than other histones although this has not been tested. In this light it is interesting that unique peptides for histone H2A.Y and H1.5 were identified in proteomic studies of the mammalian NE, suggesting that these might have higher affinity for the lamina than other histone subtypes though this also has yet to be directly tested [79].

4. Activation of Genes at the Nuclear Periphery

While most studies have focused on NE silencing, association with the nuclear periphery has also been associated with transcriptional activation. As mentioned above (section 2) the amount of dense peripheral chromatin varies in different mammalian cell types. Brain cells tend to have very little dense peripheral chromatin and the proteolipid protein (PLP) gene undergoes the transition from inactive to active while remaining at the
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periphery during oligodendrocyte differentiation [80]. A similar effect was observed for the interferon-γ locus [50] and the breast cancer ERBB-2 and osteogenesis collagen type 1 alpha 1 (COL1A1) loci are active when at the NE [81,82].

The yeast NE can also activate genes. Certain genes located proximal to NPCs are optimally activated at the periphery due to recruitment of transcription factors to these sites [83,84]. The ability of the NPC to both repress and activate genes may at least in part be due to interactions with insulator DNA sequences like the Drosophila gypsy sequences mentioned above (section 3.2). As genes flanked by such sequences are protected from the effects of nearby silencing or activating marks on chromatin, this function has also been referred to as “boundary activity”. Several NPC proteins were found to be able to function in insulator/ boundary activity using a very creative assay system [85]. In the boundary assay (Fig. 2B) a transgene was constructed in which two reporter genes were placed within a partially depreressed mating type locus with DNA sequences flanking that interact specifically with the DNA binding domain of the Gal4p transcription factor. Proteins were fused to the Gal4p DNA binding domain protein to screen for those that, when targeted to the specific flanking sequences, would be able to keep the reporter gene between them active when the second reporter was shut off due to propagation of silencing from the mating locus [85]. This screen identified the NPC-associated proteins Nup2p, Nup60p, Mlp1/2p and the Ran-GTP exchange factor Prp20p as being important for the formation of boundary activity in yeast [85,86]. Prp20p binds chromatin and in particular the H2A.Z (also called Htz1p) variant of histone H2A that is loaded by the SWR-C chromatin-remodeling complex and reported to be often associated with silenced chromatin [76]. Mass spectrometric analysis of nucleosomes associated with Prp20p and H2A.Z showed hypoacetylation on histone H4 at K5 and K8 considered to mark silent chromatin, but only moderately reduced acetylation (compared to total chromatin) at K12 and K16 that would be considered to mark active chromatin [87]. This combination of opposing epigenetic marks is consistent with an intermediate “poised” state and thus with boundary activity.
Consistent with the yeast role of NPCs in recruiting transcription factors and boundary elements, genome-wide analysis of DNA that had been co-immunoprecipitated with NPC components in yeast indicated an overall enrichment in transcriptionally active genes [88]. This contrasts with the favoring of silent marks on certain specific mammalian NPC proteins such as Nup93 [27], suggesting a division of labor within the NPC between silencing and activating functions, though there might also be differences between organisms.

5. De Novo Modification of Histones at the NE

For the endogenous loci that have been shown to move between the periphery and interior of the nucleus, the chromatin modifications at the periphery differ from those in the interior. The *Mash1* locus involved in mammalian neurogenesis is located at the periphery in ES cells, replicates late and has some histone modifications characteristic of silenced chromatin and few active marks [49]. After *Mash1* moves to the interior in neurally committed cells the timing of locus replication switches to early and histone modifications consistent with active chromatin predominate (Table 1; [49]). However, as the movement in these systems is not subject to simple experimental regulation it is not possible to determine if the change in histone modifications is determined by the different environments or is a prerequisite for affinity-directed movement of the locus to the different environment.

This distinction was clear in the LacO systems as locus movement was directed by a purely artificial affinity mechanism. Differences in histone modifications were observed such that histone H4 acetylation was reduced when the locus was at the periphery [60]. Thus the more repressive environment at the periphery must contain enzymes that deacetylate histones. This was previously shown in the case of LAP2β, where a 2-hybrid screen identified histone deacetylase 3 (HDAC3) as a binding partner [13]. HDAC3 mediates H4 deacetylation, and is linked to heterochromatic, transcriptionally inactive genomic regions. Thus recruitment of HDAC3 to the periphery by LAP2β can silence genes that come into contact with the periphery [13]. Interestingly, treatment with HDAC3 inhibitors had little effect on NE-directed gene repression in the LacO system that used LAP2β to tether the locus to the
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periphery [58], suggesting that steric constraints at the periphery or specific repressors play a greater role than recruitment to an environment rich with silencing histone deacetylases.

NE proteins can directly modify histones for activation as well as repression. One of the proteins identified in a proteomic analysis of mammalian NEs, NET43/hALP, is a histone acetyltransferase [79]. NET43/hALP localizes to mitotic chromosomes in mammalian cells by binding to the INM protein SUN1 [89]. Depletion of SUN1 results in delayed chromosome decondensation and a reduction in histone H2B and H4 acetylation and these functions are mediated by NET43/hALP [89]. Interestingly NET43/hALP appears to be upregulated during lymphocyte activation when the large amount of dense peripheral chromatin of resting lymphocytes becomes decondensed (N. Korfali, L. Florens, and E. Schirmer, unpublished observations).

6. NE Diseases and Epigenetics

Twelve different NE proteins have now been linked to human disease (Table 2) [90-94]. Principle among them is lamin A, which with nearly 200 identified mutations has now been named the most mutated gene in the human genome. Lamin diseases affect many different tissues and include muscular dystrophies [95-97], lipodystrophy [98-100], neuropathy [101,102], cardiomyopathy [103], dermopathy [104] and the aging disease progeria [105,106]. Several NETs and associated proteins also cause diseases or syndromes affecting muscle [107-110], bone [111,112], brain [113-117], skin [104] and immune cells [118]. Although each disease exhibits pathology preferentially in particular tissues, some patients have presented with overlapping pathologies. Recent observations suggest that in some cases affected individuals may harbor mutations in multiple proteins. For example one particularly debilitating case of Emery-Dreifuss muscular dystrophy is caused by mutations in both emerin and in desmin [119].

The three currently favored and partially overlapping mechanisms proposed to explain NE disease pathologies are: 1) cell damage due to mechanical instability of the nucleus, 2) disruption of signaling cascades through the NE affecting cell cycle progression,
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stem cell maintenance or differentiation, and 3) alteration of gene expression due to physical disruption of regulatory contacts at the NE.

The hypothesis that NE diseases involve genome misregulation is supported by observations that 1) lamin A-binding transcriptional regulators do not bind lamin mutants that cause disease, 2) microarray data from normal and NE mutation cells indicates a pattern of misregulation of myoD and Rb pathways in NE-related muscular dystrophies [120,121], and 3) cells from patients with NE diseases have abnormal distribution of dense peripheral chromatin. The first two observations are likely due to specific interactions with transcriptional regulators, but the alterations in chromatin are likely due to epigenetic misregulation. Specifically dense chromatin that is normally directly apposed to the NE redistributes away from the membrane in patients with lamin-related muscular dystrophy [122,123], progeria [124] and INM protein-related NE diseases such as emerin-linked muscular dystrophy [125-127]. Similar alterations in chromatin organization were observed in mouse lamin depletion models [128] and transgenic mice expressing lamin point mutants associated with cardiomyopathy [129]. Moreover, some differences in the overall positioning of chromosomes have also been observed in cells with specific lamin A mutations [92].

Progeria is caused by a lamin mutation that results in accumulation of an early pre-form with a farnesyl moiety [105,106]. Progeroid cells were analyzed for histone modifications associated with epigenetic regulation, finding that marks associated with silenced chromatin (histone H3 lysine 9 and lysine 27 methylation: H3K9Me3 and H3K27Me3) were reduced while a mark of active chromatin (histone H4 lysine 20 methylation: H4K20Me) was increased [130]. Moreover, in a female patient silencing marks on the inactive X chromosome decreased and lost their tight association with the periphery by fluorescence microscopy. A more striking effect was observed in immortalized tissue culture cells where H3K27Me3 strongly accumulated at the periphery with endogenous lamins, but dissipated in cells expressing a progeria mutation [130]. Moreover, an earlier pre-lamin A form lacking the farnesyl moiety accumulated HP1α in abundance over farnesylated pre-lamin A [131]. These types of changes were recapitulated in normally aging cells [132].
Moreover some of these heterochromatin defects could be reversed by treatment of cells with farnesyltransferase inhibitors (i.e. that reduce accumulation of the farnesylated form of lamin A that occurs in progeria) [133].

A further novel layer of epigenetic regulation may derive from nuclear structural organization at the level of nuclear shape. Nuclear lobulation is an aspect of neutrophil differentiation that increases the relative ratio of nuclear surface area to DNA [134]. This in theory would propagate silencing or activating effects from the NE over a much greater percentage of the genome. The differentiation program of neutrophils focuses most heavily on genes regulated by C/EBP, PU.1 and at later stages retinoic acid receptor (RAR) transcription factors [135] and unfortunately these genes have not been specifically mapped to determine their relationship to the periphery. Nonetheless, a recent study investigating global transcription in different blood cell lineages found that, though far fewer genes were expressed in neutrophils compared to progenitor cells, in both cases expression was concentrated in the nuclear interior [136]. Another particularly interesting observation is that terminally differentiated neutrophils withdraw completely from the cell cycle with complete silencing of E2F-mediated transcription [137]. In light of the repression of E2F-mediated transcription by overexpression of the INM protein LAP2ß via its recruitment of germ cell-less to the periphery [12] the silencing during neutrophil differentiation could well be related to the increase in the NE surface area:DNA ratio. Neutrophil lobulation is blocked in the Pelger-Huet anomaly disorder associated with mutations in the INM protein LBR [118].

7. Summary

Whether through lineage-heritable gene positioning, sequestration of transcription factors, or recruitment of transcriptional repressors and histone modifying enzymes, the NE contributes its own version of epigenetics to the fine-tuning of differentiation, stem cell maintenance and gene expression. The complexity of interactions and mechanisms for silencing at the NE are likely to increase dramatically in the future as recent NE proteomic studies in both yeast and mammals have identified many novel uncharacterized proteins at the
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NE [79,138-140] and overexpression of several of these novel INM proteins alters chromatin organization (N. Zuleger, P. Malik, L. Florens and E. Schirmer, unpublished observations). Moreover indications of tissue-specific differences in NE composition [141,142] argue for differences in NE epigenetics in different cell types. It will be particularly interesting in the future to insert the LacO loci mentioned above into different cell types that have differences in the original distribution of peripheral heterochromatin to test how the pre-existing repressive environment of the periphery varies among different cell types.

A particularly intriguing observation in analyzing the NE proteome is that the unique INM proteins indicated to be the most abundant are almost without exception lacking in enzymatic functions, yet they seem to have an incredibly large number of binding partners [143]. Moreover these binding partners appear to literally coat the surface of the INM proteins in the cell [142]. Thus it is reasonable to assume that the effects of NE proteins in epigenetic regulation derive from a combination of their variable expression in different cell types and their ability to recruit specific chromatin modifying proteins to the NE. The collosal scale of NPCs, with over 30 core proteins and many additional peripherally associated proteins forming >50 MDa complexes, could allow parts of the NPC to recruit factors that propagate silenced chromatin while others activate genes. The finding moreover that some of these proteins have boundary/insulator activity allows for active and repressed states to occur within a short stretch of chromatin and thus increases the complexity of active/inactive genes in the proximity of the NPCs. If NPCs have even a fraction of the tissue variation indicated at the NE, the range of possible combinations for varied regulation is enormous.

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Legends to Figures and Tables

Fig. 1.

Schematic of nuclear envelope organization. The nuclear envelope (NE) is a double membrane system continuous with the endoplasmic reticulum (ER). In addition to sharing proteins with the proximal ER, the outer nuclear membrane (ONM) has a few characterized unique NE proteins. The inner nuclear membrane (INM) contains many more unique integral proteins, which commonly are associated with the intermediate filament lamin polymer and chromatin. The membrane is perforated by nuclear pore complexes (NPCs), large macromolecular assemblies upwards of 50 MDa that direct transport of large molecules in and out of the nucleus. Differences in the interactions between INM proteins and their
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chromatin and chromatin-modifying interaction partners could result in different types of chromatin accumulating at the periphery. For example, lamins bind core histones (H2A:H2B:H3:H4), LBR binds to epigenetically marked histones and HP1, LAP2β binds to the histone deacetylase HDAC3, and several INM proteins bind the BAF DNA crosslinker.
Systems for assembling specific chromatin at the NE. A. In the lac operator system a gene locus is active when at the nuclear interior (left panel), but becomes repressed at the periphery (right panels). Movement to the periphery requires going through mitosis and the locus can be released from the periphery with IPTG treatment. Repression could occur through steric constraints (e.g. association with the lamin polymer) or by recruitment of transcriptional repressors (germ cell-less [gcl])/ histone modifying enzymes (HDAC3). B. Boundary activity at the NPCs yields characteristics of both silent and active chromatin. The boundary assay (bottom) places the \textit{URA3} and \textit{ADE2} genes within a partially derepressed HML (mating type) locus with \textit{ADE2} flanked by Gal4p binding sequences (gal bs). If a protein fused to the Gal4p DNA binding domain allows \textit{ADE2} expression while \textit{URA3} remains shut off, then it is
said to have boundary activity. If the cells are grown in the absence of adenine they need expression of this ADE2 gene to survive, but in the presence of the drug 5-fluoroorotic acid (5-FOA) expression from the URA3 gene becomes toxic. Thus the ability to grow in the combined absence of adenine and presence of 5-FOA when a particular NPC protein is fused to the Gal4p DNA binding domain indicates its role in boundary activity. Results using this assay suggest that the Nup60p and Mlp1/2p proteins that have previously been shown to be important for telomere tethering to the periphery are also involved in boundary activity, but in association with Nup2p and the Ran-GTP exchange factor Prp20p. Prp20p mediates the interaction with chromatin through binding “poised” histones with opposing epigenetic marks (e.g. acetylated [Ac] K12 and unmodified [Nm] K5) and the H2A.Z variant that is sometimes associated with silenced chromatin.
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Table 1. Changes in histone modifications between peripheral and central localization for the Mash1 gene.

<table>
<thead>
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<th>Mash1 periphery</th>
<th>Mash1 interior</th>
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<tr>
<td>H3K9me3</td>
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<td>+/-</td>
</tr>
<tr>
<td>H3K27me3</td>
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<td>+</td>
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<tr>
<td>H4Ac</td>
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[49]

Table 2. Diseases caused by nuclear envelope protein mutations

<table>
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<th>reference</th>
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<td>muscle</td>
<td>[95]</td>
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<tr>
<td></td>
<td>Emery-Dreifuss muscular dystrophy (EDMD) type 3 (AR)</td>
<td>muscle</td>
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<td>Limb-girdle muscular dystrophy type 1B (LGMD-1B)</td>
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<td></td>
<td>Charcot-Marie-Tooth disorder type 2B1 (CMT-2B1)</td>
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<td></td>
<td>Mandibuloacral dysplasia (MAD) type A/B</td>
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<td></td>
<td>Autosomal dominant lipoatrophy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy and leukomelanodermic papules</td>
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<td></td>
<td>Dunnigan-type familial partial lipodystrophy (FPLD2)</td>
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<td>Disorder/Condition</td>
<td>Tissue Affected</td>
<td>Reference(s)</td>
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<td>Seip syndrome (BSCL2)</td>
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<td>Dilated cardiomyopathy with conduction defect (CMD1A)</td>
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<td>[103]</td>
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<td>Restrictive dermopathy (RD)</td>
<td>skin</td>
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<td>Hutchison-Gilford Progeria syndrome (HGPS)</td>
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<td>[105,106]</td>
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<td>Atypical Werner Syndrome (AWS)</td>
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<td>Lamin B1 adult-onset autosomal dominant leukodystrophy</td>
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<td>Lamin B2 Barraquer-Simons syndrome</td>
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<td>Transmembrane Proteins</td>
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<td>Emerin Emery-Dreifuss muscular dystrophy (EDMD) type 1 (X)</td>
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<td>Lamin B-receptor Pelger-Huet anomaly (PHA)</td>
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<td>MAN1 Buschke-Ollendorff syndrome (BOS)</td>
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<td>Melorheostosis</td>
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<td>FACE-1 Restrictive dermopathy (RD)</td>
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<td>LAP2α cardiomyopathy</td>
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<td>Aladin Triple A syndrome</td>
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<td>Nup60 Infantile bilateral striatal necrosis</td>
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(AD), autosomal dominant; (AR), autosomal recessive; (X), X-linked
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References


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clinical condition linked to a novel mutation in lamins A and C with generalized
lipoatrophy, insulin-resistant diabetes, disseminated leukomelanodermic
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Hegele. Novel lamin A/C gene (LMNA) mutations in atypical progeroid

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