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The Laminopathies and the Insights They Provide into the Structural and Functional Organization of the Nucleus

Xianrong Wong and Colin L. Stewart

Regenerative and Developmental Biology Group, Institute of Medical Biology, Singapore 138648; email: xianrong.wong@imb.a-star.edu.sg, colin.stewart@imb.a-star.edu.sg

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Abstract

In recent years, our perspective on the cell nucleus has evolved from the view that it is a passive but permeable storage organelle housing the cell's genetic material to an understanding that it is in fact a highly organized, integrative, and dynamic regulatory hub. In particular, the subcompartment at the nuclear periphery, comprising the nuclear envelope and the underlying lamina, is now known to be a critical nexus in the regulation of chromatin organization, transcriptional output, biochemical and mechanosignaling pathways, and, more recently, cytoskeletal organization. We review the various functional roles of the nuclear periphery and their deregulation in diseases of the nuclear envelope, specifically the laminopathies, which, despite their rarity, provide insights into contemporary health-care issues.

INTRODUCTION

As an organelle, the nucleus has functions that extend far beyond that of a passive but permeable storage repository of the genome. The nucleus is itself highly organized, containing functionally distinct neighborhoods of nonrandomly positioned chromosomes and specialized proteinaceous subcompartments that, in concert, allow the nucleus to interpret and affect cellular decisions and outputs, thereby influencing normal cellular physiology (23). Of these nuclear subcompartments, the nuclear periphery has attracted major interest, primarily due to the numerous diseases caused by mutations in the lamins and some proteins localized to the nuclear envelope (NE).

The most prominent features of the nuclear periphery are the NE and the underlying nuclear lamina (**Figure 1**). The NE comprises a double membrane barrier consisting of the inner nuclear membrane and the outer nuclear membrane, which encompass the perinuclear space. It is traversed by the nuclear pore complexes, which control the trafficking of macromolecules between the nucleoplasm and cytoplasm. The inner and outer nuclear membranes connect with each other where they are traversed by nuclear pore complexes (49). The outer nuclear membrane also connects at multiple points with the cytoplasmic endoplasmic reticulum, rendering the endoplasmic reticulum, inner nuclear membrane, and outer nuclear membrane a continuous membrane system, with the endoplasmic reticulum lumen being contiguous with the perinuclear space (125). However, despite this contiguity, these three compartments (especially the inner and outer nuclear membranes) are functionally distinct, with each membrane system defined by the specific proteins or complexes associated with them.

Underlying the NE is the nuclear lamina, a 10–30-nm-thick filamentous meshwork, with its thickness varying between different cell types (58). The principal components of the lamina are the type V intermediate filament proteins—the lamins (44). In mammals, the lamins are grouped into two classes: type A (LaA, LaAΔ10, and LaC) and type B (LaB1, LaB2, and LaB3) (107, 139). Most adult somatic cells contain four major lamin proteins (LaA, LaB1, LaB2, and LaC). A single gene, lamin A (*LMNA*), encodes the A-type lamins LaA and LaC, which are generated by alternate splicing of a common pre-mRNA (76, 84). A minor spliced variant, LaC2, is also produced in the testis (43). Separate genes encode LaB1 and LaB2; LaB3 is also produced as a minor spliced variant of LaB2 and, as with LaC2, is found in the testis (42, 76).

In mammals, recent high-resolution light and electron microscopy studies have revealed that the A-, B-, and C-type lamins form their own spatially separate but interacting overlapping filament networks of 3.5-Å tetrameric filaments (121, 132), with LaC showing an apparent preferential association with nuclear pores (145). This makes lamina organization in mammalian somatic cells more complex and less regular than that previously observed in the frog oocyte lamina (1). How such filament networks are altered by disease-causing mutations is not yet known, although earlier investigations indicated that some *LMNA* mutations result in the failure of the lamins to assemble a visible lamina (110).

THE LAMINOPATHIES AND NUCLEAR ENVELOPATHIES

The principal stimulus generating interest in the NE and lamina was the discovery that some 30 inherited diseases and anomalies—including cardiomyopathies, muscular dystrophies, conditions affecting white fat and skeletal homeostasis, and premature-aging-like syndromes—are caused by mutations in the *LMNA* gene and in genes encoding some of the NE-associated proteins (143). The largest of this group of diseases, the primary laminopathies, is associated with *LMNA* mutations. The secondary laminopathies are caused by mutations in the gene encoding the enzyme ZMPSTE24, the endoprotease essential for the posttranslational maturation of pre-LaA to mature LaA (148).

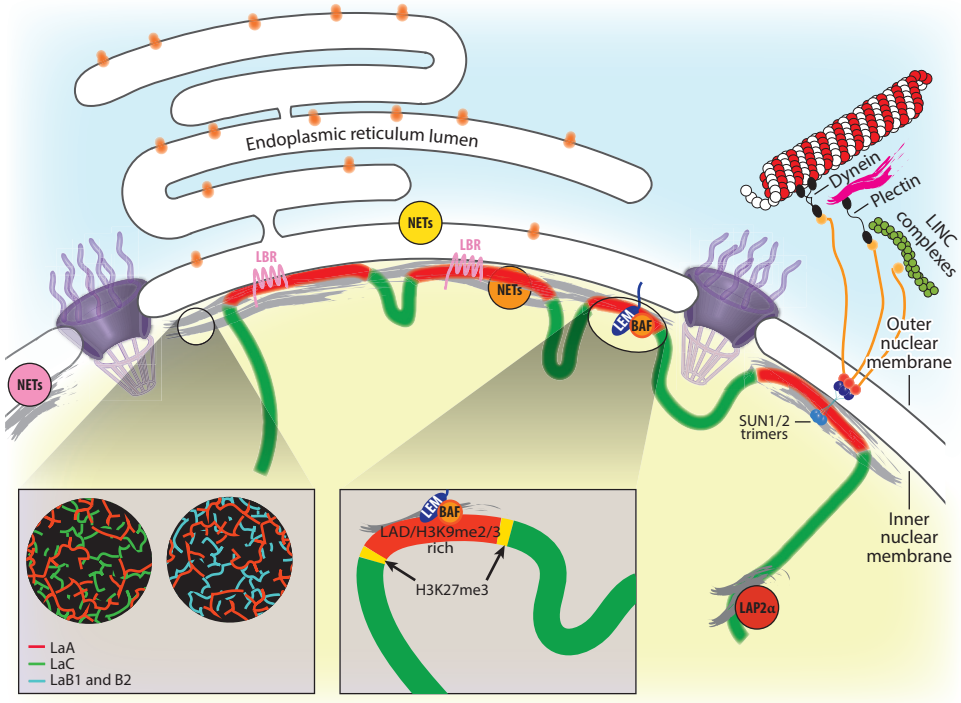

Figure 1

Functional organization at the nuclear periphery. The nuclear periphery is composed of the nuclear envelope and the underlying lamina meshwork. The nuclear envelope comprises the inner and outer nuclear membranes, which are contiguous with the endoplasmic reticulum. The outer nuclear membrane is similar to the endoplasmic reticulum in terms of protein composition (with ribosomes occupying the outer nuclear membrane but not the inner nuclear membrane); the inner nuclear membrane anchors a subset of nuclear envelope transmembrane proteins (NETs) by the underlying lamina. The lamina comprises separate but overlapping meshworks of the four lamin isoforms, as illustrated by the inset on the left. The lamina maintains nuclear integrity as well as an anchoring platform for inner nuclear membrane proteins and chromatin. The protein composition at the nuclear periphery has been profiled in different cell types and various NETs (69), many of which have unknown nuclear functions. Other inner nuclear membrane proteins are relatively well characterized, including the BAF-binding LEM-domain proteins and the lamin B receptor (LBR) (*pink eight-pass transmembrane protein*), which are implicated in heterochromatin anchorage at the periphery (see **Table 1**). The peripheral heterochromatin, termed lamina-associated domains (LADs), is enriched in di- and trimethylation of histone H3 on lysine 9 (H3K9me2/3) throughout and H3K27me3 at the borders, as illustrated by the inset on the right. The function of lamins extends beyond the nucleus, as they anchor the linker of nucleoskeleton and cytoskeleton (LINC) complexes comprising the SUN- and KASH-domain proteins, thereby directly influencing cytoskeletal networks.

The primary *LMNA* laminopathies are classified into three groups. The largest consists of diseases affecting striated muscle, which include the autosomal dominant form of Emery–Dreifuss muscular dystrophy (AD-EDMD), dilated cardiomyopathy (DCM), and limb-girdle muscular dystrophy 1B. These diseases are caused mostly by missense mutations, approximately 450 of which have been documented (2), and which are found throughout the 12 exons of the *LMNA* gene. This group also includes a very rare peripheral neuropathy (R298C), a recessive form of Charcot–Marie–Tooth disease (AR-CMT2A) that causes motor nerve demyelination with muscle wasting in the limbs (29).

The second group of laminopathies mainly influences white fat distribution and skeletal development. The two diseases are Dunnigan-type familial partial lipodystrophy (FPLD2) and mandibuloacral dysplasia (13, 99). The third group, which has attracted the most interest, is the premature-aging or progeroid syndromes, Hutchinson–Gilford progeria syndrome (HGPS), and a few cases of atypical Werner's syndrome (17, 28, 37). The secondary laminopathies result in either perinatal lethality (restrictive dermopathy) or mandibuloacral dysplasia with lipodystrophy. These conditions are due to different mutations in ZMPSTE24. Mice lacking ZMPSTE24 live for a few months after birth but develop many progeroid features, including a tendency for their ribs to fracture (41, 104).

In contrast to the A-type laminopathies, mutations in the B-type lamins are linked to only two diseases, possibly because both B-type lamins are required during fetal development, particularly in regulating nuclear migration during neurogenesis of the central nervous system. The exception is adult-onset autosomal dominant leukodystrophy, which arises relatively late in life and is caused by duplication of the *LMNB1* gene, which results in demyelination of neurons in the central nervous system. LaB1 has also been implicated in enhancing a susceptibility to neural tube defects (59, 102).

In addition to the laminopathies, mutations in at least 12 NE-associated proteins result in disease. The NE genes associated with known diseases or anomalies are summarized in **Table 1**. The pathologies of many NE diseases overlap with some of the laminopathies, although deletion or loss of at least two NE proteins (LAP2α and SUN1) results in a significant amelioration of *LMNA*-induced DCM (16, 22). The overlap in pathologies resulting from mutations in the NE proteins and the lamins suggests that the lamins and some NE proteins form an interlocking functional network (62).

Here, we do not discuss the clinical aspects (pathology, treatments, etc.) of the laminopathies and nuclear envelopathies, as this topic has been covered in many recent reviews. However, the diseases arising from mutations in these proteins have stimulated major interest in the functional architecture of the nucleus, revealing surprising and novel findings about nuclear organization and functions beyond that of being a mere repository of the genome.

DEVELOPMENTAL AND TISSUE-SPECIFIC VARIATION OF THE LAMINS AND NUCLEAR ENVELOPE PROTEIN EXPRESSION

In mammals, lamin expression is developmentally regulated. All nucleated mammalian cells express at least one B-type lamin, whereas A-type lamins are absent during the early pre- and postimplantation embryonic stages and in embryonic stem cells, with these types expressing high levels of LaB1 and LaB2 (124). A-type lamin expression then reappears as different tissues form in the postimplantation embryo, with some tissues not expressing A-type lamins until after birth, such as the central nervous system, crypt cells in the gut epithelium, and white blood cells (113, 123). Surprisingly, the expression of lamins in embryonic stem cells appears to be nonessential, as embryonic stem cells and their derivatives that lack all three lamins proliferate normally in culture, maintain euploidy, and differentiate into multiple cell types, including those with fibroblastic, cardiac, and neuronal phenotypes (66).

During murine development, A-type lamin expression is also dispensable; mice that lack both LaA and LaC are indistinguishable from normal siblings at birth, possibly because of the shared redundancy with the lamin B receptor (LBR) that is expressed (or even re-expressed) in cells engineered to lack *Lmna* (123). Problems arising from the loss of *Lmna* or from specific mutations appear after birth in mice, with loss of *Lmna* resulting in postnatal death within three weeks, which correlates to some extent with the normal silencing of LBR expression in many postnatal tissues.

Table 1 The nuclear envelopathies (for additional details, see Reference 62)

Protein	Protein function	Disease/anomaly
Emerin (encoded by *EMD*)	Associated with the nuclear envelope, may regulate β-catenin nuclear entry and MKL1 nuclear localization	X-linked Emery–Dreifuss muscular dystrophy
Man1 (encoded by *LEMD3*)	Regulates TGF-β signaling by modulating Smad phosphorylation, required for the development of the vascular system	Buschke–Ollendorff syndrome, excessive bone nodule formation
Lap1/Traf3 (encoded by *TOR1AIP1*)	Interacts with torsin, LULL1, and emerin	Myopathy exacerbated by EMD loss
Lem2/Net25 (encoded by *LEMD2*)	Chromatin organization, MAP/AKT signaling	Homozygote nulls are embryonic lethal; progeroid symptoms result from missense mutations
Lap2 (encoded by *TMPO*)	Chromatin organization, telomere maintenance	Dilated cardiomyopathy, reduced epidermal proliferation; its loss ameliorates LMNA-induced muscular dystrophy and dilated cardiomyopathy
Torsin	AAA+ ATPase that interacts closely with Lap1	Dystonia in the central nervous system, steatohepatitis
Lamin B receptor (encoded by *LBR*)	Reduced sterol reductase activity, heterochromatin organization	Pelger–Huët anomaly, Greenberg dysplasia
Nesprin-1 (encoded by *SYNE1*)	LINC complex tethers nucleus to cytoskeleton, required for nuclear migration during central nervous system development	Limb-girdle muscular dystrophy, autosomal recessive cerebellar ataxia type 1
Nesprin-2 (encoded by *SYNE2*)	LINC complex tethers nucleus to cytoskeleton, variants are required for nuclear migration during central nervous system development	Emery–Dreifuss muscular dystrophy
Nesprin/Kash4	Interacts with microtubule motor proteins, required for nuclear positioning in cochlear hair cells	Mutations result in deafness
Sun1	Anchors LINC complex to the inner nuclear membrane, regulates microRNA synthesis during muscle regeneration	Its loss ameliorates *LMNA*-induced muscular dystrophy and dilated cardiomyopathy; missense mutations are associated with muscular dystrophy
Baf (encoded by *BANF1*)	Postmitotic nuclear reassembly, chromatin organization	Néstor–Guillermo progeria

By contrast, human embryonic fibroblasts that lack *LMNA* are difficult to propagate, and during embryogenesis, loss of *LMNA* may have severe consequences that lead to preterm lethality (135). The exceptions are in neurons of the central nervous system, where LaA is largely absent due to translational inhibition by the microRNA miR-9, which binds to the 3′ end of the longer LaA transcript and not to the shorter LaC transcript (64).

Less is known about the cell and tissue variation of the B-type lamins. Human and murine fibroblasts with reduced levels of LaB1 undergo senescence (34, 120). In vivo LaB1 levels decline as keratinocytes mature in the skin epidermis (34, 120). By contrast, LaB2 levels do not change as cells senesce, and loss of LaB2 has no effect on fibroblast proliferation (34). Unlike the A-type lamins, the B-type lamins are not mutually redundant during embryogenesis, as both are required for

normal development of the central nervous system (20, 137). Loss of LaB1 reduces the cell number in and layering of the cerebral cortex and leads to hypothalamic abnormalities and a reduction in cerebellar size due to abnormal neural migration. LaB2 is necessary for nuclear migration in developing neurons during central nervous system development, and the loss of LaB2 results in a smaller cerebellum (147). In adults, B-type lamin expression appears to be nonessential in some tissues, such as the skin epidermis and liver (146). These findings reveal that an absolute dependence on lamin expression in mammals varies among different cell types and that early embryos, including pluripotent cells derived from them and some of their differentiated derivatives, may not require any of the lamins for their proliferation and differentiation (66). The derivation of mouse lines with conditional (floxed) alleles (140) is defining which adult tissues overtly depend on the A-type lamins. Both the adult liver and exocrine pancreas require A-type lamins to function, with the *Lmna*-null males developing steatohepatitis and pancreatitis, respectively (36, 70). Adult cardiomyocytes also depend on the A-type lamins, probably to protect the nuclei from the mechanical stresses incurred by contractions at rates of 600 cycles per minute. However, the skin epidermis and gut epithelium can apparently dispense with the A-type lamins and still function without any overt effect, possibly due to compensatory expression of the gene encoding LBR (123, 140).

During their synthesis, LaA, LaB1, and LaB2 undergo carboxymethylation, followed by the sequential addition and removal of hydrophobic (farnesyl and geranyl) residues and two endoproteolytic cleavage steps that remove terminal peptides to produce the mature lamins. Why LaA undergoes such extensive posttranslational processing is unclear, as mice expressing mature LaA (in which all pre-LaA processing was bypassed) exhibit no discernible pathology (25). Furthermore, mice with only mature LaC appear normal, revealing a high degree of redundancy between the two A-type lamins (40). LaC-only mice live 10% longer than normal mice even though they are obese and moderately insulin resistant and carry an increased tumor burden. Consequently, it has been proposed that the different splice products of *LMNA* may have a role in metabolic fuel partitioning and influence metabolic energy preferences between fat and carbohydrate utilization, although the mechanism is unclear (81). Nevertheless, the importance of LaA posttranslational farnesylation cannot be ignored, as disruption of the processing due to either *LMNA* mutations or loss of ZMPSTE24, which results in the retention of farnesylated LaA, causes progeria and perinatal lethality, respectively.

With the B-type lamins, farnesylation of LaB2 is not essential for normal development, although it is essential for LaB1. Embryos expressing nonfarnesylated LaB1 have lower levels of LaB1, and in the nuclei of the migrating neurons, LaB1 farnesylation is required to retain chromatin at the NE, with detached chromatin being associated with perinatal death (65).

THE PHYSICAL FUNCTIONS OF THE LAMINS

The lamins, particularly the A-type lamins, have a structural role in that they maintain nuclear shape and rigidity and confer cellular resistance to mechanical strain (71, 73). The ability to assemble into higher-order structures and meshworks is instrumental in the upkeep of A-type lamins' roles in governing nuclear structural integrity, since the inclusion of specific mutations that prevent the assembly of the lamins into a lamina or the loss of the A-type lamins results in distortion of the typically oval nuclear shape to varying degrees (110, 126). An extreme form of natural NE distortion is found in granulocytes lacking LaA (100). In fibroblasts deficient in A-type but not B-type lamins, the nuclei are more deformable and less resistant to mechanical strain, such that repetitive stretching results in the cells becoming physically weaker and susceptible to mechanically induced rupture that leads to necrosis or apoptosis (71).

The mutations causing muscular dystrophy and cardiomyopathy are distinct from those affecting adipose tissues. While mutations affecting striated muscles are scattered throughout the *LMNA* gene—including the head and rod domains that are important for high-order assemblies—mutations associated with FPLD2 are congregated at the immunoglobulin-fold domain, which harbors the majority of binding sites for protein partners. Additionally, with respect to the immunoglobulin fold, whereas disease mutations affecting striated muscles tend to be located in the interior, potentially leading to the destabilization of the lamin protein, mutations causing FPLD2 cluster within a small positively charged patch on the surface (31, 117). Importantly, FPLD2-associated mutations show no effect on the structure of the immunoglobulin fold; however, a reduction of the surface charge, as induced by missense mutations, may compromise interactions with key binding partners (85). Unlike both wild-type *LMNA* and FPLD mutants, the expression of many EDMD- and DCM-associated *LMNA* alleles consistently fails to restore nuclear stiffness to LaA/LaC-deficient cells, leading to nuclear fragility and NE rupture (113).

The dynamics and repercussions of NE rupture events have been more rigorously investigated in a cell migration context. In one study, cells were made to migrate through constricted channels (109). NE rupture was observed as the cell nucleus passes through the constriction, with rupture incidence increasing with nuclear lamin depletion. The rupture of the NE led to an uncontrolled exchange of nucleocytoplasmic content, resulting in DNA damage that was then repaired as the rupture was sealed by the endosomal sorting complexes required for transport III (ESCRT-III) machinery (**Figure 2**). This study highlighted the importance of both NE resealing and DNA repair in reestablishing cellular health, since cell survival was compromised only when both processes were jeopardized. Conceivably, NE rupture events would occur at a higher frequency in more fragile nuclei, such as those harboring *LMNA* mutations affecting striated muscles. Notably, while migration through a constriction in this study represents a one-off event, cells in skeletal and cardiac muscles chronically experience forces that may be capable of inducing NE ruptures of more fragile nuclei, potentially shifting the balance away from cellular homeostasis as rupture frequency exceeds DNA repair kinetics. In support of this conjecture, anecdotal evidence of in vivo rupture—including the presence of nucleus-localized mitochondria, the electron microscopy documentation of NE discontinuities, and the consequently increased apoptosis—was observed in skeletal muscle and cardiac tissue from mice and patients with EDMD and DCM (52).

Additionally, different tissues express A-type lamins at levels in direct proportion to the overall stiffness of the tissue, with softer tissues such as brain and fat expressing lower LaA levels, whereas stiff tissues, such as cartilage and bone, express higher levels. The tissue variation in lamin levels may also prevent nuclear distortion and disruption associated with the degree of physical stress to which different tissues are subjected (128) (see the section titled Mechanotransduction).

The lamins can have much farther-reaching effects on the mechanical properties of the whole cell rather than just the nucleus, in that mutation or loss of *LMNA* also reduces cytoplasmic stiffness and compliance (72, 74), as well as affecting centrosome and nuclear positioning during cell migration (14). Although the precise molecular basis of these effects remains unclear, it may well be that lamin mutations disrupt the linker of nucleoskeleton and cytoskeleton (LINC) complexes, which tether the interphase nuclei to the three different cytoskeletal networks, and this in turn has a knock-on effect on the organization of the cytoskeletal networks and cytoplasmic organization (86).

Linker of nucleoskeleton and cytoskeleton (LINC) complex: a complex consisting of the SUN- and KASH-domain proteins at the NE that tethers the interphase nucleus to the cytoskeleton

Chromosome territories: regions of the nucleus preferentially occupied by specific chromosomes; the boundaries between different territories are regions of genome interaction

THE NUCLEAR PERIPHERY AND CHROMATIN ORGANIZATION

Rudimentary measurements in the nineteenth century led to the idea that chromatin is functionally organized within the cell nucleus and to the concept of chromosome territories, in which

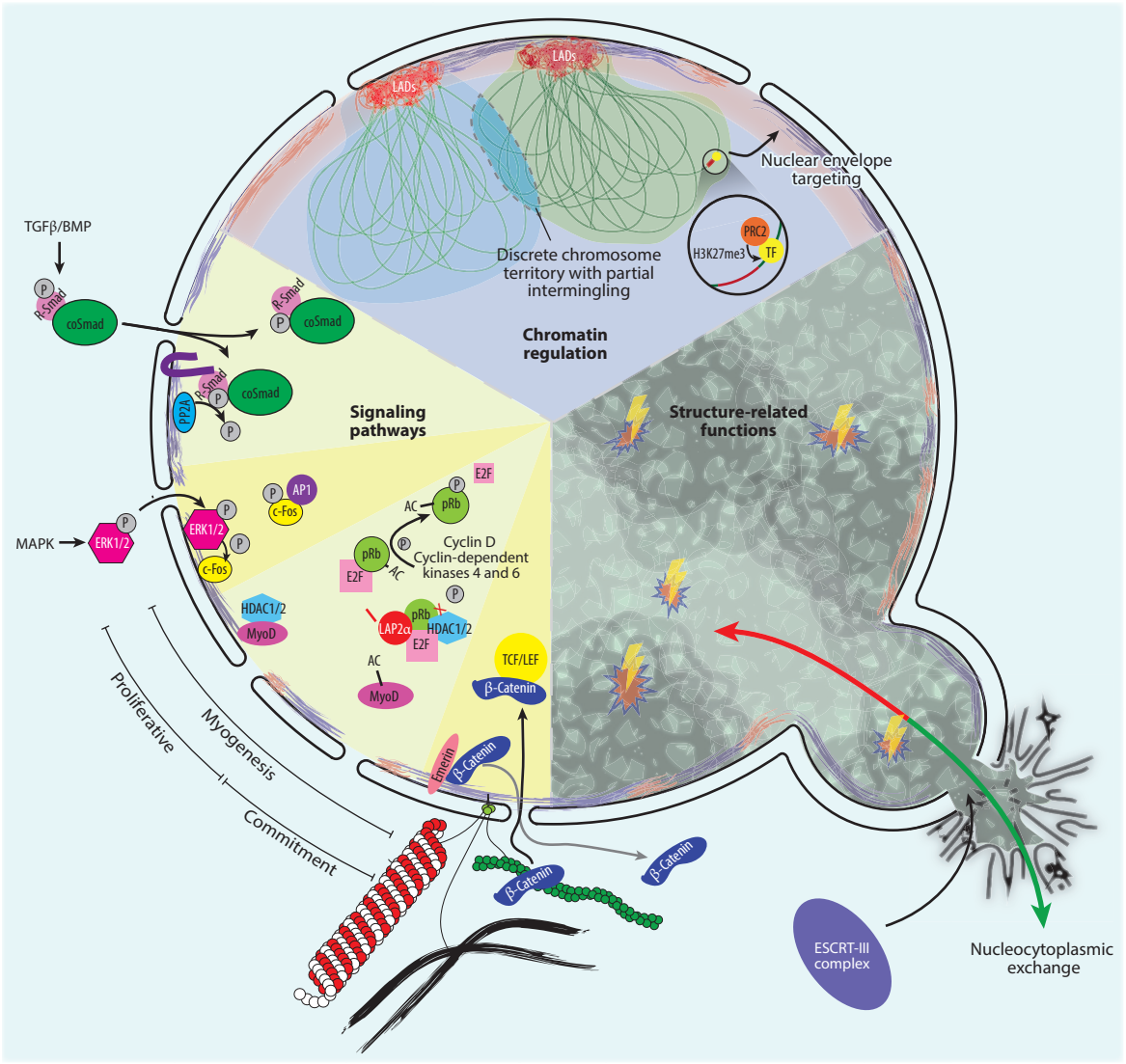

Figure 2

The diverse roles of nuclear lamins. These roles are broadly classified into gene expression through chromatin regulation (*lilac background*), modulation of signaling pathways (*yellow background*), and more structure-related functions (*teal background*). In the area related to chromatin regulation, chromosomes occupy distinct territories, as illustrated by the two chromosomes with different shadings demarcating the chromosome territories. Lamina-associated domains (LADs) (*red*) are anchored at the nuclear periphery via interactions with the lamin B receptor (LBR) and A-type lamins, potentially mediated by middleman/adaptor proteins. Additionally, the instruction for heterochromatin targeting to the periphery depends on the local epigenetic signature, with di- and trimethylation of histone H3 on lysine 9 (H3K9me2/3) and H3K27me3 being important for such targeting to and retention at the nuclear periphery. A-type lamins, H3k9me2/3, and H3K27me3 are also important in the clustering and further compaction of LADs at the nuclear periphery, as depletion of lamin A/C or perturbations to H3K9me2/3 or H3K27me3 disrupt LAD clustering. The area related to modulation of signaling pathways shows some of the many pathways that are regulated by lamins. In the area related to more structural functions, the nuclear lamins govern nuclear integrity. Nuclear envelope rupture can occur as cells migrate through a confined passage or in the presence of mechanical perturbations, such as those induced during skeletal or cardiac muscle contraction. This rupture is more evident in cells that are deficient in the lamins or express mutant forms of the lamins. A ruptured nuclear envelope compromises compartmentalization, and nucleocytoplasmic exchange occurs, resulting in DNA damage that is then rapidly repaired, concomitant with nuclear envelope resealing by the endosomal sorting complexes required for transport III (ESCRT-III) machinery.

chromosomes occupy distinct nuclear volumes. This idea was first proposed in 1885 and was eventually proven in the late twentieth century (23).

The development of chromosome conformation capture allowed the study of noncontiguous chromatin interactions (53). The Hi-C variant of this technique enabled the mapping of genome-wide inter- and intrachromatin contact frequencies and demonstrated that interphase chromosomes adopt a fractal globule configuration (75). In this configuration, chromosomes fold into local self-interacting structural domains known as topologically associating domains (TADs), which are maintained by chromatin architectural proteins such as CTCF and cohesin at TAD boundaries; this local organization occurs iteratively over the entire chromosome, leading to the fractal globule configuration (32, 75) (**Figure 2**). The fractal globule configuration prevents knot formation and allows easy local unfolding and refolding of DNA for local genic regulation and cell replication (93). TADs segregate based on their transcriptional status into active (A) and inactive (B) compartments, with A compartments mostly occupying the nuclear interior and B compartments associated with transcriptionally repressive nuclear compartments at the NE and the periphery of the nucleoli (75). As such, the study of chromatin at the NE, now widely known as lamina-associated domains (LADs), is attractive because the NE aids in establishing interphase chromosome topology and hence overall genome organization. Moreover, the nuclear lamina is a transcriptionally repressive compartment and so presents a manipulative model for transcriptional regulation via spatial organizational changes relative to the lamina (79).

DNA adenine methyltransferase identification (DamID): a technique that identifies which parts of the genome interact with chromatin proteins, as in mapping which genomic regions interact with the lamina

The introduction of DNA adenine methyltransferase identification (DamID), which identifies genome-wide lamina–chromatin interactions, made the study of LADs feasible and broadened our understanding of chromatin regulation and dynamics at the lamina. This technique has enabled a wide range of genome-wide LAD mapping studies across various cell types and species (105). In mammalian cell lines, LADs range from 10 kb to 10 Mb in size and make up a third of their respective genomes (136). Although they are relatively gene poor, LADs are replete with developmentally regulated genes, reinforcing the hypothesis that LAD dynamics serves as a mode for regulating developmental transcription programs (3, 105). As an example, when LADs were profiled across neural development, a potential core organizational architecture was identified, onto which discrete cell type–specific differences were superimposed, such that genes within domains that attain lamina association are mostly repressed, and genes within domains that detach from the lamina are activated or poised for expression (105). The latter observation suggested a model in which transcriptional competence and nuclear lamina attachment could be uncoupled. More specifically, a genomic locus could remain heterochromatinized upon detachment from the lamina and, similarly, could become heterochromatinized prior to lamina attachment.

The above findings raise the question of how a genomic region might be mechanistically recruited to or away from the nuclear periphery. It has become clear that it takes mutual effort from both ends—genomic and lamina—for the appropriate targeting of genomic loci to the nuclear periphery.

Initial studies aimed to identify genomic zip codes that direct a locus to the nuclear lamina. In one such study, Zullo et al. (150) uncovered lamina-associating sequences enriched in GA dinucleotides. When integrated into the genome, these sequences recruited ectopic loci to the nuclear lamina. However, a study by Guelen et al. (51) found that the sequence motif is generally not enriched in LADs; notably, this study involved only two variable LADs, and therefore any generalization of the results is premature. Nonetheless, Zullo et al. (150) identified a cell type–specific transcription factor (ZBTB7B, also known as THPOK), a histone deacetylase (HDAC3), and an inner nuclear membrane protein (Lap2β) that mediate de novo interactions with the nuclear lamina. Importantly, ZBTB7B, a transcription factor recognizing GA_n sequences, features a BTB–POZ domain that recruits Polycomb repressive complex 2 (PRC2) to chromatin, thereby

initiating trimethylation of histone H3 on lysine 27 (H3K27me3) (11). As such, both ZBTB7B and HDAC3 appear, in a concerted manner, to induce a local heterochromatic state that then drives the locus to the nuclear lamina. Consistently, and in contrast to the scarcity of GA dinucleotides within LADs, comparative bioinformatic analyses have found that the bodies of LADs and LAD borders are highly correlated with the heterochromatin marks H3K9me2/3 and H3K27me3, respectively (55). These findings suggest that the epigenetic state may be the ultimate factor in determining relegation to the lamina, whereas lamina-associating sequences and their cognate protein partners may simply act as molecular switches, initiating and subsequently promoting a local chromatin signature favorable for lamina association.

Proximity-dependent biotin identification (BioID): a proximity-based labeling technique to identify protein–protein interactions under physiological conditions

Indeed, a similar study identified lamina-targeting fragments enriched in binding motifs for the DNA-binding protein Yin Yang 1 (YY1), which, when recruited to a transgene cassette consisting of an array of LacO repeats via LacO–LacI tethering, induced local H3K27me3 and subsequent lamina targeting (55). Moreover, the transgene cassette appeared to be preenriched in H3K9me2/3, possibly due to the repetitive nature of the transgene cassette (i.e., LacO arrays) (55). The gain in H3K27me3 thus sets up a local epigenetic signature reminiscent of LADs. Perturbations to H3K27me3 or H3K9me2/3 by RNA interference–mediated knockdown or pharmacological inhibition of EZH2 (necessary for H3K27me3) or G9a (involved in H3K9 methylation) consistently prevented appropriate targeting of the transgene cassette (55). Coincidently, H3K9 methylation is also important for the lamina association of the β-globin locus (*HBB*) in human cells and for a transgene array and endogenous LADs in *Caenorhabditis elegans* (8, 131).

To add another layer of complexity, lamina targeting may be dependent on middleman/adaptor proteins—proteins that interface with chromatin and the nuclear periphery. One such example is CEC-4, an inner nuclear membrane protein in *C. elegans* that binds to mono-, di-, and trimethylated H3K9 via its chromodomain (47). Genetic ablation of CEC-4 resulted in the loss of proper lamina association of a transgene cassette and endogenous LADs, a phenotype reminiscent of genetic mutants devoid of the H3K9 methyltransferases MET-2 and SET-25 (47, 131). Similarly, PRR14, another such adaptor protein, contains a nuclear lamina–targeting domain and a separate domain that binds HP1α, a protein recognizing the H3K9me2/3 modification that is enriched in LADs (108).

More recently, a high-throughput methodology that merges DamID and proximity-dependent biotin identification (BioID) was used to map the microproteome of LADs, thereby identifying candidate proteins at the nuclear lamina–chromatin interface (24). While DamID provides a means to identify DNA sequences associated with the NE, BioID identifies protein interactors and near neighbors. Fusing a catalytically inactive form of the DpnI enzyme (m6A-tracer) that recognizes GATC adenine methylation with BirA resulted in a method to explore protein interactomes within DamID-defined LADs, thereby allowing high-throughput screening of putative linker proteins that serve as middlemen in organizing LADs to the nuclear lamina.

Perhaps the most obvious proteins on the peripheral end are the lamins and a few fairly well-characterized inner nuclear membrane proteins, such as LBR, Lap2β, and emerin. Lamins bind both DNA and chromatin in vitro (50) and are involved in peripheral chromatin attachment in many organisms. In *Drosophila melanogaster*, deletion of the sole B-type lamin resulted in detachment of a transgene array from the periphery (67). By contrast, the expression of a lamin carrying the Y59C point mutation, which is linked to AD-EDMD in humans, in *C. elegans* led to inappropriate peripheral sequestration of a locus harboring a myogenic gene, thereby compromising differentiation of the muscle lineage (89). Strangely, however, deletion of all lamin genes in mouse embryonic stem cells had little effect on lamina chromatin contacts genome-wide or on the cells' viability and ability to differentiate into different lineages (66). This indifference to lamin deletion may be explained by the redundant roles of additional heterochromatin tetherers. Genetic

studies in mice revealed that total displacement of heterochromatin at the nuclear periphery can be achieved only in the absence of both A-type lamins and LBR (123). This total detachment is not an artificially induced phenomenon; in photoreceptor rod cells of nocturnal animals, where neither LBR nor A-type lamins are expressed, it results in an inverted chromatin configuration in which heterochromatin clusters in the nuclear interior, while euchromatin demonstrates a more peripheral disposition. This configuration is presumed to allow for better night vision by decreasing light scattering. Intriguingly, the ectopic expression of LBR but not LaC in photoreceptor rod cells was sufficient to prevent the inverted chromatin configuration in rod cells (123). This finding suggests that A-type lamins may effect heterochromatin attachment via mediator proteins such as Lap2, emerin, and various other poorly characterized NE transmembrane proteins, some of which influence genome architecture upon ectopic expression, as well as the middleman proteins, as discussed above (27, 112).

Since nuclear anchorage exerts structural constraints on the overall genome topology, *LMNA* mutations could conceivably result not only in chromatin changes at the periphery but also in secondary genome organizational perturbations. Indeed, genome disorganization has been documented in various laminopathies from enhanced LaA (R453W associated with AD-EDMD) binding to transcriptional start sites of myogenic genes, thereby preventing muscle differentiation to a more global disorganization, as observed in progeria (91, 106).

Recently, three separate but similar studies reported on genome perturbations in relation to *LMNA*-associated DCM (7, 30, 115). All of these studies detailed genome organizational changes in mutant cardiomyocytes derived from induced pluripotent stem cells and compared them with isogenic controls, albeit to different degrees, and all of them identified therapeutic targets (designed based on knowledge of genome organizational changes) that ameliorated the conductive defects associated with DCM. Yet one of these studies noted that the extent of the perturbations (compartment changes) was minor, with only 1.2% of the genome exhibiting chromatin compartment changes (from B to A compartments or vice versa, with the former dominating these changes) (7). For a few loci, this study also confirmed that the switch from B to A compartments represented a detachment from the nuclear lamina. The authors noted that the majority of genes exhibiting chromatin compartment changes did not alter their gene expression status compared with isogenic control cells. Both observations therefore suggested an incompatibility in the role of A-type lamins in chromatin organization and challenged the validity of the chromatin hypothesis as the molecular basis of the laminopathies. It is, however, important to note that these studies relied on techniques based on population measures, which tend to obscure significant cell-to-cell variability, and the 1.2% observed changes may well be an underrepresentation of the overall perturbation (83). Additionally, chromatin perturbations may occur without necessarily exhibiting compartment changes, such as the effects on the integrity of compaction in TAD cliques (higher-order heterochromatin assembly of long-range inter-TAD interactions within the same compartment), as has been recently suggested, with this phenomenon dependent on LaA and LaC as well as the epigenetic marks H3K27me3 and H3K9me2/3 (83, 95). Transcriptional competence and nuclear lamina attachment can also be uncoupled such that, upon detachment from the lamina, a gene does not necessarily increase transcriptional competence immediately (105). The upregulation of these poised genes could require a subsequent switch from a heterochromatic state to a euchromatic state. It is therefore tempting to propose that the lamina acts as a scaffold to protect and reinforce the heterochromatic status of LADs.

Obviously, many unresolved issues remain. One of the most striking subjects would perhaps be the mechanistic means of achieving a tissue- and cell type–specific LAD configuration and hence genome organization. While the local epigenetic state might be the deterministic factor for peripheral targeting, a comprehensive analysis of variable LADs across multiple cells types

could conceivably uncover lamina-associating sequences and binding motifs that will consequently lead to the identification of cell type–specific molecular partners that locally initiate the switch to a heterochromatic state favorable for peripheral targeting. Additionally, the identification and characterization of middleman/adaptor proteins and tissue-specific NE transmembrane proteins that are capable of influencing genome architecture may shed light on both tissue-specific genome architecture and its possible links to laminopathies that are likewise highly tissue specific.

NUCLEAR LAMINS AND SIGNALING

Apart from directly regulating chromatin dynamics, the lamina also influences gene expression by serving as a platform for the docking of transcriptional factors and downstream signaling molecules. Such sequestration of transcriptional factors spatially restricts and hence limits their activities by segregating them from their cognate promoters.

An example of restrictive sequestration at the NE is R-Smad–Man1 perinuclear interactions. R-Smads are the transcriptional effectors of the TGFβ and BMP pathways (88) (**Figure 2**). Upon activation of these pathways, R-Smads are phosphorylated, oligomerize with Smad4, and are subsequently translocated into the nucleus, where they regulate transcription (39). The physical interaction of R-Smads with Man1 directly antagonizes TGFβ and BMP signaling by indirectly regulating Smad phosphorylation, which in the absence of Man1 results in the R-Smads being retained within the nucleus, where they cause transcriptional overactivity and, in mice lacking Man1, disrupt vascular development (22). Additionally, A-type lamins at the nuclear periphery may act as a scaffold for protein phosphatase 2A (PP2A)–mediated inhibition of TGFβ signaling (134). Recent studies have found that, via previously unknown mechanisms, TGFβ signaling is elevated in the hearts of $Lmna^{H222P/H222P}$ mice, a murine model for EDMD with DCM (15, 134). Moreover, the aberrant hyperactivation of TGFβ signaling led to an abnormal overactivation of yet another signaling cascade, the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway (15). Whether these *LMNA* mutations affect Smad function directly in some manner or indirectly through effects on Man1 remains to be established.

The MAPK/ERK pathway is critically involved in converting external information into intracellular signaling events that, in many mammalian cell types, promote cellular growth and proliferation. Active ERK1/2 may interact and colocalize with LaA at the nuclear lamina, where it more efficiently activates c-Fos, a member of the AP-1 family of transcription factors that is also anchored at the nuclear periphery via direct interactions with LaA (46). The activation of c-Fos by ERK1/2 phosphorylation releases c-Fos from the NE, after which it heterodimerizes with other AP-1 family members, such as c-Jun, to initiate transcription (61). Serum-starved quiescent cells with low AP-1 activity consistently exhibit a perinuclear localization of c-Fos (61). The reintroduction of serum releases c-Fos from the NE, restoring AP-1 function and hence allowing cellular proliferation. Moreover, enhanced proliferation is a cellular phenotype of *LMNA*-null cells that results in part from the loss of the isolative regulation of c-Fos (61).

Numerous studies have found that abnormally activated ERK signaling is associated with EDMD and DCM. Most prominently, the hearts of $Lmna^{H222P/H222P}$ mice display aberrantly overactivated ERK1/2 prior to the manifestation of cardiomyopathy. The chronic systemic treatment of these mice with an ERK1/2 inhibitor, at least prior to the development of DCM, temporarily blocks cardiomyopathy progression (96).

LaA and LaC are also important in regulating two highly interdependent pathways: cell cycle control and DNA damage repair (DDR) (**Figure 2**). In regulating the former, LaA and LaC provide an additional layer of regulation to the retinoblastoma protein (pRb). This protein regulates

cellular proliferation and differentiation through its inhibitory actions on the transcription factor E2F-DP (68). Under nonproliferative conditions, pRb binds to E2F-DP in a hypophosphorylated state. In proliferating cells, it becomes hyperphosphorylated and consequently releases E2F-DP, leading to the transcription of target genes required for S phase progression. The direct binding of nucleoplasmic LaA and Lap2α, a spliceoform of the protein encoded by the *TMPO* gene, results in the accumulation of hypophosphorylated pRb, possibly by sterically hindering the action of cyclin-dependent kinases and additionally via LaA-dependent PP2A dephosphorylation of pRb (33, 134). Moreover, Lap2α, together with LaA, can occupy promoters of E2F-DP target genes, thereby directly inactivating their transcription (33).

LaA has both direct and indirect influences on the regulation of DDR. DDR pathways are highly complex, involving a series of steps from DNA damage sensing to transduction and finally execution, in which repair is initiated, mostly concomitant with cell cycle arrest, until the damage is restored. However, if the damage is beyond repair, growth arrest or apoptosis is initiated. Although a variety of repair mechanisms specific to different classes of DNA damage have been identified, homologous recombination (HR) and nonhomologous end joining (NHEJ), two disparate repair pathways for DNA double strand breaks (DSBs), have been extensively characterized due to their particular toxicity to cells (144). One of the earlier DDR events is the phosphorylation of the histone variant H2AX around the DNA break, which facilitates the recruitment of other factors that further modify the local chromatin and promote local enrichment of yet other factors, in particular 53BP1 and BRCA1, two key proteins in regulating the balance between HR and NHEJ (10, 12, 99). While BRCA1 facilitates end resection and the recruitment of RAD51, which is essential for homology search and strand invasion during HR, 53BP1 inhibits end resection and simultaneously promotes the local enrichment of proteins required for NHEJ (119). This raises the question of how a decision between HR and NHEJ is made. Recent studies have used capture Hi-C, a variant of the chromosome conformation capture technique, to measure relative interaction frequencies between DSBs before and after DSB induction. In contrast to NHEJ-prone intergenic DSBs, DSBs within active genes are enriched in RAD51 and exhibit clustering in G1 phase. It was hypothesized that genic DSB clustering provides a means to sequester and prime these breaks for faithful HR repair in S phase. Furthermore, DSB clustering is dependent on SUN2, a LINC complex component and FMN2 actin organizer. Moreover, the LINC complex has been implicated in DSB mobility in several other systems, including yeast and 53BP1-occupied and unprotected telomeres in higher eukaryotes (82, 127). Although the effects on HR and NHEJ resulting from mutations in and loss of A-type lamins have been widely documented (as discussed below), how the same lamina defects, which potentially influence LINC complex functions, affect DDR choice remains unclear.

In the absence of LaA and LaC, there is a marked decrease in 53BP1 at radiation-induced DNA breaks attributable to the decrease in the global levels of the protein (111). Coincident with global 53BP1 loss in *LMNA*-deficient cells is a notable increase in CTSL, a cysteine protease directly responsible for the decrease in 53BP1 (111). Therefore, the loss of *LMNA* was implicated in the transcriptional upregulation of CTSL, resulting in the global loss of 53BP1. LaA and LaC bind directly to the 53BP1 Tudor domain, protecting 53BP1 from proteasomal degradation and thereby maintaining a steady nucleoplasmic pool of 53BP1 for rapid recruitment during DNA damage (45). Additionally, depletion of A-type lamins compromises HR as a result of transcriptional repression of the *BRCA1* and *RAD51* genes (111). The discovery of a novel chemical tool that specifically perturbs endogenous LaA/C–RAD51 binding leads to proteasomal degradation of RAD51, suggesting that, similar to LaA/C–53BP1 binding, LaA/C binding to RAD51 protects it from degradation, possibly by sequestration (111). Consistent with the role of

LaA in regulating and promoting HR and NHEJ, *Lmna*-null fibroblasts exhibit signs of genome instability, as illustrated by high incidences of aneuploidy, increased chromosome breaks, and persistence of γ-H2AX foci, indicating unrepaired DNA (48).

Progeroid syndromes are associated with genome instability. *Zmpste24*-deficient cells display high incidences of aneuploidy, DNA damage, and chromosome abnormalities, with mice exhibiting hypersensitivity to DNA-damaging agents (77). Additionally, recruitment of both 53BP1 and RAD51 to DNA lesions is impaired in fibroblasts from *Zmpste24*-null mice and in HGPS fibroblasts, resulting in defective DDR (78). Because the ectopic expression of an uncleavable and hence permanently farnesylated pre-LaA (or progerin in wild-type cells) induces the same DDR defects, both proteins may act in a dominant negative fashion to perturb DNA damage response and repair. Notably, the existence of the nucleoplasmic pool of LaA is absolutely dependent upon Lap2α expression, and coincidentally, Lap2α is reduced in HGPS cells, thereby obliterating the pool of nucleoplasmic LaA (18, 97, 138). It is conceivable that the dominant negative effects of pre-LaA and/or progerin on DDR arise in part due to the elimination of the nucleoplasmic LaA/C pool, which affects the protein stability of both RAD51 and 53BP1 and therefore also affects DDR. On a related note, accelerated telomere attrition is commonly observed in progeria, with Lap2α, together with nucleoplasmic LaA and LaC, localizing to and conferring protective effects on telomeres. In HGPS, however, telomere end protection is lost because Lap2α (and nucleoplasmic LaA and LaC) are displaced from telomeres (18).

Apart from its regulatory effect on proliferation, pRb also influences the cellular differentiation of several tissues (114). As such, it functions throughout the regenerative spectrum, from stem cell proliferation to commitment and differentiation. This is perhaps best demonstrated in the process of myogenesis, which begins as myoblasts exit the cell cycle and are committed to differentiate by initiating transcriptional changes. This decision to commit involves a highly regulated interplay of factors involved in the regulation of proliferation and differentiation, and essential to this process are myogenic regulatory factors, including MyoD and myogenin (4, 142).

The myogenic regulatory factors feature three structural domains, of which the basic helix-loop-helix (bHLH) domain, by allowing binding to specific DNA sequences to regulate muscle-specific gene transcription, is the main contributor of myogenesis (4). Notably, there is ample evidence suggesting an interplay between MyoD and pRb (**Figure 2**). In a proliferative state, MyoD is bound by HDAC1 and HDAC2, which maintain MyoD in a hypoacetylated state, preventing its binding to DNA, presumably via interactions with LaA (5). As differentiation is initiated, the cells exit the cell cycle, and pRb becomes hypophosphorylated and binds LaA and Lap2α. Additionally, hypoacetylated pRb also binds HDAC1 and HDAC2, thereby drawing them away from MyoD (5). This results in an accumulation of acetylated MyoD, which then induces the initial stages of myogenesis via binding to downstream target promoters. Concurrently, expression of LaA and LaC is upregulated to provide for a positive feedback loop in inhibiting proliferation via hypophosphorylated pRb binding (5). Peculiarly, through unknown mechanisms, this coregulation of MyoD and pRb is dependent on both emerin and LaA/C, so that when emerin is lost in X-linked EDMD or in the presence of certain dominant missense mutations in *LMNA* (AD-EDMD), myogenesis is perturbed and associated with uncoordinated phosphorylation and acetylation events (5, 92).

The canonical Wnt signaling pathway (also called the Wnt/β-catenin pathway) epitomizes the active participation of NE proteins in regulating the nuclear accumulation of key effector signaling proteins, with the pathway being perturbed by various laminopathies (**Figure 2**). Central to this pathway is the regulation of β-catenin (19). The pathway is activated by Wnt proteins binding to the frizzled receptors, which induce changes culminating in the cytoplasmic accumulation of β-catenin, which is otherwise destined for proteasomal degradation. Undegraded β-catenin translocates into the nucleus, where it acts as a cofactor for the TCF/LEF family of transcriptional

factors in inducing gene transcription programs that instruct stem and progenitor cell renewal, proliferation, and differentiation. Nuclear β-catenin is found both in the nucleoplasm and at the nuclear lamina, and several studies have implicated emerin and the LINC complex in the accumulation of β-catenin in the nucleus, where it binds TCF/LEF transcription factors in effecting gene transcription (87, 98, 133).

The involvement of emerin in regulating nuclear β-catenin dynamics stems from correlative in vitro studies of emerin overexpression and studies of EDMD cells lacking emerin (87). Overexpression of emerin depleted nuclear β-catenin while concomitantly increasing the cytoplasmic β-catenin pool; by contrast, fibroblasts from X-linked EDMD patients lacking emerin showed an inverse effect. This anticorrelative effect may depend on the adenomatous polyposis coli (APC)–like domain of emerin, which directly binds β-catenin, since the overexpression of a truncated form of emerin lacking the APC domain did not result in β-catenin loss from the nucleus. However, the molecular subtleties behind emerin's ability to induce the export of β-catenin are not understood. The regulation of nuclear β-catenin levels by the LINC complex may involve aspects of mechanosensing, since the induction of mechanical forces or strains leads to a LINC complex–dependent accumulation of β-catenin in the nucleus (98, 133). This effect is, however, dependent on an intact linkage from lamina-anchored SUN proteins to nesprins and finally to the cytoskeleton because the force-dependent accumulation of nuclear β-catenin is abrogated (*a*) in the absence of SUN proteins, (*b*) in the absence of functional LINC complexes as a result of the expression of dominant negative SUN or KASH domains, and (*c*) when cells experience cytoskeletal disruptions as a consequence of pharmacological treatment (98, 133). It is still unclear, however, whether specific SUN- or KASH-domain proteins are involved or these proteins have redundant or even opposing roles in regulating nuclear β-catenin dynamics. Nonetheless, such studies are beginning to expand our understanding of the mechanisms behind Wnt/β-catenin pathway dysregulation reported in accelerated aging.

Indeed, studies have suggested that the absence of the transcriptionally active form of β-catenin causes abnormal epidermal stem cell proliferation in the hair follicles of the *Zmpste24*$^{-/-}$ model of premature aging (38). Similarly, there is diminished nuclear accumulation of β-catenin in HGPS patient fibroblasts and in basal keratinocytes of an HGPS murine model, which has been proposed to be the underlying cause of perturbed epidermal development (122). One study showed that the Wnt pathway is also disrupted in *Lmna*Δ9 progeroid mouse and HGPS patient cells, albeit through a reduction in the nuclear localization of the Wnt-regulated Lef1 transcription factor (57). This inhibition results in a compromised ability to produce at least one extracellular matrix (ECM) component, type I collagen, via decreased Lef1 occupancy at the promoter of the *Col11A1* gene. Consequently, the lack of a functional ECM compromised the growth of postnatal fibroblasts and vascular integrity (57). The importance of a functional ECM in progeria was corroborated in one study by the lack of progeroid phenotypes in the *Zmpste24* chimeric mouse model, in which the mice develop normally while keeping similar proportions of *Zmpste24*-null (pre-LaA-accumulating) and Zmpste24-expressing (mature-LaA-containing) cells (26). In this study, ECM production by *Zmpste24*-expressing cells presumably enabled the survival of *Zmpste24*-null cells.

Importantly, in the cell fate commitment of mesenchymal stem cells, there is an inverse relationship between the canonical Wnt pathway and PPARγ, the main inducer of adipogenesis. While the Wnt pathway favors osteoblastogenesis, PPARγ favors adipogenesis, and the two fates are mutually exclusive. This inverse relationship is a result of the mutual negative inhibition of the two pathways; the Wnt pathway inhibits PPARγ expression, with PPARγ promoting the proteasomal degradation of β-catenin (21, 94). Given the involvement of emerin, nesprin-2, and potentially many other NE proteins in regulating the Wnt pathway, and the alterations in adipose tissue in some laminopathies, such as FPLD and HGPS, it will be interesting to see, in these

diseases, whether the nuclear architecture or composition changes in a way that influences the Wnt pathway, thereby dysregulating the formation of adipose tissue.

The contribution of the lamina to regulating signaling pathways is likely much more extensive than what is presented here, as many more NE-resident proteins uncovered in proteomics screens that have links to signaling remain undercharacterized. Our current understanding of the lamina and its associated proteins' influences on signaling pathways may merely represent the tip of an iceberg.

MECHANOTRANSDUCTION

As discussed above, it is becoming increasingly difficult to draw a clear distinction between gene expression and the structural and mechanical disease models for laminopathies, with the concept of mechanotransduction marrying the two. Mechanotransduction refers to the process whereby mechanical stimuli (the structural model) sensed by cells are converted to biochemical or genetic outputs (the gene expression model), resulting in specific cellular responses (90). Since most biochemical signaling pathways culminate with the nuclear entry of signaling molecules, the nucleus is central in mechanotransduction. Notably, with respect to the nucleus, mechanotransduction can be bidirectional: Physical stimuli from the extracellular environment induce biochemical signals in the nucleus, and mechanical changes emanate from the nucleus and result in cytoplasmic and/or extracellular changes (see the section titled Nuclear Lamins and Signaling). Additionally, different avenues for propagating the initial physical stimulus dictate the speed at which a response is generated, with mechanical forces transmitted directly through the cytoplasm to the nucleus via the LINC complex being the fastest mode of signal propagation (30 μm/s as opposed to 1–2 μm/s for diffusion- or motor-based propagation) (90). Importantly, mechanotransduction through the LINC complexes may regulate a variety of signaling pathways, including SUN1 regulation of Drosha and hence its effects on microRNA levels, which then regulate the expression of myogenic proteins (60, 80, 118, 130). As such, the LINC complexes have garnered tremendous interest.

The effects of physical stimuli on gene expression were derived from studying the differentiation of mesenchymal stem cells on varying substrates (128). Mesenchymal stem cell differentiation into specific lineages was favored when cultured on ECMs with mechanical properties matching those of the desired tissue, and there was a correlation between the ratio of A- to B-type lamin levels and tissue stiffness. Furthermore, mesenchymal stem cells can fine-tune LaA levels based on the stiffness of the matrix in which they reside. Differentiation along a desired lineage was most optimal when both ECM elasticity and LaA expression levels matched those of the specific tissue. How a combination of ECM elasticity and optimal LaA expression eventually affects gene transcription and cell fate decision is unclear, although such transcriptional changes could be induced by several non-mutually-exclusive mechanisms.

The fastest mechanism for inducing transcriptional changes is undoubtedly the transmission of force directly to the underlying DNA. The ability to mechanically induce conformational as well as transcriptional changes in DNA was assessed using an engineered cassette consisting of an array of LacO repeats (for live visualization of the locus via eGFP–LacI binding) and the *DHFR* gene (129). In the presence of an intact actomyosin cytoskeleton and functional nucleocytoskeletal coupling, the application of shear stresses on integrins via magnetic beads resulted in a rapid strain-induced locus decompaction and a subsequent increase in transcription of the *DHFR* gene proportional to the stretching force. However, it is unclear how specificity could be established via this mechanism if it is used for the regulation of endogenous genes.

Alternatively, a transmission of force can induce changes in biochemical signaling pathways that in turn lead to changes in gene expression, such as Wnt signaling, of many possibly affected

pathways. As mentioned, cells grown on substrates of differing elasticity fine-tune LaA levels, which leads to changes in cell fate decisions. Notably, growing mesenchymal stem cells on stiff substrates promoted osteogenesis, while growing the same cells on softer substrates promoted adipogenesis (128). Although increasing LaA expression on a stiffer ECM was suggested to serve as a protective mechanism against nuclear rupture, it concurrently reinforces the high tensile stress from the ECM that is exerted on the cytoskeletal fibers through the LINC complex. This high-tension state may lead to the eventual translocation of β-catenin into the nucleus, inducing transcriptional changes that inhibit PPARγ expression, therefore favoring osteogenesis. By contrast, when grown on a soft matrix, β-catenin is excluded from the nucleus due to low cellular tensile stress with decreased nuclear translocation, which leads to adipogenesis, consistent with the Wnt pathway inhibiting adipogenesis (94).

Changes in LaA levels, conformation, or even posttranslational modifications may also lead to overall organizational changes in the genome. As mentioned, peripheral heterochromatin anchorage at the NE depends on LaA, LaC, and LBR. While LBR is sufficient for this function, LaA and LaC require mediator proteins to achieve the same role. Therefore, mechanically induced changes (possibly mediated through the LINC complexes) to the levels of LaA and LaC, their conformations, or their posttranslational modifications can give rise to changes in their interaction with protein interactors/middleman proteins that may mediate LAD binding and hence affect LAD organization and gene expression.

Although it has been gaining prominence, the concept of mechanotransduction, especially in influencing gene expression changes, is still at its infancy. Hopefully, existing and emerging genomic technologies such as DamID, Hi-C, and chromatin immunoprecipitation can be used in conjunction with novel mechanical stimulation techniques to unravel both local subtleties and global changes in genome organization subject to force induction.

THERAPIES

The discovery of the numerous diseases linked to mutations in the lamins and other NE proteins initiated searches for ways to treat or at least ameliorate these diseases. For the cardiomyopathies arising from the *LMNA* and *EMD* mutations, MAPK inhibitors have modest effects in delaying DCM progression (96). Currently available procedures rely on pacemakers; ultimately, if the disease progresses, a heart transplant is the only effective cure, making lamin-induced DCM one of the major reasons for heart transplantations (141). For FPLD2, treatment with the adipokine leptin or its analogs can, in some cases, lead to improved metabolism (103).

With a greater understanding of which biochemical pathways are altered by the *LMNA* mutations, new therapeutic pathways are being explored, particularly with regard to HGPS, presumably in the hope that any therapeutic effective at treating HGPS may then be applied to improving normal aging. Many attempts using different compounds, including mTOR inhibitors, farnesyltransferase inhibitors, and new compounds arising from various screens (e.g., remodellin), have been tested on progeric mouse models, with limited to modest effects (6, 54). Recently, interest has focused on using the latest gene editing procedures, centering on the CRISPR/Cas9 system, to manipulate the mutated alleles and either restore the mutation to the wild-type sequence or delete the mutated allele (116). Unfortunately, using such approaches with the laminopathies would currently be complicated by challenges in delivering sufficient amounts of the therapeutic to the affected tissues. First, available methods using adeno-associated virus delivery are expensive, and it is unclear whether the right serotypes exist to deliver the therapeutic to all affected tissues. Second, it is not clear how efficiently CRISPR/Cas9-mediated HR, which would be needed to repair the mutation, would work in nonproliferating tissues such as cardiomyocytes

(101). Third, deletion or inactivation of the mutated allele (nearly all laminopathies arise from dominant missense mutations) may not work because DCM and EDMD can arise from lamin haploinsufficiency (9). A possible and elegant alternative for treating HGPS would be to suppress A-type expression but retain C-type expression by using microRNAs specific to the A-type lamins (149). This approach would suppress expression of the disease-causing LaA while preserving LaC expression as it normally occurs in the central nervous system, as mice that express only LaC appear to be normal. Finally, genetic manipulation or biochemical interference of more tractable proteins that interact with the lamins may also offer routes to halting lamin-induced disease progression; for example, loss of either SUN1 and Lap2α, neither of which is lethal when deleted, markedly improved the life spans of mice with *Lmna*-induced disease (16, 22).

CONCLUDING REMARKS AND RELEVANCE TO CONTEMPORARY HEALTH-CARE ISSUES

Nuclear lamins are undoubtedly far more intricately involved in diverse cellular roles than previously thought, with the lamina and NE proteins showing not only tissue- and cell-specific patterns of expression but also patterns that change during development. Mutations in the lamin and NE genes cause a multitude of diseases. While laminopathies are considered rare, it is becoming increasingly evident that they account for a sizable proportion of more common contemporary health-care issues. For instance, 0.5–5% of patients with DCM, a major cause of heart failure, show *LMNA* pathogenic variants, and when considering cases of DCM with atrioventricular conduction disorders, this proportion rises to 33%, making *LMNA* the second-most-commonly-mutated gene (out of at least 30 potentially linked genes) in DCM (63). Additionally, a high prevalence of laminopathies is observed in patients with metabolic syndrome, a major health hazard of the modern world that is characterized by abdominal adiposity, impaired fasting glucose, and hyperinsulinemia (35). Importantly, although HGPS is a truly rare genetic disease, affecting 1 in 4 million worldwide, it serves as a model for the deterioration of tissues and organ systems that occurs with normal aging, including alopecia, loss of subcutaneous fat, and significant cardiovascular abnormalities and failure (56). The study of laminopathies will provide deep insights into the etiology of modern health-care issues, as well as possibly providing novel therapeutic interventions, and may, in the near future, help to demystify the aging process.

SUMMARY POINTS

1. The nucleus can no longer be considered a porous bag that serves merely to contain the genome.
2. The nuclear envelope (NE) and lamina are now recognized to have many significant cellular functions, including regulating signaling pathways and their transcription factors, organizing chromatin, and modulating DNA repair mechanisms and cytoskeletal function.
3. The interphase nucleus, through the NE-associated LINC complex, is physically tethered to the cytoskeletal networks. This tethering is required for nuclear positioning, determining organelle distribution, regulating cell migration, DNA damage responses, and perhaps mechanotransduction (i.e., how cells sense their physical and mechanical environment and respond by changes in gene expression).

4. The underlying lamina is important for maintaining nuclear shape, in protecting the nucleus from mechanical stresses, and as a scaffold in regulating chromatin organization. It is also required for the correct localization of many nuclear proteins regulating gene expression and DNA repair.
5. The laminopathies are caused by mutations in the lamins and other NE proteins. Although they are rare, the study of the molecular pathology of these diseases is providing insights into more common afflictions, such as obesity, cardiovascular disease, and aging.

FUTURE ISSUES

1. A consensus is emerging that the lamina has primarily structural roles, protecting the nucleus from mechanical stresses and functioning as a scaffold for the correct localization of NE proteins that regulate many biochemical pathways. Future research will be directed at understanding how these different functions are integrated, as it is becoming apparent that lamin mutations alter the expression levels of some NE proteins, with these changes underlying some of the molecular pathologies caused by the mutations. This suggests that the lamina and NE include a highly integrated cross-regulatory network of interacting proteins.
2. Determining the functions of the lamina may eventually lead to a deeper understanding and molecular description of mechanotransduction.
3. Answers should emerge as to the precise role of the lamina and NE in organizing chromatin, especially heterochromatin, and whether this organization is essential for gene regulation.
4. The LINC complex is of increasing interest in terms of how it integrates nuclear functions with the cytoskeleton. These functions go beyond signaling and mechanotransduction and reveal how the nucleus is a key organizer of the cytoskeleton and is important in regulating many cytoskeletal functions, such as nuclear positioning during development, cell migration, and organelle distribution within the cell.

DISCLOSURE STATEMENT

C.L.S. is a founding member of Neuvocor, which is developing therapeutics to treat laminopathies.

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