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DNA Damage and Lamins

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Abstract

The spatial and temporal organization of the genome has emerged as an additional level of regulation of nuclear functions. Structural proteins associated with the nuclear envelope play important roles in the organization of the genome. The nuclear lamina, a polymeric meshwork formed by lamins (A- and B-type) and lamin-associated proteins, is viewed as a scaffold for tethering chromatin and protein complexes regulating a variety of nuclear functions. Alterations in lamins function impact DNA transactions such as transcription, replication, and repair, as well as epigenetic modifications that change chromatin structure. These data, and the association of defective lamins with a whole variety of degenerative disorders, premature aging syndromes, and cancer, provide evidence for these proteins operating as caretakers of the genome. In this chapter, we summarize current knowledge about the function of lamins in the maintenance of genome integrity, with special emphasis on the role of A-type lamins in the maintenance of telomere homeostasis and mechanisms of DNA damage repair. These findings have begun to shed some light onto molecular mechanisms by which alterations in A-type lamins induce genomic instability and contribute to the pathophysiology of aging and aging-related diseases, especially cancer.

Keywords

Lamins; Laminopathies; Hutchinson–Gilford Progeria Syndrome (HGPS); DNA repair; Cathepsins; Vitamin D; DNA damage response; Telomeres; Nonhomologous end-joining; Homologous recombination; Farnesyltransferase inhibitors; Reactive oxygen species; 53BP1; Genomic instability

Introduction

The human genome is organized into different levels of complexity. The DNA is packaged into nucleosomes to form the chromatin fiber, which in turn can acquire different structures and states of compaction. Chromatin compaction is fundamental for fitting 2 m of DNA in humans into the small volume of the nucleus. Another level of complexity is the 3D nuclear organization of the genome, which is responsible for maintaining the territories occupied by the different chromosomes, as well as the accumulation of repressive chromatin or heterochromatin at the nuclear periphery [1–3]. Alterations in nuclear architecture and

chromatin structure are associated with disease, thus providing evidence of the importance of genome organization for genome function [4–6].

In recent years, the nuclear lamina has arisen as a key structure in the organization of genome function. The nuclear lamina is a filamentous structure under the inner nuclear membrane composed of A-type and B-type lamins and lamin-associated proteins, which provide a scaffold for tethering chromatin and protein complexes to specific subcompartments [7–11] (Fig. 1). This compartmentalization is brought about by the interaction of the nuclear lamina with large domains throughout the genome, known as lamina-associated domains (LADs), which vary in size between 0.1 and 10 Mb. LADs are usually gene-poor regions enriched in repressive chromatin marks and demarcated by boundaries including insulators, CpG islands, and gene promoters oriented away from the lamina [12]. The organization of the genome orchestrated by the nuclear lamina is thought to be essential for the proper regulation of nuclear functions, including gene transcription and silencing, DNA replication and repair, positioning of nuclear pore complexes, chromatin remodeling, and nuclear envelope breakdown and reassembly during mitosis [6, 13, 14].

A-type and B-type lamins are type V intermediate filament proteins with a characteristic central α -helical coiled coil rod domain flanked by non-helical globular N-terminal “head” and C-terminal “tail” domains [15] (Fig. 2). While B-type lamins are constitutively expressed in all cell types and during all stages of development, A-type lamins are only expressed after the onset of cell differentiation. In the mouse, expression of A-type lamins is not observed until embryonic day 12. The B-type lamins class includes lamin B1, encoded by the *LMNB1* gene, and lamins B2 and B3 that result from alternative splicing of the *LMNB2* gene. In contrast, four A-type lamins (lamins A, C, A₁₀, and C2) proteins result from alternative splicing of a unique *LMNA* gene [16]. A-type and B-type lamins contain a -CAAX motif at their C-terminus, which undergoes farnesylation and carboxymethylation shortly after synthesis [17,18]. This posttranslational modification is thought to facilitate anchoring of lamins to the inner nuclear membrane. In the case of lamin A, further processing of the protein takes place by the metalloprotease Zmpste24, which removes 15 residues at the C-terminus, including the farnesylated cysteine [19, 20], rendering mature lamin A (Fig. 2). This processing step is crucial for the proper function of lamin A, and alterations in this process result in severe nuclear abnormalities linked to disease, as discussed below. In addition, different studies have shown that the C-terminal domain of A-type lamins contains the binding sites for most lamin-binding proteins, as well as chromatin [13, 21, 22].

In recent years, interest in lamins has increased due to the association of hundreds of mutations in the *LMNA* gene with over a dozen degenerative disorders, broadly termed laminopathies, which include muscular dystrophies, neuropathies, lipodystrophies, and a variety of premature aging syndromes. In addition, alterations of A-type lamins function have been associated with physiological aging and cancer [4, 23, 24]. The connection between A-type lamins and aging came primarily from the discovery that the fatal premature aging disease Hutchinson Gilford Progeria Syndrome (HGPS) is caused by a mutation in the *LMNA* gene that results in the expression of a mutant dominant-negative prelamin A isoform known as “progerin” [25–27]. Progerin contains an internal deletion of 50 amino acids near

the C-terminus, which removes the second cleavage site by Zmpste24. Thus, a farnesylated form of the protein accumulates, which is toxic for the cell.

Interestingly, the mouse knockout for Zmpste24 exhibits similar phenotypes as human patients with HGPS [20, 28], representing a good model to study progeria. The fact that progerin accumulates in fibroblasts from old individuals has implicated A-type lamins also in physiological aging [29, 30]. Furthermore, a link between A-type lamins and cancer has been established by studies showing that their expression is altered in many types of malignancies, which is often associated with increased aggressiveness [31–34]. Methylation-induced silencing of the *LMNA* gene is a major event in leukemia, lymphomas, and small cell lung cancer, while overexpression is associated with colon carcinoma. Thus, expression of mutant forms of A-type lamins as well as changes in their expression can lead to disease states. Although the specific molecular mechanisms affected by defects in lamins remain poorly understood, various lines of evidence have linked laminopathies with increased genomic instability. Here, we provide an overview of the advances made in recent years elucidating the roles of lamins, primarily A-type lamins, in mechanisms of DNA repair and maintenance of genome integrity.

Genomic Instability

Genomic instability is defined as the tendency of the genome to acquire mutations and epimutations as well as alterations in gene or chromosome dosage when processes involved in maintaining and replicating the genome become dysfunctional. Maintaining the stability and the correct sequence composition of the three billion bases that form our genome is critical for a faithful transmission of genomic information. Our genome is under constant attack by endogenous and exogenous agents [35]. As much as 10^5 lesions in DNA can occur per cell per day. DNA damage can result from side products of our normal metabolic activities such as free radicals and reactive oxygen (ROS) and reactive nitrogen species, as well as from environmental factors such as UV radiation, X-rays, and chemical compounds [36]. In addition, deficiencies in DNA replication or loss of telomere function can result in DNA lesions, primarily DNA double-strand breaks (Fig. 3). These dangerous lesions are repaired primarily by nonhomologous end-joining (NHEJ) or homologous recombination (see below). Improper or inefficient repair of DNA damage causes mutations, chromosomal translocations, or loss of genetic information that could ultimately lead to a large number of human syndromes, including premature aging, various cancer predispositions, and genetic abnormalities [36, 37]. A whole body of evidence has also implicated alterations in nuclear architecture and chromatin structure (epigenetic changes) in the acquisition of genomic instability. Furthermore, defects in the integrity of mitochondria profoundly impact on the stability of our genome, by generating ROS that damage DNA and oxidize proteins. Importantly, genomic instability increases with age and represents one of the highest risks for aging-related diseases, including cardiovascular diseases and cancer [38, 39]. Thus, understanding the molecular mechanisms behind the age-related acquisition of genomic instability is an active area of research, which will be fundamental to learn how to counteract its pathogenic effects.

The DNA Damage Response

The cell has evolved a complex pathway, termed DNA damage response (DDR), to sense, signal, and ultimately repair DNA lesions that arise from endogenous or exogenous insults [37, 38, 40–42]. A variety of DNA repair mechanisms, with specificity towards different categories of DNA damage have been identified. These include base-excision repair, nucleotide-excision repair (NER), transcription-coupled repair, homologous recombination (HR), and NHEJ. HR and NHEJ, the main mechanisms responsible for the repair of DNA double-strand breaks (DSBs), are considered to compete for repair substrates and be mutually exclusive [37, 43, 44]. These pathways have been extensively characterized due to the fact that DSBs are particularly dangerous to the cell [45]. HR repairs the damage with great fidelity and requires both resection of the 5' ends around the break and the presence of a homologous template. In contrast, NHEJ is an error-prone mechanism of DNA repair, which involves end ligation of broken DNA. While the HR repair mechanism is active during S and G2 phases of the cell cycle, NHEJ seems to be active throughout the cell cycle [46].

A growing list of factors has been clearly implicated in the DDR. These include sensors of the lesions such as the MRN complex (made up of the factors MRE11, NBS1, and RAD50), which recruits and activates the kinase ATM at sites of damage [47, 48]. ATM is considered a master regulator that phosphorylates a whole variety of factors involved in DDR and in the activation of cell cycle checkpoints [49, 50]. One of the earlier events in DDR is phosphorylation of the histone variant H2AX in the chromatin surrounding the break [51, 52] (Fig. 4). The presence of nuclear foci labeled with phospho-H2AX (γ -H2AX) indicates sites of DNA damage, and the kinetics of formation and resolution of these foci is widely used to identify defects in DNA repair. In turn, these events facilitate the recruitment of additional factors such as MDC1 [53], and ubiquitin and SUMO ligases that introduce further changes in the chromatin surrounding the break [54–58]. These changes facilitate the recruitment of downstream effectors of DNA DSB repair mechanisms. Two effectors have been recently in the spotlight due to their key role in regulating the balance between HR and NHEJ to repair DSBs: BRCA1 and 53BP1. BRCA1 promotes HR by facilitating end-resection and RAD51 recruitment, which is essential for homology search and strand invasion during recombination. In contrast, 53BP1 facilitates the recruitment of proteins necessary for NHEJ such as Ku70/Ku80, DNA-PKcs, and XRCC4/Ligase IV, while inhibiting end-resection [59, 60] (Fig. 4). Thus, the DDR is a complex pathway that mobilizes and recruits a whole variety of nuclear proteins to sites of DNA damage. Activation of this pathway can halt cell cycle progression until the damage is restored, or initiate mechanisms of growth arrest or cell death if the damage is beyond repair [40, 41]. A number of studies in the last few years have found that defects in different steps in the DDR occur in laminopathies.

Evidence for Defective DNA Repair in Laminopathies

Several lines of evidence have linked laminopathies with increased genomic instability. In particular, the expression of A-type lamin mutants has been associated with impairment in the ability of cells to properly repair DNA damage and maintain telomere homeostasis.

Defects in DNA repair were first reported in the premature aging laminopathy HGPS and the *Zmpste24*^{-/-} mouse model of progeria. Fibroblasts from HGPS patients and *Zmpste24*^{-/-} mice have increased levels of basal DNA damage, showing chromosome aberrations and increased sensitivity to DNA-damaging agents [28, 61], as well as a permanently activated DDR [30]. As a consequence of the defects in DNA repair, *Zmpste24*^{-/-} mice exhibit increased sensitivity to ionizing radiation (IR). When exposed to γ -irradiation, 80 % of knockout mice died within 12 days, in contrast to only 20 % of control mice [61]. Later studies indicated that the accumulation of unrepairable DNA damage in laminopathies such as HGPS and Restrictive Dermopathy is in part due to elevated levels of ROS and greater sensitivity to oxidative stress [62]. As such, the presence of ROS scavengers reduced the basal levels of DNA damage, thus suggesting that these types of compounds might help ameliorate the phenotype in some laminopathies. Similarly, the laminopathy mandibuloacral dysplasia type A (MADA), caused by the homozygous R527H mutation in the *LMNA* gene, is characterized by genomic instability. Fibroblasts from MADA patients exhibit defects in their ability to repair DNA damage, including reduced activation of p53 and its downstream targets, increased chromosome damage, and presence of residual γ -H2AX foci characteristic of unrepaired DNA lesions [63].

A whole variety of molecular mechanisms have been proposed to contribute to the defects in DNA repair and the increased genomic instability in progeria. The landmark study by Liu and colleagues [61] demonstrated that progeria cells exhibit delayed recruitment of 53BP1 and RAD51 to γ -H2AX-labeled DNA repair foci after IR. Later studies showed that HGPS and *Zmpste24*^{-/-} fibroblasts have an aberrant accumulation of Xeroderma Pigmentosum group A (XPA), a protein involved in NER, at sites of DNA lesions [64]. Binding of XPA also activates ATM- and ATR-dependent signaling cascades that contribute to proliferation arrest, and thus could contribute to premature senescence in these cells. Interestingly, depletion of XPA by siRNA in progeria cells partially restored the recruitment of HR factors Rad50 and Rad51 to sites of DNA damage [61, 64]. However, the partial rescue indicated that additional mechanisms contribute to the DNA repair deficiencies in these cells. Some proposed mechanisms include the absence of the nuclear DNA-PK holoenzyme, a key factor in NHEJ repair that is linked to premature and physiological aging [65], as well as defects in chromatin-modifying activities. In particular, loss of components of the NuRD complex and decreased levels of the histone acetyltransferase Mof were observed in progeria cells and associated with impaired recruitment of DNA repair factors and increased basal DNA damage [66, 67]. The decrease in Mof levels in *Zmpste24*^{-/-} cells correlates with lower global levels of H4K16 acetylation, a histone mark associated with chromatin compaction, and decreased recruitment of DNA repair factors such as 53BP1. The importance of this histone modification is demonstrated by the restoration of 53BP1 recruitment upon ectopic expression of Mof [67]. Overall, these reports provide a strong correlation between mutations or alterations in the processing of A-type lamins and a hindered ability to maintain the integrity of the genome. In addition, they have started to shed some light into putative molecular mechanisms behind the increased genomic instability in progeria. Elucidating the specific steps during these processes could bring about new possibilities for treatment of progeria, other laminopathies, and cancer.

Role of A-Type Lamins in Telomere Maintenance

Telomere dysfunction due to excessive attrition of telomeric DNA repeats or to defects in telomere-binding proteins known as the “shelterin” complex is sensed by the cell as DNA damage, which activates DDR and checkpoint pathways [68]. Dysfunctional telomeres, which can be easily visualized by the colocalization of γ -H2AX with a telomere DNA probe, are aberrantly repaired by the NHEJ pathway, which results in toxic chromosome end-to-end fusions [69] (Fig. 5). Telomere dys-function leads to cell death or a permanent growth arrest, which can be bypassed by inactivation of tumor suppressor mechanisms, contributing to genomic instability.

There is evidence in the literature that A-type lamins associate with telomeres, putatively via telomere-binding proteins [70, 71]. This association has been shown to be important for the proper distribution of telomeres within the 3D nuclear space. A-type lamins-deficient cells exhibit accumulation of telomeres towards the nuclear periphery during interphase [72]. In addition, lamins have an impact on the mobility of telomeres in the nucleus [73]. Monitoring telomere dynamics in human fibro-blasts revealed that HGPS cells have reduced mobility with respect to normal fibro-blasts. Moreover, loss of A-type lamins increases telomere dynamics. These changes could contribute to the alterations in telomere metabolism observed in lamindeficient cells [74]. HGPS fibroblasts are characterized by faster telomere attrition during proliferation [75, 76]. This was shown to be a direct consequence of the expression of progerin, as hematopoietic cells from HGPS patients, which do not express progerin, do not show this phenotype. In contrast, no differences in telomere length were observed in *Zmpste24*^{-/-} fibroblasts, although whether or not *Zmpste24*^{-/-} telomeres are dysfunctional due to uncapping remains to be tested. Furthermore, *Lmna*^{-/-} mice exhibit a modest but consistent decreased telomere length when compared to wild-type littermates, as well as an increased frequency of chromosomes that lack telomere signals, presenting with signal-free ends [72]. Further evidence for a role of A-type lamins in telomere biology was the finding that proliferative defects on human fibroblasts expressing lamin A mutants are rescued by telomerase [77].

The above data indicate that A-type lamins participate in the localization and mobility of telomeres within the nucleus, as well as in the maintenance of telomere homeostasis. Recent studies show that A-type lamins also participate indirectly in the aberrant processing of dysfunctional telomeres by NHEJ, which leads to chromosome end-to-end fusions. Expression of a dominant-negative version of the telomere-binding protein TRF2 (TRF2^{B M}) leads to uncapping of telomeres and massive appearance of chromosome end-to-end fusions in most cell types. However, in *Lmna*^{-/-} mouse embryonic fibroblasts (MEFs), the extent of chromosome fusions in response to TRF2^{B M} was greatly reduced [78] (Fig. 5). As discussed below, this defect was linked to the degradation of 53BP1, a protein that is essential for NHEJ of dysfunctional telomeres [79].

Novel Aspects of DNA Double-Strand Repair Revealed by Loss of A-Type Lamins

The generation of a mouse model deficient in A-type lamins (*Lmna*^{-/-}) revealed important information about the function of these structural nuclear proteins at the cellular and organismal level. However, a recent study showed that the *Lmna*^{-/-} mouse model is not a complete knockout [80] and that low levels of a truncated form of the protein is produced lacking exons 8–11. These mice develop to term with no obvious abnormalities, but they exhibit severe postnatal growth retardation, dying at around 5–6 weeks of age. *Lmna*^{-/-} mice develop defects in skeletal and cardiac muscles, a phenotype characteristic of muscular dystrophy. At the cellular level, perturbations of the nuclear envelope, loss of heterochromatin from the nuclear periphery, epigenetic alterations, and nuclear fragility are observed, concomitant with mislocalization of emerin, an inner nuclear membrane protein linked to Emery–Dreifuss muscular dystrophy (EDMD) [81]. The *Lmna*^{-/-} mouse model has been utilized to improve our understanding of the role of A-type lamins in DNA repair, signaling through the DDR, and telomere maintenance.

Recent studies have shown that *Lmna*^{-/-} MEFs exhibit signs of genomic instability illustrated by increased aneuploidy, higher incidence of chromosome and chromatid breaks, and basal levels of unrepaired DNA, as shown by the presence of γ -H2AX foci. As discussed above, *Lmna*^{-/-} MEFs also present with decreased telomere length [72]. These data indicate that A-type lamins deficiency affects the ability of cells to properly repair DNA damage and maintain genome integrity. However, given the great variety of diseases associated with the different mutations/loss of A-type lamins, it is tempting to speculate that different types of alterations in these proteins would impact different aspects of the DDR and specific mechanisms of DNA repair. Establishing which steps in the DDR each type of alteration in lamins affects could provide new avenues for targeted treatment. In the case of cancer for example, knowing if tumor cells in which the *LMNA* promoter is silenced by DNA methylation are defective in DNA repair and more sensitive to DNA-damaging agents, would provide valuable information towards the use of specific cancer therapeutics.

Mechanisms Behind Genomic Instability in *Lmna*^{-/-} Mice

One of the mechanisms behind genomic instability in A-type lamins-deficient cells is the loss of the DNA repair factor 53BP1. Studies monitoring the ability of A-type lamins-deficient cells to sense DNA damage did not find any defects in the activation of DDR [78]. ATM-dependent phosphorylation of H2AX (γ -H2AX) and phosphorylation of p53 at Ser15 were not affected in *Lmna*^{-/-} MEFs, and the kinetics of formation and resolution of γ -H2AX ionizing radiation-induced foci (IRIF) was indistinguishable between *Lmna*^{+/+} and *Lmna*^{-/-} MEFs [78]. In contrast, this study demonstrated a marked decrease in the accumulation of 53BP1 at IRIF in *Lmna*^{-/-} MEFs when compared to *Lmna*^{+/+} at all postirradiation times tested. Importantly, this deficiency is due to a marked decrease in the global levels of 53BP1 protein, and not to failed recruitment, since a similar number of 53BP1 IRIF formed although at a much lower intensity [78] (Fig. 6). Further evidence for a role of A-type lamins in the stabilization of 53BP1 protein came from experiments showing that acute depletion of A-type lamins by lentiviral transduction with shRNAs leads to decreased levels

of 53BP1 protein but not transcript levels. Interestingly, reconstitution of either lamin A, lamin C, or both, into *Lmna*^{-/-} MEFs, rescued normal levels of 53BP1.

Loss of 53BP1 and Defects in NHEJ in *Lmna*^{-/-} Mice

The DNA repair factor 53BP1 has been shown to be important for long-range NHEJ processes such as class-switch and *V(D)J* recombination, as well as processing of dysfunctional telomeres [79, 82–84]. In addition, 53BP1 participates in the repair of short-range DNA DSBs by inhibiting end-resection and facilitating the recruitment of the NHEJ DNA repair machinery [85, 86].

The decreased levels of 53BP1 protein in *Lmna*^{-/-} MEFs suggest that these cells could be deficient in long-range and short-range NHEJ. The reduced ability of lamin A/C-deficient cells to promote chromosome end-to-end fusions upon telomere dysfunction is consistent with a defect in long-range NHEJ [78] (Fig. 5). In addition, studies were performed to assess putative defects in short-range NHEJ by monitoring the kinetics of repair of IR-induced DNA DSB by neutral comet assays. This assay examines the presence of unrepaired DNA by combining DNA gel electrophoresis with fluorescence microscopy to visualize migration of DNA fragments from individual agarose-embedded cells. The comet head contains high-molecular weight and intact DNA, and the comet tail contains the leading ends of migrating fragments. Olive moment, calculated as the product of the amount of DNA in the tail and the mean distance of migration in the tail, is a measure of unrepaired DNA [87] (Fig. 7). The comet assay showed a typical biphasic mode of repair for *Lmna*^{+/+} MEFs, with a fast phase of repair corresponding to classical NHEJ, and a slower phase of repair that is associated with alternative mechanisms of repair [88]. In contrast, *Lmna*^{-/-} MEFs exhibited profound defects in the fast phase of DNA DSB repair [78, 89]. Importantly, reconstitution of 53BP1 into *Lmna*^{-/-} cells rescued the fast phase of repair of IR-induced DNA DSBs, as well as the processing of dysfunctional telomeres by NHEJ [78]. Altogether, these findings indicate that 53BP1 deficiency is a major contributor of the defects in long-range and short-range NHEJ observed in lamins-deficient cells.

How Do A-Type Lamins Regulate 53BP1 Levels?

A search for mechanisms by which A-type lamins affect DNA DSB repair revealed a role for cathepsin L (CTSL) in regulating 53BP1 protein stability. CTSL is a cysteine protease ubiquitously expressed in tissues that is synthesized as an inactive zymogen which undergoes autoproteolytic processing within the lysosomal/endosomal compartment to produce the mature active form [90]. In addition to its classical localization in the lysosomes, CTSL has been found in the nucleus [91] and secreted into the extracellular space [92]. Extracellular CTSL has been reported in numerous types of cancer and is often associated with increased invasiveness and metastasis [93–95].

The first association between CTSL and A-type lamins was established in *Zmpste24*^{-/-} mice, which present with a marked increase in CTSL transcript levels [28]. Studies on *Lmna*^{-/-} mice showed a marked increase in the levels of CTSL mRNA and protein, suggesting that loss of A-type lamins induces transcriptional upregulation of CTSL or increased stability of its transcripts [89]. Moreover, the increase in CTSL is directly

responsible for the decrease in 53BP1 protein levels, since depletion of CTSL via shRNAs restored 53BP1 protein levels in *Lmna*^{-/-} MEFs. Importantly, depletion of CTSL rescued the fast phase of repair of IR-induced DNA DSBs as well as NHEJ of dysfunctional telomeres. Interestingly, depletion of both 53BP1 and CTSL prevented restoration of NHEJ, demonstrating that the rescue is due to stabilization of 53BP1 (Fig. 8).

The regulatory effect of CTSL on 53BP1 and thus in DNA repair is not restricted to lamin A/C-deficient cells. A recent study shows that loss of the tumor suppressor DNA repair factor BRCA1 leads to upregulation of CTSL and degradation of 53BP1 protein [96]. Interestingly, activation of CTSL-mediated degradation of 53BP1 allows the bypass of the characteristic growth arrest induced by BRCA1 loss. Moreover, increased levels of nuclear CTSL were observed in human tumors with the poorest prognosis, including sporadic triple negative breast cancers and tumors from women carrying germline mutations in the BRCA1 gene. Importantly, upregulation of CTSL was correlated with decreased levels of 53BP1 in these tumors. These studies suggest that upregulation of CTSL activity is a novel mechanism contributing to genomic instability in some laminopathies and some types of cancer, which could be targeted with therapeutic purposes.

Defects in HR Upon Loss of A-Type Lamins

Based on the theory of competition between NHEJ and HR for the repair of DNA DSBs [86, 97], one could envision that loss of 53BP1-dependent NHEJ in lamin A/C-deficient cells is compensated by an increase in HR. However, studies in MCF7 cells carrying a reporter construct for monitoring HR demonstrated that depletion of A-type lamins leads to a 40 % decrease in HR, despite a marked downregulation of 53BP1 [78]. Experiments aiming to gain insight into mechanisms behind this defect revealed that depletion of lamins A/C results in transcriptional repression of the BRCA1 and RAD51 genes, which encode proteins essential for HR [78]. Previous reports had shown transcriptional repression of BRCA1 and RAD51 under stresses such as hypoxia, via formation of p130/E2F4 repressor complexes at their promoters [98, 99]. Interestingly, loss of A-type lamins is associated with increased degradation of the Rb family members pRb and p107, with only a minor effect on p130 [100, 101]. Importantly, in lamin A/C-deficient cells, repression of BRCA1 and RAD51 genes was linked to the status of Rb family members, with repression requiring p130 and occurring in the context of pRb and p107 deficiency. These data suggest activation of a repressive mechanism in lamin A/C-deficient cells, whereby altering the balance of the pocket proteins facilitates the association of p130 with E2F4, leading to transcriptional inhibition of certain genes (Fig. 8). However, given the role of A-type lamins in nuclear compartmentalization, it is also possible that lamin A/C deficiency alters the nuclear localization of BRCA1 and RAD51 genes, which might in turn contribute to their transcriptional repression. Although many more studies are needed to fully understand the consequences of lamins dysfunction for genomic stability and DNA repair, the data so far support a role for A-type lamins in the maintenance of mechanisms of DNA DSB repair. A-type lamins regulate HR indirectly by impacting on transcriptional regulation of key factors in this process, BRCA1 and RAD51. On the other hand, lamin A/C deficiency activates CTSL-mediated degradation of 53BP1, contributing to defects in long-range and short-range NHEJ.

Development of Therapeutic Strategies for Laminopathies

The progress made in recent years about the molecular mechanisms contributing to the pathogenesis of laminopathies has expedited the search for therapeutic strategies that could ameliorate these diseases. Preclinical studies on mouse models of laminopathies have identified farnesyltransferase inhibitors (FTIs) and prenylation inhibitors, such as statins and bisphosphonates, as beneficial treatments for certain lamin-related diseases [102, 103]. Cell-based assays have also suggested that rapamycin, scavengers of ROS, and vitamin D could have a protective role against increased genomic instability brought about by lamin dysfunction.

Farnesyltransferase Inhibitors, Statins and Bisphosphonates

Several lines of evidence indicate that the farnesylated forms of prelamin A and progerin cause the phenotypes associated with progeroid syndromes [104]. FTIs block prelamin A and progerin farnesylation and translocate these proteins from the nuclear envelope to the nucleoplasm [23, 105, 106]. FTI treatment of cells from HGPS knock-in and *Zmpste24* knockout mouse models, as well as HGPS and Face1/*Zmpste24*-deficient human cells improved nuclear abnormalities, albeit not being efficient in reducing defects in DNA repair. In addition, treatment with FTIs ameliorates the disease phenotypes of *Zmpste24*^{-/-} mice and improves their longevity, although there is considerable variation in the effect in different progeria mouse models [107, 108]. Based on these exciting results, the first clinical drug trial for children with progeria was started on May 2007. The FTI lonafarnib has proven some effectiveness for progeria, with patients showing improvement in rate of weight gain, hearing, bone structure, and flexibility of blood vessels [109]. For information see <http://www.progeriaresearch.org/>. However, the improvements were minor and the FTI treatment is far from curing the syndrome. Thus, the main challenge in the field of progeria is to find additional strategies to improve such dramatic phenotypes, which requires a detailed understanding of the molecular pathways affected.

In 2008, a study showed that some prelamin A undergoes geranylgeranylation when farnesylation is inhibited with FTIs [103]. This can explain the marginal efficiency of FTIs improving the overall progeria phenotype. Interestingly, a combination of two common drugs blocking several steps of the farnesyl pyrophosphate and geranylgeranyl pyrophosphate biosynthetic pathway, statins and aminobisphosphonates, have been shown to significantly improve aging-like symptoms and longevity in *Zmpste24*^{-/-} mice [103]. Interestingly, these drugs improved nuclear morphology and also DNA damage, as assessed by decreased levels of γ -H2AX foci. These studies indicate that these drugs are likely to be more efficient than FTIs in ameliorating the phenotype of progeria patients. A follow-up trial is now in place in 45 children with HGPS being treated with lonafarnib in combination with a bisphosphonate (zoledronate) and a statin (pravastatin), with the hope that this regimen will be more efficient than FTI alone.

Novel Strategies: Vitamin D, Rapamycin, and ROS Scavengers

The identification of CTSL-mediated degradation of 53BP1 as a contributor to genomic instability in *Lmna*^{-/-} fibroblasts prompted the exploration of strategies to inhibit this

protease, with the goal of rescuing the DNA repair defects in these cells. As expected, treatment of A-type lamins-deficient cells with cathepsin inhibitors rescued the levels of 53BP1, as well as defects in DNA damage repair [89]. Studies in colon cancer models had previously shown that vitamin D exerts an inhibitory effect on cathepsins. The mechanism underlying this effect seems to be the transcriptional upregulation by vitamin D receptor (VDR) of cystatin D, an endogenous inhibitor of cathepsins, including CTSL [110]. Consistent with this notion, treatment of *Lmna*^{-/-} MEFs with vitamin D inhibited CTSL-mediated degradation of 53BP1, rescued long-range and short-range NHEJ events, while causing a reduction in the percentage of cells presenting with nuclear morphological abnormalities and basal unrepaired DNA damage [89]. These cell-based studies suggest that vitamin D could represent a novel, safer, and cost-effective therapeutic strategy for lamin-related diseases that present with defects in DNA repair and genomic instability. Preclinical studies with vitamin D alone or in combination with FTIs or prenylation inhibitors in mouse models of laminopathies need to be performed to test the efficacy of such therapeutic regimens.

A recent report suggested that rapamycin, an immunosuppressant drug, reduces nuclear defects in fibroblasts from HGPS patients and prolongs cellular lifespan. The mechanism behind this improvement is the increased clearance of toxic progerin by autophagy [111]. It will be interesting to determine whether rapamycin is capable of reducing disease phenotypes and extending life in mouse models of laminopathies.

New evidence has also linked lamin dysfunction with increased generation of ROS and reduced levels of antioxidant enzymes [112, 113]. In turn, persistent ROS can oxidize some cysteine residues in lamins, causing alterations in the structure of the nuclear lamina [113]. The accumulation of ROS could play a role in the increased levels of DNA damage and the genomic instability observed in lamin-deficient cells. This notion is supported by the fact that treatment of progeroid cells with the ROS scavenger *N*-acetyl cysteine reduces the amount of unrepairable DNA damage [62]. Thus, compounds that reduce the levels of ROS in the cell could represent yet another strategy to reduce genomic instability in lamin-related diseases [114].

Concluding Remarks

In recent years, interest in A-type lamins has increased due to the association of alterations of these structural nuclear proteins with a variety of human diseases, including severe premature aging syndromes and cancer. The molecular mechanisms contributing to the phenotypes of lamin-related diseases are beginning to be uncovered. New evidence indicates that A-type lamins play a key role maintaining telomere localization, structure, length and function, keeping in check the levels of ROS, as well as regulating the levels of factors participating in cell cycle regulation (Rb family members) and DNA repair (53BP1, BRCA1, RAD51, XPA). Understanding how lamins maintain genome integrity will surely provide new therapeutic strategies towards laminopathies and potentially cancer.

Abbreviations

DDR	DNA damage response
DSB	Double-strand break
EDMD	Emery–Dreifuss muscular dystrophy
FTI	Farnesyltransferase inhibitor
HGPS	Hutchinson–Gilford progeria syndrome
HR	Homologous recombination
IR	Ionizing radiation
IRIF	Ionizing radiation-induced foci
LAD	Lamin-associated domain
MADA	Mandibuloacral dysplasia type A
MEF	Mouse embryonic fibroblast
NER	Nucleotide-excision repair
NHEJ	Nonhomologous end-joining
ROS	Reactive oxygen species
shRNA	Short hairpin RNA

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Fig. 1.

A-type lamins function as a scaffold that ensures nuclear function. A-type lamins are a special class of intermediate filaments that form the nuclear lamina, a protein meshwork underlying the inner nuclear membrane, which can also extend throughout the nucleoplasm. They serve as a scaffold for tethering chromatin and proteins to specific nuclear subcompartments. A whole variety of functions have been ascribed to A-type lamins including processes such as DNA replication, transcription, and DNA repair. For the most part, the molecular mechanisms behind these functions remain poorly understood

Fig. 2.

Schematic representation of lamin A structure and posttranslational processing. Lamins consist of a central rod domain, flanked by a globular head and a globular tail domain. Lamin A is synthesized as a prelamina A precursor which undergoes processing of its C-terminus. The C-terminal -CAAX motif (*blue*) undergoes farnesylation (*green*), cleavage of the last three amino acids (*red*), followed by carboxymethylation (*purple*) of the new terminal cysteine. A second cleavage by the metalloprotease Zmpste24 (*red*) removes another 15 amino acids, including the farnesyl group, rendering mature lamin A. Sites of interaction with gene transcriptional regulators such as Rb family members, lamin-associated proteins such as LAP2 α , inner nuclear membrane proteins such as emerin, or chromatin have been described that involve primarily the C-terminal part of the protein and the central rod domain

Fig. 3.

Processes essential for maintenance of genome stability. Maintenance of telomere homeostasis and mechanisms of DNA DSB repair is critical for genome stability. DSBs resulting from telomere dysfunction or exogenous or endogenous insults are repaired primarily by two mechanisms: nonhomologous end-joining (NHEJ) and homologous recombination (HR)

Fig. 4.

Important factors in the DNA damage response (DDR). The presence of a DNA DSB is sensed by the MRN complex, which recruits and activates ATM, a master regulator of DDR. ATM phosphorylates H2AX, which targets MDC1 and other mediator factors to the break. These factors attract RNF8 and RNF168 which ubiquitylate histones around the break. These histone modifications facilitate recruitment of factors that participate in DNA DSB repair such as 53BP1 and BRCA1. 53BP1 promotes recruitment of the NHEJ machinery and leads to end-joining of the break. In contrast, BRCA1 promotes end-resection of the break, which initiates the complex process of recombination. The presence of DSBs can be easily visualized by performing immunofluorescence, as shown for γ -H2AX ionizing radiation-induced foci (IRIF)

Fig. 5.

A-type lamins are essential for NHEJ of dysfunctional telomeres. **(a)** Telomere dysfunction due to overexpression of a dominant-negative version of the telomere-binding protein TRF2 causes chromosome end-to-end fusions, a process that requires a functional NHEJ mechanism of repair. **(b)** Graph shows that loss of A-type lamins reduces significantly the frequency of chromosome end-to-end fusions by NHEJ. **(c)** Table shows the total number of fusions observed as well as the percentage of metaphases with fusions in *Lmna*^{+/+} and *Lmna*^{-/-} MEFs. These data demonstrated an unprecedented role for A-type lamins in NHEJ of dysfunctional telomeres

Fig. 6. Loss of A-type lamins impacts on 53BP1 stability and IRIF formation. Immunofluorescence showing a marked decrease in global levels of 53BP1 protein in *Lmna*^{-/-} MEFs and reduced intensity of 53BP1 labeling of DNA repair foci at 1 h postirradiation. Thus, loss of A-type lamins decreases the accumulation of 53BP1 protein at DNA repair foci, which explains reduced NHEJ in lamin-deficient cells

Fig. 7.

Lamin-deficient cells exhibit defects in the repair of DNA DSBs induced by IR. (a) Images of neutral comet assays. *Lmna*^{+/+} and *Lmna*^{-/-} MEFs were irradiated and subjected to single cell electrophoresis at different times postirradiation. Note how the comet tail decreases over time due to repair of DSBs. (b) Olive moment measures unrepaired DNA damage. A bimodal form of DNA DSB repair is clearly observed in *Lmna*^{+/+} fibroblasts, with the fast phase corresponding to NHEJ occurring within the first hour after DNA damage. *Lmna*^{-/-} fibroblasts exhibited a greatly reduced rate of the fast component of DSB repair, indicating that these proteins play a role in the NHEJ repair mechanism

Fig. 8.

Model of roles of A-type lamins in DNA repair. Loss of A-type lamins upregulates CTSL expression, resulting in elevated protein levels both in the nucleus and in the lysosomes. CTSL participates in the degradation of 53BP1, and the Rb family members pRb and p107, favoring the formation of p130/E2F4 repressor complexes, which in turn inhibit BRCA1 and RAD51 gene expression. The decrease in 53BP1 hinders NHEJ and the decrease in BRCA1 and RAD51 impairs HR repair. Other factors are likely to contribute to genomic instability in different laminopathies such as the accumulation of XPA at DSBs, the deficiency in DNA-PK, the increase in generation of ROS, epigenetic alterations, and loss of telomere homeostasis (not shown in the model). Importantly, vitamin D treatment inhibits CTSL-mediated degradation of 53BP1 and upregulates transcription of BRCA1 and RAD51, thus providing a potential therapeutic strategy