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Loss of A-type lamins and genomic instability

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Research performed in the last few years has revealed important roles for the spatial and temporal organization of the genome on genome function and integrity. A challenge in the field is to determine the molecular mechanisms involved in the organization of genome function. A-type lamins, key structural components of the nucleus, have been implicated in the maintenance of nuclear architecture and chromatin structure. Interestingly, alterations of A-type lamins lead to defects in DNA replication and repair as well as gene transcription and silencing. Elucidating the functions of these proteins is a topical subject since alterations of A-type lamins are associated with a variety of human diseases, ranging from muscular dystrophies and premature aging syndromes to cancer. Here, we discuss novels roles for A-type lamins in the maintenance of telomere structure, length and function as well as in the stabilization of a key DNA damage response factor. These studies support the notion that increased genomic instability due to defects in telomere biology and DNA repair contribute to the pathogenesis of lamin-related diseases.

Key words: A-type lamins, DNA damage response, genomic instability, nuclear organization, telomeres

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Introduction

The human genome is organized into different levels of complexity. Packaging of DNA into different chromatin states and 3D nuclear organization of the genome are emerging as additional levels of regulation of genome function.[1-3] Changes in nuclear architecture and chromatin structure are associated with disease. For example, alterations of nuclear morphology in tumor cells have remained the gold standard for cancer diagnosis.[4] However, for the most part, the functional interplay between genome organization and function is poorly understood both in normal tissue homeostasis and during tumorigenesis.

A recent study has shown that large domains of the human genome interact with the nuclear lamina. These lamina-associated domains (LADs), which vary in size between 0.1 and 10 megabases, are gene-poor regions enriched in repressive chromatin marks. The boundaries of LADs are sharply demarcated by putative insulators such as the CTCF protein, CpG islands, and gene promoters oriented away from the lamina.[5] A-type lamins—lamins A and C—are intermediate filament proteins that form part of the nuclear lamina and a nucleoplasmic network. They are thought to play a scaffolding role for tethering chromatin to specific subcompartments, which in turn serves to organize nuclear processes.[6-8] In fact, depletion of A-type lamins or expression of mutant forms of the proteins leads to defects in chromatin remodeling and in the 3D organization of the genome, as exemplified by loss of heterochromatin from the nuclear periphery.[9,10] Interestingly, changes in the expression of A-type lamins are observed in leukemias, lymphomas, small cell lung and ovarian cancer, as well as colon carcinoma, often associated with poor prognosis.[11-13] The cellular mechanisms affected by these malignancy-associated alterations of A-type lamins are only beginning to be unraveled.[14]

Alterations of telomere biology and defects in repair of DNA damage are among the leading causes for genomic instability, and clear contributors to aging and cancer phenotypes. Loss of telomere

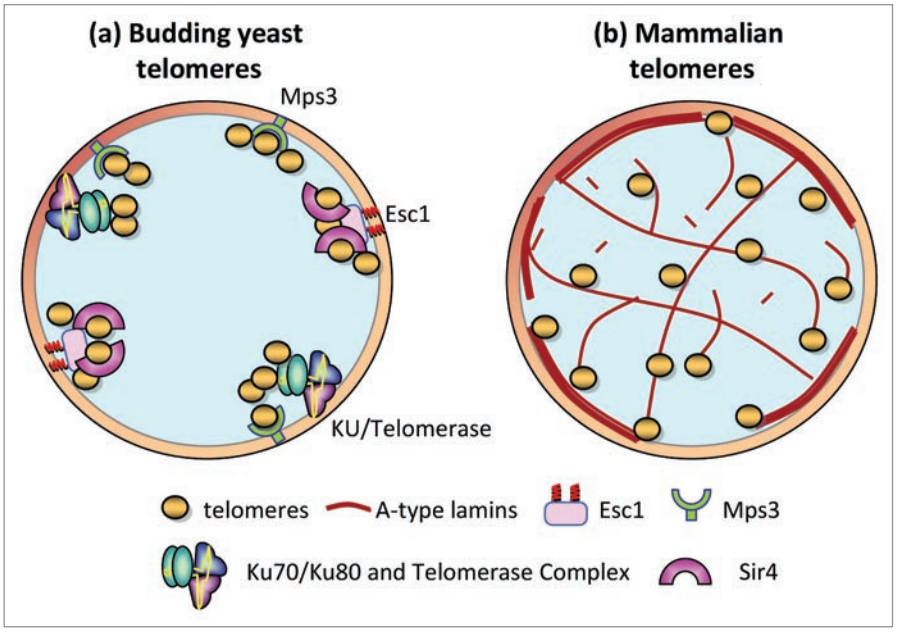


Figure 1. Nuclear organization of telomeres. (A) In budding yeast, association of telomeres to the nuclear envelope is mediated by two redundant mechanisms. One involves yKu70/Ku80 heterodimer, which tethers telomeres to the nuclear periphery via interaction with the telomerase complex Est1/Est2Tlc1, and the SUN domain protein Mps3. In the second mechanism, Sir4 serves as a bridge between telomeres and the nuclear membrane protein enhancer of silent chromatin 1 (Esc1). (B) In mammals, telomeres are distributed throughout the entire nucleoplasm. A-type lamins, key structural components of the nuclear matrix, contribute to their localization in the 3D nuclear space.

Do not distribute.

integrity by attrition of telomere repeats below a critical threshold, or by defects in the binding of telomeric proteins is sensed by the cell as DNA double-strand breaks (DSBs), which activates checkpoint pathways.[15] The DNA-damage response (DDR) can be considered a signal transduction pathway where the DNA damage is detected by "sensors" that trigger the activation of a transduction system composed of protein kinases and a series of "mediators", which in turn activate "effectors" that delay cell cycle progression until the damage is removed.[16,17] DNA damage repair occurs via high fidelity repair by the homologous recombination (HR) pathway, and the less accurate non-homologous end-joining (NHEJ) pathway. In the case of dysfunctional telomeres, the primary DDR is activation of the NHEJ pathway, leading to chromosome end-to-end fusions.[18] Telomere dysfunction leads to a permanent growth arrest, which can be bypassed by inactivation of tumor suppressor mechanisms, contributing to genomic instability. Similarly, defects in the repair of DNA lesions have a profound impact on the stability of the genome. DNA double-strand breaks are especially dangerous because their inefficient repair can result in genetic translocations, deletions, chromosome fusions and loss of genomic information, leading in certain cases to cellular transformation.[19-21]

In recent years, various lines of evidence have linked laminopathies with increased genomic instability. In particular, the expression of A-type lamins mutant isoforms has been associated with defective DNA repair.[22,23] Our recent findings reveal that loss of A-type lamins impacts on the maintenance of telomeres and a proper DNA damage response.

Nuclear Organization of Telomeres

Telomeres are nucleoprotein structures that protect the ends of eukaryotic chromosomes.[15,24,25] A minimal length of telomeric DNA repeats and proper binding of specialized proteins such as shelterin complex components and DNA repair factors are required for the maintenance of telomere structure and function.[26] Similarly, the acquisition of a heterochromatic structure at mammalian telomeres is critical for the control of telomere homeostasis.[27] The importance of telomere compartmentalization for telomere function has been clearly demonstrated in yeast.[28] In *Saccharomyces cerevisiae* or budding yeast, telomeres are clustered in 3–8 foci at the nuclear periphery.[29,30] At least two redundant mechanisms have been identified that mediate tethering of telomeres to the periphery. One mechanism involves yKu70/Ku80 heterodimer, which associates with the telomerase complex Est1/Est2/Tlc1, and an integral inner nuclear membrane protein of the SUN domain family, Mps3.[31,32] A second mechanism involves the histone deacetylase Sir4, which binds to the inner nuclear membrane-associated protein Esc1 (Enhancer of silent chromatin 1)[33-35] (**Fig. 1**). Importantly, disruption of the tethering of telomeres to the nuclear periphery leads to deprotection and hyperrecombination of telomeres,[32] and derepression of subtelomeric genes.[28] In the case of Sir4, inactivating mutations also involve telomere shortening,[36] suggesting a link between telomere localization at the nuclear periphery and maintenance of length homeostasis.

Mammalian telomeres do not accumulate at the nuclear periphery, except during meiosis.[37] They are distributed throughout the entire nucleoplasm in G_1 and S phases of the cell cycle, while assembling in the center of the nucleus during G_2 in preparation for mitosis[38] (**Fig. 1**). Interactions between telomeres and the nuclear matrix have been proposed to determine their localization in the nuclear space.[39,40] Tracking 3D trajectories of fluorescently labeled telomeres in a broad time range has provided new information about telomere dynamics within the nucleus. At short time scales, the diffusion of telomeres is anomalous, while at longer time scales a normal diffusion is observed with a wide distribution of diffusion constants. This transient anomalous diffusion was explained by the existence of a local binding or obstruction mechanism to telomere mobility.[41] To date, the molecular mechanisms that orchestrate nuclear tethering and localization of mammalian telomeres,

and their relevance for telomere metabolism remain unknown.[42]

Interestingly, the 3D organization of telomeres is altered in tumor cells,[38,43] and in senescent cells that present defects in the nuclear lamina.[44] This data suggests a relationship between changes in nuclear distribution of telomeres and alterations of telomere metabolism observed during senescence and immortality. A recent study in our laboratory has shown that A-type lamins bind to mouse telomeres and participate in their nuclear compartmentalization.[45] Embryonic fibroblasts from *LMNA* null mice exhibit changes in the nuclear distribution of telomeres towards the nuclear periphery and away from the nuclear center. This result was unexpected, since lamins are highly enriched at the nuclear periphery. However, while B-type lamins are exclusively found at the nuclear periphery, lamins A and C are proposed to form part of a filamentous meshwork that expands throughout the entire nucleoplasm. We speculate that A-type lamins actively participate in the distribution of telomeres throughout the nucleus (**Fig. 2**). In the absence of A-type lamins, proteins at the nuclear periphery such as B-type lamins, inner nuclear membrane proteins or nuclear pore complex proteins, could undertake the tethering of telomeres. In this model, the nuclear periphery would represent a default pathway for telomere distribution, resembling telomere localization in yeast, which do not express lamins. This model is supported by a study in human cells showing that intranuclear lamina structures colocalize with telomeres in human mesenchymal stem cells, and that expression of mutant isoforms that accumulate in the nucleoplasm increased the association of telomeres to lamins in these cells.[44] This study supports the idea that A-type lamins help tether telomeres throughout the 3D nuclear space.

Prospect studies need to characterize the molecular determinants of the association of A-type lamins with telomeres. A-type lamins can bind directly to DNA-chromatin and indirectly via their interaction with lamin-associated proteins such as LAP-2α, emerin and MAN1.[6,46] Although we found that A-type lamins bind telomeric sequences by chromatin immunoprecipitation, we do not know whether the interaction is direct or mediated by lamin-associated proteins. LAP-2α is of special interest given that it binds to telomeres during nuclear reassembly after mitosis.[47] In addition, LAP-2α mediates the interaction of Rb with A-type lamins, contributing to the stabilization of Rb family function,[48] and is the only member of its family that is localized throughout the nucleoplasm. All these characteristics make LAP-2α a good candidate for mediating telomeres tethering to A-type lamins. From the telomere end, it is possible that components of the shelterin complex associate with A-type lamins or lamin-associated proteins localized at the nucleoplasm. Alternatively, A-type lamins might recognize heterochromatic features at the telomere. Heterochromatin Protein 1 (HP1) has been shown to form a complex with A-type lamins and LAP-2α,[49] and therefore could participate in the tethering of heterochromatic domains such as telomeres and centromeres to the scaffold of A-type lamins (**Fig. 2**).

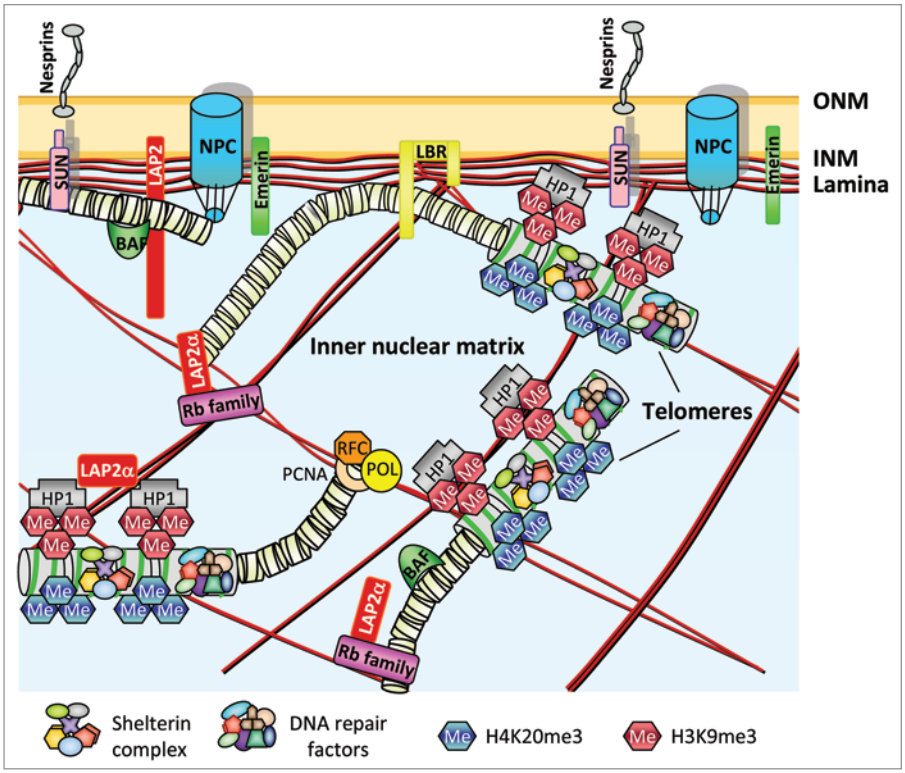
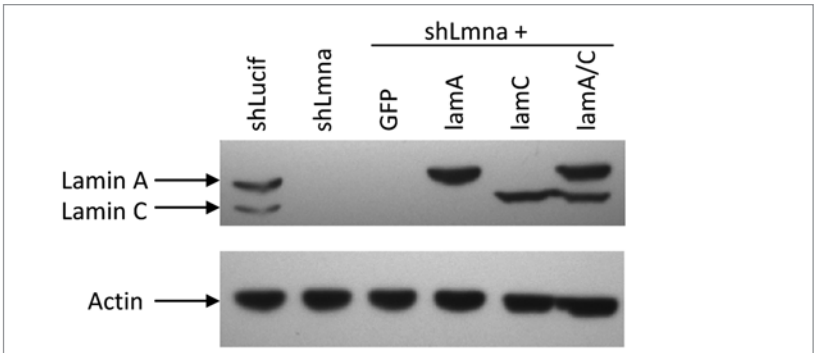


Figure 2. A-type lamins as a scaffold for tethering telomeres. We propose that telomeres associate with A-type lamins either directly or via lamin-associated proteins such as LAP-2α. Shelterin complex components or heterochromatic features at the telomere might mediate the tethering. The scaffold provided by the lamins actively participates in the nuclear distribution of telomeres. In this model, the nuclear periphery represents a default pathway for telomere localization, which is revealed upon loss of A-type lamins.

A-Type Lamins and Telomere Structure, Length and Function

The first evidence supporting a role for A-type lamins in telomere biology came from studies of patients with Hutchinson Gilford Progeria Syndrome. HGPS or progeria is a premature aging disease caused by a mutation in the *LMNA* gene that generates a truncated lamin A isoform known as progerin, which is toxic for the cell.[50,51] HGPS fibroblasts were shown to undergo faster telomere attrition during proliferation than normal counterparts.[52,53] Fibroblasts from HGPS patients and aged individuals also present defects in epigenetic marks characteristic of constitutive heterochromatin, although the effect on telomeres was not tested.[54,55] The mechanism by which mutation in the *LMNA* gene resulting in the expression



	Average telomere length	% tel<10Kb	% tel>80Kb
shLucif	**43.1 ± 23.5**	4.7	7.2
shLmna	**38.3 ± 22.4**	7.6	5.0
shLmna + GFP	**39.6 ± 23.4**	7.2	5.6
shLmna + lam A	**43.5 ± 24.7**	4.8	7.2
shLmna + lam C	**46.5 ± 24.3**	4.4	10.2
shLmna + lam A/C	**47.9 ± 25.7**	3.1	11.1

Figure 3. A-type lamins play a role in maintenance of telomere length. (A) Western blot to detect the levels of lamins A/C upon lentiviral transduction of wild-type MEFs with constructs carrying a shRNA specific for lamins A and C (shLmna), or a shLucif control (left two lanes). The right four lanes show levels of lamins after retroviral transduction of lamins A/C-depleted MEFs with cDNAs for GFP, lamin A, lamin C or both. Note how lentiviral transduction with shLmna efficiently depletes A-type lamins, and retroviral transduction with cDNAs for lamins A and C rescues the cellular levels of the proteins. (B) Q-FISH analysis to measure telomere length of MEFs after depletion of A-type lamins (compare shLmna and shLucif), and after reconstitution of lamin A, lamin C or both into lamins A/C-depleted MEFs. Note the decrease in average telomere length upon depletion of A-type lamins after only five passages of the cells in culture, and the telomere elongation induced by reconstitution of A-type lamins.

of progerin leads to telomere shortening remains unknown. Additional evidence of a crosstalk between A-type lamins and telomeres was provided by studies showing that telomerase rescues proliferative defects of human fibroblasts expressing lamin A mutants,[56] and that senescence-associated alterations of the nuclear lamina are accompanied by aggregation of telomeres to the nuclear lamina.[44]

Despite the importance of telomere maintenance for cancer progression, the impact that the loss of A-type lamins that characterizes certain tumor types has on telomere biology remained undetermined. Our study using *LMNA* knockout mouse fibroblasts as a model revealed that A-type lamins play a key role in the maintenance of telomere structure, length and function. Telomeres in *Lmna*$^{-/-}$ mice are consistently shorter than the corresponding wild-type controls, and exhibit an increase in signal-free ends (loss of telomeric signals).[45] Furthermore, acute depletion of A-type lamins by shRNAs specific for depletion of lamins A and C, leads to telomere shortening after only a few passages of the cells in culture as determined by Quantitative Fluorescence In Situ Hybridization (Q-FISH) with a telomeric probe. Most importantly, reintroduction of either lamin A, lamin C, or both by retroviral transduction of A-type lamins-depleted cells rescues the telomere shortening phenotype to varying degrees (**Fig. 3**). Reintroduction of lamins lead to a significant increase in average telomere length, as well as a decrease in the pool of short telomeres and an increase in the pool of long telomeres. These data indicate that A-type lamins play a key role in the control of telomere length. However, we do not know the mechanism behind the telomere shortening phenotype observed in A-type lamins-deficient cells. The levels of telomerase activity and the binding of the shelterin complex components TRF1 and TRF2 were not affected by the loss of A-type lamins. Similarly, we did not observe any evidence of aberrant recombination at telomeres, which could explain the loss of telomeric sequences. It is possible that the binding of other shelterin complex components or DNA repair factors with a function at the telomere could be defective in *LMNA* null cells. Alternatively or concomitantly, loss of A-type lamins might hamper the accessibility of telomerase or other proteins implicated in telomere metabolism, especially factors implicated in telomere replication.

Maintenance of a heterochromatic structure at telomeres is also critical for the control of telomere length. Previous studies demonstrated that loss of heterochromatic features such as methylation of histones H3 and H4 at different lysine residues and methylation of subtelomeric DNA results in a pronounced telomere elongation phenotype.[42,57-59] In most cases, telomere elongation correlated with an increase of telomere sister chromatid exchange (T-SCE) events, characteristic of the activation of Alternative Lengthening of Telomeres (ALT) pathway of telomere maintenance.[60,61] We found that loss of A-type lamins results in decreased levels of the heterochromatic mark H4K20me3-histone H4 trimethylated at lysine 20. This defect is likely due to the fact that Rb family members, which have a known role in the stabilization of this chromatin modification, are targeted to degradation by the proteasome upon loss of A-type lamins.[62] However, contrary to the telomere elongation phenotype characteristic of Rb-deficient cells[63,64] and H4K20me3-deficient cells (Suv4-20 h dn),[65] the loss of Rb or decrease in H4K20me3 was not sufficient to trigger telomere elongation in the context of A-type lamins deficiency. Thus, A-type lamins, or a process regulated by these proteins, are necessary for the elongation of telomeres upon loss of Rb family members and decrease of

H4K20me3. Future experiments need to determine if ALT is the mechanism activated upon loss of Rb family members, and if A-type lamins inhibit recombination at telomeres.

A-Type Lamins and DNA Repair

Expression of mutant lamin A isoforms is associated with defective DNA repair. Fibroblasts from HGPS patients and from a mouse model of progeria generated by depletion of the metalloproteinase responsible for the maturation of prelamin A (*Zmpste24* knock-out)[66] have alterations in the DDR.[22] In particular, these cells exhibit increased DNA damage and chromosome aberrations and are more sensitive to DNA-damaging agents. At a molecular level, HGPS and *Zmpste24*$^{-/-}$ MEFs showed a delayed recruitment of p53 binding protein 1 (53BP1) to phosphorylated histone H2AX (γ-H2AX)-labeled DNA repair foci upon induction of DNA damage, and a delayed disappearance of these foci. *Zmpste24*$^{-/-}$ MEFs also showed impaired recruitment of Rad51 to sites of DNA damage leading to a delayed checkpoint response and defective DNA repair.[22] Furthermore, ectopic expression of mutant forms of lamin A in the presence of wild-type lamins A/C diminished the cellular ability to form γ-H2AX-labeled DNA repair foci in response to mild doses of cisplatin or UV irradiation, and mislocalized ATR kinase, a key sensor in DDR.[67] More recent studies have demonstrated that fibroblasts from HGPS patients and from *Zmpste24*$^{-/-}$ MEFs display an activated DNA damage response, as evidenced by enhanced γH2AX, and activation of the p53 pathway.[55,68] All these reports suggest that unprocessed prelamin A and truncated lamin A act in a dominant negative fashion to perturb DNA damage response and repair. Elucidating the specific steps during these processes that are affected in the different laminopathies could bring about new possibilities for treatment.

In spite of the progress made in understanding how specific mutations in lamins affect DNA repair, the impact that complete loss of A-type lamins has in the DNA damage response remains unknown. Our recent study shows that *LMNA* null fibroblasts exhibit signs of genomic instability: higher incidence of chromosome and chromatid breaks, increased numbers of signal free ends (telomere loss), and basal levels of unrepaired DNA, as shown by the presence of cells labeled with γH2AX. These data indicate that not only mutant forms of lamins, but also depletion of A-type lamins affects the ability of cells to properly deal with DNA damage. Nevertheless, the different mutations and the changes in expression of A-type lamins are expected to have different consequences for nuclear function, given the variety of diseases associated with the different alterations. Establishing for example, 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 lamins as targets for cancer therapeutics.

Loss of telomere integrity activates a classical DDR characterized by the activation of the ATM/p53 pathway and the formation of DNA damage foci at telomeres.[69] These telomere dysfunction-induced foci (TIF) contain many DNA damage response proteins, including γ-H2AX, 53BP1, MDC1 and MRN complex, and are established as a read-out of telomere damage. Activation of ATM/p53 is followed by the inappropriate repair of dysfunctional telomeres by the NHEJ repair pathway, leading to chromosome end-to-end fusions.[70] Two recent studies have shown that deprotected telomeres are more mobile and sample larger territories within the nucleus than functional telomeres.[71,72] According to Dimitrova et al.[71] 53BP1 plays an active role in chromatin dynamics, such that it facilitates the association and fusion of dysfunctional telomeres that might be far away within the nucleus. Our study shows that the chromosome end-to-end fusions resulting from dysfunctional telomeres induced by the expression of a dominant negative TRF2 protein require A-type lamins, providing the first link between loss of A-type lamins and defective NHEJ repair. In addition, we found that loss of A-type lamins leads to decreased levels of 53BP1, due to destabilization of the protein.[45] The decrease in 53BP1 levels is likely to be responsible for the low efficiency of end-to-end fusions upon telomere dysfunction in A-type lamins-deficient cells, although this point remains to be clearly demonstrated. It is possible that in addition to maintaining 53BP1 stability, A-type lamins play an active role in the DDR and in the 53BP1-mediated regulation of mobility and NHEJ of dysfunctional telomeres.

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 premature aging syndromes and cancer. The molecular mechanisms contributing to the phenotypes of lamin-related diseases are only beginning to be uncovered. We have summarized here recent data indicating that loss of A-type impacts telomere structure, length and function as well as the stability of 53BP1, a key factor in the DNA damage response also implicated in NHEJ of dysfunctional telomeres. Future studies need to determine if loss of A-type lamins affects the maintenance of telomeres and the stability of 53BP1 in human cells, contributing to the genomic instability that drives malignancy. In addition, it must be determined if alterations of these processes play a role in the pathophysiology of the different laminopathies resulting from mutations in the *LMNA* gene.

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