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DNA repair defects and genome instability in Hutchinson-Gilford Progeria Syndrome

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Abstract

The integrity of the nuclear lamina has emerged as an important factor in the maintenance of genome stability. In particular, mutations in the *LMNA* gene, encoding A-type lamins (lamin A/C), alter nuclear morphology and function, and cause genomic instability. *LMNA* gene mutations are associated with a variety of degenerative diseases and devastating premature aging syndromes such as Hutchinson-Gilford Progeria Syndrome (HGPS) and Restrictive Dermopathy (RD). HGPS is a severe laminopathy, with patients dying in their teens from myocardial infarction or stroke. HGPS patient-derived cells exhibit nuclear shape abnormalities, changes in epigenetic regulation and gene expression, telomere shortening, genome instability, and premature senescence. This review highlights recent advances in identifying molecular mechanisms that contribute to the pathophysiology of HGPS, with a special emphasis on DNA repair defects and genome instability.

Pathophysiology of HGPS

Mutations in the *LMNA* gene, encoding lamin A and C, result in defects in DNA replication and repair, gene transcription and silencing, positioning of nuclear pore complexes, chromatin remodeling, and nuclear envelope breakdown and reassembly during mitosis [1–4]. To date, more than 400 mutations in the *LMNA* gene have been associated with over a dozen degenerative disorders including neuropathies, muscular dystrophies, lipodystrophies, and premature aging disorders. Among the most severe of these disorders are Hutchinson-Gilford Progeria Syndrome (HGPS) and Restrictive Dermopathy [5–7]. HGPS is a rare but devastating disease in which patients appear normal at birth but develop severe growth abnormalities within two years. Children with HGPS have characteristics associated with premature aging, and generally die in their teens predominantly due to cardiovascular complications associated with atherosclerosis (e.g., myocardial infarction or stroke) [8–11]. Restrictive Dermopathy is even more severe, with growth retardation and reduced movement *in utero*, leading to death soon after birth [7]. Both diseases are caused by

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mutations that disrupt the normal processing of prelamin A to lamin A, thereby causing lamin A precursors to accumulate. The processing of prelamin A is a multistep process initiated by farnesylation of prelamin A at its C-terminal 'CaaX' ('a', aliphatic residues; 'X', any residue) motif. Next, -aaX is cleaved by endopeptidases Rce1 (Ras-converting enzyme 1) or Zmpste24, and the newly-terminal cysteine is carboxymethylated by the enzyme Icmt (isoprenylcysteine carboxyl methyltransferase) [12]. Finally, a second cleavage by Zmpste24 removes fifteen additional amino acids, including the farnesylated and carboxymethylated cysteine, generating mature lamin A.

Most reported HGPS cases carry the same *de novo* heterozygous silent mutation (c. 1827C>T, G608G) in exon 11 of *LMNA* [13,14]. This mutation activates a cryptic mRNA splice site that results in the deletion of 50 amino acids including the final Zmpste24 cleavage site near the C-terminus of prelamin A, yielding a permanently farnesylated and carboxymethylated dominant protein named "progerin" [5,6]. Progerin alters nuclear architecture, releases heterochromatin from the nuclear periphery, changes epigenetic regulation, signaling and gene expression, disrupts telomeres, causes genome instability and leads to premature senescence [15–23]. Restrictive Dermopathy is caused by prelamin A accumulation due to missense mutations in *LMNA*, or loss of Zmpste24 function [7].

In the few years since HGPS was genetically mapped [13,14], we have gained significant knowledge about its pathophysiology [8,9,11,24] and the cellular and molecular mechanisms of HGPS-associated tissue degeneration and premature aging [23]. In addition, a number of therapeutic strategies have been identified that ameliorate the phenotype of mouse models of the disease [25,26]. Studies in fibroblasts derived from HGPS patients and several mouse models of progeria, including transgenic mice carrying the G608G-mutated human *LMNA* gene on a bacterial artificial chromosome (BAC) [24], *Zmpste24*^{-/-} mice [27], and *Lmna*^{G609G} mice carrying the 'knocked-in' human mutation [22,28] have been instrumental in identifying pathways altered in HGPS [29]. Farnesyltransferase inhibitors (FTIs) prevented both the onset and late progression of cardiovascular disease in mouse progeria models, motivating the first clinical trial for HGPS patients [30–33]. Two additional drugs, statin (pravastatin) and bisphosphonate (zoledronate), were later included to block alternative prenylation pathways [32]. Patients showed improved weight gain over a two-year period, and improved vascular status, bone structure, and audiological status [33,34]. FTIs also extended mean survival of HGPS patients by 1.6 years [34]. Other potentially beneficial treatments, studied so far only in cells and animal models, include rapamycin [35], sodium salicylate [22], pyrophosphate [28] and Icmt (isoprenylcysteine carboxyl methyltransferase) inhibitors [36].

Despite these advances, our ability to ameliorate the effects of progerin expression in patients is limited. This is in part due to the fact that progerin expression has global effects, disrupting a variety of signaling cascades, as well as genome structure and function. Progerin aberrantly activates the NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) and Notch signaling pathways, decreases Wnt/ β -catenin function, and leads to loss of Rb (retinoblastoma) family proteins and decreased levels of the longevity factor Sirtuin 1 [23]. Notably, progerin expression also causes DNA repair defects, accumulation of DNA damage and genome instability, and premature senescence

[15,17,37]. New findings discussed below provide significant insight into the genomic consequences of progerin expression, and mechanisms that may protect HGPS patients from cancer.

Genome instability in HGPS

Genome instability is a hallmark of both aging and cancer [38,39]. Genome instability is caused by impaired sensing, signaling, or repair of DNA damage caused by agents either external (ionizing radiation, chemicals, UV) or endogenous (free radicals, DNA replication errors, or DNA crosslinkages) [40]. Telomere dysfunction, due to loss of telomeric DNA repeats below a critical threshold or loss of telomere capping, also contributes to genome instability. Telomere dysfunction and deficiencies in the repair of DNA double-strand breaks (DSBs) are among the leading contributors to genome instability in aging cells [41–43]. DSBs are repaired by two main mechanisms: non-homologous end-joining (NHEJ) and homologous recombination (HR). HR repairs DSBs with great fidelity by using the sister chromatid as the template for recombination [44]. By contrast, NHEJ is an error-prone mechanism that is also responsible for ‘repairing’ lost telomeres via chromosome end-to-end fusions.

Fibroblasts from HGPS patients or from several mouse models of progeria exhibit hallmarks of genome instability including elevated basal levels of γ H2AX, a phosphorylated histone marker of unrepaired DNA damage [17,45–47]. New studies discussed below reveal defects in multiple pathways including DSB repair [17], telomere function [42,48,49], reactive oxygen species and oxidative stress [50], and epigenetic regulation.

Defects in DNA double-strand break repair

The phenotypes of *Zmpste24*^{−/−} and HGPS fibroblasts indicate deficiencies in both the DNA damage response (DDR) and mechanisms of DSB repair. For example, progeroid fibroblasts accumulate basal levels of DNA damage, as shown by the presence of γ H2AX-positive foci, concomitant with a persistent activation of DDR checkpoint kinases (ATM [ataxia telangiectasia mutated], ATR [ataxia telangiectasia and Rad3-related protein], Chk1 [checkpoint kinase 1]), and higher sensitivity to agents that cause DSBs [17,19,47]. Bone marrow cells from *Zmpste24*^{−/−} mice are also more likely to show genome instability in the form of aneuploidy. These mice are highly sensitive to whole-body irradiation (80% of *Zmpste24*^{−/−} mice die by 12 days post-IR, compared to 20% of wild-type controls), due to proposed delays in recruiting RAD51 and 53BP1 (Tumor suppressor p53-binding protein 1) to γ H2AX-positive DNA repair foci [17]. Notably, RAD51 and 53BP1 represent two different repair pathways. RAD51 recruitment to DNA breaks is essential for homology search and strand invasion during HR [51], whereas 53BP1 promotes recruitment of the NHEJ machinery. Therefore, these studies suggested that both pathways (HR and NHEJ) could be compromised in progeroid human and mouse cells. Consistent with NHEJ defects, Liu et al. reported that HGPS patient-derived fibroblasts and vascular smooth muscle cells (SMCs) differentiated from HGPS induced pluripotent stem cells (iPSCs) exhibit reduced expression of the DNAPK holoenzyme, consisting of DNA-PKcs/Ku70/Ku80 [52]. However, a recent study also in SMCs differentiated from HGPS iPSCs revealed an activation of NHEJ [53]. Importantly, progerin accumulation stimulates a powerful

suppression of PARP1 (Poly-ADP-ribose polymerase 1) [53], which normally suppresses NHEJ [54]. As a result, most HGPS SMCs activate the error-prone NHEJ repair pathway during S-phase, leading to mitotic catastrophe and cell death [53]. This study elucidates a molecular pathway underlying the progressive SMC loss in HGPS patients.

Other studies in *Zmpste24*^{-/-} and HGPS cells linked DNA repair defects with aberrant accumulation of the nucleotide excision repair protein XPA (Xeroderma Pigmentosum, complementation group A) at DNA lesions; this is significant because XPA activates ATM- and ATR-dependent signaling cascades that contribute to proliferation arrest [55]. This study also reported that the recruitment of Rad50 and Rad51 to the DSB sites is impaired in human HGPS and *Zmpste24*-deficient cells. In addition, HGPS cells exhibit a delay in the recruitment of phospho-NBS1 and MRE11, components of the MRN complex that are necessary for HR [56]. Furthermore, MEFs from *Zmpste24*^{-/-} mice have defects in ATM-KAP-1 signaling and DNA damage-induced chromatin remodeling, both of which normally contribute to the recruitment and retention of DNA repair proteins at heterochromatic lesions [57].

Altogether these studies indicate that the mechanisms underlying genomic instability in HGPS are complex, and involve alterations in the expression and recruitment of a variety of DNA repair factors to sites of DNA damage. These alterations compromise the two main mechanisms of DNA DSB repair, HR and NHEJ, leading to activation of checkpoints and proliferation arrest (Figure 1). Despite these advancements, we still have limited information about the molecular mechanisms by which progerin expression disrupts the expression or recruitment of DNA repair components.

Another huge question in this field is why HGPS patients have no predisposition to cancer, given their elevated levels of DNA damage and genome instability [58]. Intriguing new evidence suggests that neoplastic transformation and invasiveness is reduced in cells that either accumulate prelamin A or express progerin. De la Rosa et al. generated *Zmpste24* mosaic mice, which develop normally and keep similar proportions of *Zmpste24*-proficient and *Zmpste24*-deficient cells throughout their life [59]. *Zmpste24*-deficient cells accumulate prelamin A while *Zmpste24*-proficient cells express mature lamin A. They reported that prelamin A accumulation does not affect tumor initiation and growth in response to carcinogenic protocols such as topical DMBA-TPA application to induce skin papillomas, or urethane injection to generate lung adenomas. However, they found that *Zmpste24* mosaic mice have a decrease incidence of infiltrating oral carcinomas induced by administration of 4-nitroquinoline-1-oxide (4-NQO) compared to control mice. Accordingly, silencing of *ZMPSTE24* by siRNA in tumor cell lines from oral, breast, and lung cancer caused a significant decrease in their invasive potential in the presence of prelamin A [59]. These results support the potential of cell-based and systemic therapies for progeria and highlight *ZMPSTE24* as a new anticancer target. Fernandez et al. identified another protective mechanism to oncogenesis in HGPS patient cells [60]. This study found that in HGPS cells, oncogenic dedifferentiation and neoplastic transformation were inhibited, concomitant with an altered pattern of chromatin binding by BRD4 (Bromodomain containing 4), which suppresses proliferation and metastasis. Further work is needed to

understand this phenomenon, and its potential relevance to normal aging, during which both progerin expression [19] and the likelihood of cancer increase.

Telomere dysfunction

Dysfunctional or deprotected telomeres are recognized as DSBs, leading to activation of the DDR and aberrant ‘healing’ of chromosome ends by NHEJ [61], producing end-to-end fusions and other chromosomal aberrations [62]. In normal cells, telomere shortening below a critical threshold triggers a state of permanent growth arrest known as replicative senescence. HGPS patient cells show accelerated telomere shortening during proliferation in culture [42,49], accompanied by premature entry into senescence. In addition, ectopic expression of progerin in normal fibroblasts leads to accumulation of DNA damage at telomeres and a proliferation arrest with characteristics of senescence [48,63]. Notably, expression of telomerase improves proliferation and extends lifespan of HGPS cells by decreasing progerin-induced DNA damage signaling and activation of p53 and Rb (retinoblastoma) pathways, which mediate the onset of premature senescence [48,64]. These studies suggest that telomere dysfunction underlies genomic instability and premature senescence in progerin-expressing cells, and that protection of telomeres by telomerase ameliorates these phenotypes. Intriguingly, other studies have shown that telomere-driven cellular senescence correlates with activation of the cryptic splice donor site that produces progerin, while immortalization with telomerase reduces progerin transcription [63]. Thus, the forced elongation of telomeres by telomerase exerts a beneficial effect by reducing progerin production. In addition, an increase in progerin transcripts and protein levels was observed in normal fibroblasts as a result of telomere dysfunction caused by ectopic expression of a mutant version of the telomere binding protein TRF2 (telomeric repeat binding factor 2) [63]. Overall, these studies suggest an unfortunate relationship in which progerin hinders telomere maintenance, and telomere dysfunction promotes progerin production (Figure 1). Dysfunctional telomere-dependent triggering of progerin expression would explain at least in part why cells from old individuals express progerin.

Despite all these important findings, the molecular mechanisms responsible for telomere attrition/dysfunction upon progerin expression or for progerin production upon telomere dysfunction remain poorly understood. A study in primary fibroblasts carrying *LMNA* mutations (R133L or L140R) provided some insights by showing a significant reduction in the levels of telomere-binding proteins that form the shelterin complex in these cells, primarily in TRF2 levels [65]. Loss of TRF2 function disrupts the formation of protective t-loops at telomeres, triggering telomere shortening, the DNA damage response, chromosome instability, and premature senescence [66]. Interestingly, TRF2 associates with lamin A and other lamin-associated proteins, but not with progerin [67,68]. The association of lamin A with TRF2 was recently shown to stabilize the formation of t-loops within interstitial telomeric sequences, known as interstitial t-loops (ITLs) [68]. Two conditions, reduction of lamin A/C levels in normal fibroblasts or progerin expression in HGPS fibroblasts, are associated with fewer ITLs and profound telomere loss. Thus, decreased TRF2 function could be one of the mechanisms behind telomere dysfunction in cells with an aberrant nuclear lamina, causing disruption of protective t-loops at telomeres and at interstitial telomeric sequences, as well as telomere shortening and damage. Moreover, studies in cells

from *Lmna*^{-/-} mice provided more clues about how progerin expression leads to telomere dysfunction. *Lmna*^{-/-} fibroblasts exhibit altered distribution of telomeres in the 3D nuclear space, defects in telomere structure, length, and function, and a profound increase in telomere mobility (unpublished) [69,70]. By contrast, the mechanism by which telomere dysfunction promotes progerin production is an enigma. Characterizing this relationship in depth will be very important for understanding telomere instability during aging and aging related diseases.

Epigenetic alterations

Disrupted chromatin organization and epigenetic misregulation are both thought to contribute to genome instability in HGPS cells. Compared to normal fibroblasts, HGPS fibroblasts have reduced levels of HP1 α (Heterochromatin protein 1 alpha), involved in heterochromatin maintenance; reduced levels of histone H3 trimethylated at lysine 27 (H3K27me3), a mark of facultative heterochromatin, due to downregulation of histone methyltransferase EZH2; and lower levels of histone H3 trimethylated at lysine 9 (H3K9me3), a mark of pericentric constitutive heterochromatin [16]. In addition, HGPS cells exhibit increased levels in the trimethylation of histone H4 at lysine 20 (H4K20me3), an epigenetic mark for constitutive heterochromatin [16] (Figure 1). A genome-wide study of HGPS cells revealed more complex changes in H3K27me3, correlating with gene density, in certain regions of the genome [71]. In particular, gene-rich regions tended to gain H3K27me3, whereas this mark was reduced in gene-poor regions, which also tended to detach from the lamina, suggesting a functional relationship.

Intriguingly, another study showed that in *Zmpste24*^{-/-} MEFs, which express unprocessed prelamin A, and in HGPS cells, accumulating progerin, there is protection of the histone methyltransferase Suv39h1 from proteasomal degradation. This protection was associated with an increase in H3K9me3 levels and with defects in DNA repair, since Suv39h1 depletion restored DNA repair capacity and delayed senescence in progeroid cells [72]. These conflicting findings about H3K9me3 [16,72] may relate to differences in the age or gender of HGPS donors, or the number of times their cells were passaged in culture. At early passage, progeroid cells have high levels of H3K9me3 due to stabilization of Suv39h1 protein levels [72]. However, at later passages, H3K9me3 decreases due to reduced transcription of Suv39h1 [16,72]. Thus caution and accuracy are essential when comparing HGPS fibroblasts to normal cells. The importance of Suv39h1 upregulation to the progeria phenotype was demonstrated in mice doubly deficient for both *Zmpste24* and Suv39h1 (*Zmpste24*^{-/-}/*Suv39h1*^{-/-}): these mice had improved body size, bone mineral density and lifespan (prolonged almost 40%) compared to *Zmpste24*^{-/-} mice [72].

Defects in other chromatin modifiers including the histone acetyltransferase Mof (acetylates H4K16) and the NuRD (Nucleosome Remodeling Deacetylase) complex, which deacetylates histones and maintains gene repression, have been reported in progeroid cells [73,74]. Inhibiting histone deacetylation, or overexpression of Mof, each rescues the recruitment of DNA repair factors to sites of DNA damage in *Zmpste24*^{-/-} cells. These manipulations also ameliorate aging-associated phenotypes of *Zmpste24*^{-/-} mice [74]. Moreover, several NuRD complex components including RBBP4 (retinoblastoma binding

protein 4), RBBP7 and HDAC1 (histone deacetylase 1) were significantly reduced during proliferation of HGPS cells in culture, and this reduction was progerin-dependent [73]. Importantly, the loss of NuRD components was linked to DNA repair defects, since depletion of RBBP4/7 in HeLa cells led to accumulation of unrepaired DNA damage (γ H2AX foci). In addition, reduced expression of NuRD subunits preceded DNA damage accumulation in HGPS cells [73]. Interestingly, cells from normally aged individuals had significant reduced levels of RBBP4, RBBP7 and HDAC1 proteins, suggesting that loss of NuRD function is also a feature of physiological aging [73].

Overall, these studies strongly correlate progeria-associated mutations in *LMNA* with changes in chromatin-modifying activities and hindered maintenance of genome integrity. Since most epigenetic changes are reversible, therapeutic targeting of chromatin modifications represents a potential strategy for decreasing genome instability and ameliorating cell degeneration during pathological and physiological aging. However, a comprehensive analysis of chromatin alterations that drive genomic instability in HGPS is needed to identify master epigenetic regulators that might be targeted therapeutically.

Oxidative stress

Fibroblasts from patients with different laminopathies including HGPS have mitochondrial phenotypes, elevated levels of reactive oxygen species (ROS) [75], and greater sensitivity to oxidative stress than normal fibroblasts [50]. Higher ROS levels correlate with proliferation defects, but not with nuclear shape abnormalities. HGPS cells also show deficiencies in the repair of DSBs induced by ROS. Interestingly, ROS scavengers such as N-acetyl cysteine (NAC) reduced basal levels of DNA damage in HGPS cells, eliminated unrepairable ROS-induced DSBs, and improved proliferation, suggesting that oxidative stress is a major contributor to genomic instability and proliferation defects in these cells [50,76,77].

Recently, SILAC (stable isotope labeling with amino acids in culture) analysis revealed reduced levels of mitochondrial oxidative phosphorylation proteins in cells from HGPS patients, compared to healthy subjects [78]. In addition, this study presented evidence of mitochondrial dysfunction in fibroblasts isolated from adult progeroid mice (*Lmna*^{G609G/G609G} knock-in and *Zmste24*^{-/-} mice). Analysis of tissues from these mouse models showed that mitochondrial dysfunction is more pronounced in older mice, and in homozygous *Lmna*^{G609G/G609G} versus heterozygous *Lmna*^{G609G/+} mice. Thus, mitochondrial dysfunction in progeria mice is time- and dose-dependent. Thus, therapies aimed at restoring mitochondrial function or reducing ROS levels may benefit HGPS patients. Interestingly, a combination of isoprenylation inhibitors (FTI-277 or pravastatin, plus zoledronate) used in ongoing HGPS clinical trials restored mitochondrial function in progeroid mouse cells [78]. Thus, combined clinical treatment with FTIs, statins and bisphosphonates is likely beneficial because it targets many different pathways altered in progeria, including mitochondrial dysfunction.

Deciphering the normal functions of A-type lamins

Studies of genome instability in patients with HGPS or other laminopathies have started to shed light on the normal genomic roles of A-type lamins. In addition, the study of cells

depleted of lamin A/C has been instrumental to identify new functions of these proteins in the compartmentalization of genome function, and in the maintenance of genome integrity. For example, the three-dimensional localization of telomeres in the nucleus is disrupted in lamin A/C-deficient cells, accompanied by telomere shortening or loss [69,70,79]. In addition, fibroblasts from a homozygous nonsense *LMNA* mutation patient showed increased plasticity of the nuclear envelope and hypermobility of telomeres in the nuclear space [80]. In contrast, HGPS fibroblasts exhibited an overall reduction in nuclear dynamics. Interestingly, inhibitors of prenylation (FTIs) in HGPS cells, or lamin A/C silencing in normal fibroblasts confirmed that these changes are due to abnormal or reduced lamin A/C expression [80].

Loss of lamin A/C also hinders mechanisms of DNA DSB repair. For example, depletion of lamin A/C activates cathepsin L-mediated degradation of 53BP1 [81] and reduces expression of two important DNA repair proteins: BRCA1 (Breast cancer 1) and RAD51 [82,83]. As a consequence, lamin A/C-deficient cells show deficiencies in NHEJ (53BP1 loss) and HR (BRCA1 and RAD51 down-regulation), with an increased frequency of chromosome breaks/aberrations in metaphase spreads, and accumulation of unrepaired DNA damage after exposure to ionizing radiation [82,83]. Thus, loss of lamin A/C recapitulates some of the phenotypes seen in cells that express progerin (HGPS) or accumulate prelamin A (*Zmpste24*^{-/-}). This suggests that genome instability in progeria is due at least in part to loss of lamin A/C function. Interestingly, cells from mice that lack *LMNA* exon9 (*Lmna*^{9/9}) had shortened telomeres and epigenetic changes but no defects in DNA repair, and maintained normal levels of 53BP1, BRCA1 and RAD51 [84]. These results suggest it will be possible to identify functional regions within lamin A that are specifically relevant to genome stability, and identify reduced levels of 53BP1, BRCA1 and RAD51 as potential markers of genome instability in laminopathy patients.

Despite this progress, important mechanistic questions about lamins and DNA repair remain open. For example lamins A/C might function at the level of transcription (expression of DNA repair genes), as a protein support network (bind or scaffold DNA repair factors) or at the level of signaling (DNA damage response signaling). Alternatively mutant lamin A/C proteins might interfere with DNA damage signaling or repair by any number of mechanisms ranging from steric hindrance to epigenetic misregulation. More studies are needed to understand how lamins contribute to the DNA damage response and help maintain genome integrity.

Conclusions and perspectives

The mechanisms of genome instability in laminopathies are not yet fully understood, and may depend on the specific *LMNA* mutation. As discussed above, defects in DNA repair and telomere maintenance, epigenetic changes and oxidative stress all contribute to genome instability in laminopathies such as HGPS, identifying new potential therapeutic targets. Studies in mouse models of progeria have identified compounds that ameliorate a number of phenotypes. FTIs, statins and bisphosphonates, and IcmT inhibitors (among others) extend the lifespan of progeria mice to different degrees. Despite these advances, our ability to reduce the profound systemic effects of progerin expression in humans remains limited,

emphasizing the need for new and better treatments. The challenge is that progerin affects many pathways. Since progerin is dominant, emphasis should be focused on strategies to induce progerin clearance. This strategy could not only ameliorate the pathophysiology of HGPS, but may also improve the health of the aging population.

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Figure 1. Genome instability in progeria cells

In normal cells, dispersed networks of A-type lamin filaments support the inner nuclear membrane (INM) and extend throughout the nucleoplasm. Chromosomes have long and functional telomeres, normal levels of histone modifications, and can recruit DNA repair factors to DNA double-strand breaks. By contrast progerin accumulates at the inner nuclear

membrane and eventually recruits normal lamins, collapsing their networks. Progeria cells suffer telomere shortening and deficient recruitment of factors needed for homologous repair (HR) and non-homologous end joining (NHEJ) mechanisms of repair. This leads to the accumulation of phosphorylated H2AX (γ H2AX, indicated by yellow 'P'), a marker of unrepaired DNA damage. Progeria cells accumulate ROS, which damages DNA, and misregulation of histone modifications (Ac, acetylation; Me, methylation; Ub, ubiquitinylation) also contributes to genomic instability.