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REVIEW

Chromatin epigenetics and nuclear lamina keep the nucleus in shape: Examples from natural and accelerated aging

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As the repository of genetic information, the cell nucleus must protect DNA integrity from mechanical stresses. The nuclear lamina, which resides within the nuclear envelope (NE), is made up of lamins, intermediate filaments bound to DNA. The nuclear lamina provides the nucleus with the ability to deal with inward as well as outward mechanical stimuli. Chromatin, in turn, through its degrees of compaction, shares this role with the nuclear lamina, thus, ensuring the plasticity of the nucleus. Perturbation of chromatin condensation or the nuclear lamina has been linked to a plethora of biological conditions, that range from cancer and genetic diseases (laminopathies) to aging, both natural and accelerated, such as the case of Hutchinson-Gilford Progeria Syndrome (HGPS). From the experimental results accumulated so far on the topic, a direct link between variations of the epigenetic pattern and nuclear lamina structure would be suggested, however, it has never been clarified thoroughly. This relationship, instead, has a downstream important implication on nucleus shape, genome preservation, force sensing, and, ultimately, aging-related disease onset. With this review, we aim to collect recent studies on the importance of both nuclear lamina components and chromatin status in nuclear mechanics. We also aim to bring to light evidence of the link between DNA methylation and nuclear lamina in natural and accelerated aging.

KEYWORDS
aging, epigenetics, HGPS, nuclear lamina, nuclear mechanics

INTRODUCTION

The cell nucleus is the most important organelle present in eukaryotic cells, being the gatekeeper of genetic information by shielding the DNA from every kind of insult. It is straightforward to think, then, that the impairment or the loss of the well-ordered and organized structure of the nucleus can have serious consequences on DNA function and topology.

The nuclear lamina is the structure responsible for the strength of the cell nucleus. Positioned underneath the inner nuclear membrane (INM) of the nuclear envelope (NE), nuclear lamina is composed of a class of intermediate filaments, collectively called nuclear lamins. These interact, directly or indirectly, with chromatin (Maurer & Lammerding, 2019). The cell nucleus is not only the repository of genetic information, but it is also profoundly involved in the process of cell mechanotransduction, that is the translation of mechanical cues perceived by the cell into biochemical signals (Ingber, 2006). Mechanotransduction has been studied for more than 20 years now, and one of the first works on the topic dates back to the late 90s when Maniotis et al. were able to stretch nuclei of endothelial cells by stretching the cells with a glass microneedle (Maniotis et al., 1997). Later, Emmanuel Farge showed that

the Armadillo protein, the homolog of Beta Catenin in *Drosophila Melanogaster*, underwent nuclear translocation if *Drosophila* embryos were subjected to a constraint force, which induced, in turn, the expression of the *Twist* gene (Farge, 2003). Engler and colleagues, then, demonstrated that differentiation fate in mesenchymal stem cells (MSCs) depends on both substrate stiffness and a working cytoskeleton (Engler et al., 2006). Indeed, nuclear lamins connect the nucleoskeleton with the cytoskeleton by interacting with the LINC complex, a protein complex (made up of the superfamilies of proteins Nesprin and Sun) that spans the NE (Crisp et al., 2006). In this way, lamins have a central role in the mechanotransduction process that involves the NE (Isermann & Lammerding, 2013). More recently, it has been shown that Nesprin proteins can discriminate between partial as well as complete epithelial-mesenchymal transitions (EMTs) (Déjardin et al., 2020), that is, biological states where cells totally lose (complete EMTs) or partially lose (partial EMTs) epithelial markers to acquire mesenchymal ones (Aiello et al., 2018). In this study, differential EMTs were experimentally induced in Madin-Darby Canin Kidney cells (MDCKs) by wound healing as well as stimulation with hepatocyte growth factor (HGF) (Canever et al., 2021). Each kind of induced EMTs triggered a specific change in Nesprin proteins' tensional status, which permits nuclear translocation of Beta Catenin (Déjardin et al., 2020). Thus, it is clear that transmitting forces from outside of the cell towards the inside of the nucleus can regulate gene expression.

Chromatin, the complex of DNA and histone proteins, can be present in the cell nucleus either in an opened and loosened status, called euchromatin, or in a closed and compacted one, known as heterochromatin. These two states can be recognized by the presence/absence of post-translational modifications on specific aminoacidic residues of the histones (Bannister & Kouzarides, 2011), which affect three-dimensional (3D) chromatin organization from the dense and compact fibers of heterochromatin to the decompacted structure of euchromatin. Also, the methylation of the DNA, occurring at the cytosine of the CpG dinucleotide, is important for chromatin condensation (Mazzio & Soliman, 2012).

Variations of chromatin condensation status could have multiple consequences on cell viability and functionality. These can be exemplified by the epigenetic-driven change in gene transcription. Indeed, a condensed status of chromatin spanning gene promoters results in the inaccessibility of transcriptional machinery and consequent gene silencing, while open chromatin favors gene transcription. However, it is becoming clear that the epigenetic and 3D status also of non-coding DNA is important to preserve cell functionality and human health (Janssen et al., 2018). In this regard, heterochromatin and epigenetic dysfunctions at non-coding repetitive sequences of centromere have been correlated with mitotic defects, chromosomal instability, and ICF syndrome (immunodeficiency, centromeric instability, and facial dysmorphism) (Barra & Fachinetti, 2018; Costa et al., 2016; Pappalardo & Barra, 2021). Heterochromatin also preserves genome integrity by preventing repetitive sequences to recombine and being incorrectly repaired by homologous recombination when damaged (reviewed in Janssen et al., 2018). In addition, in the last years there has been an increased number of publications showing that, by playing with the global nuclear ratio between hetero- and euchromatin, the physical properties of the nucleus itself and how it responds to mechanical stimuli change (Furusawa et al., 2015; Krause et al., 2013; Shimamoto et al., 2017). With this in mind, chromatin and its three-dimensional status should not be regarded only as a way to regulate gene expression and organize the genome to preserve its integrity. It also is a physical mass with a dynamic structure whose volume and density can change. The nuclear membrane has to adapt to chromatin changes, and vice versa, to preserve genome stability and function. Any alterations of this dynamic equilibrium can jeopardize cell nucleus physical properties and, consequently, functions. This scenario should be carefully considered in the study of natural and healthy aging, a biological process involving the increasing reduction of cellular functions at multiple levels (Campisi, 2013), and of pathological and accelerated aging, such as the Hutchinson-Gilford Progeria Syndrome (HGPS). In both cases, indeed, chromatin domains are rearranged and acquire new subnuclear localizations (Chandra et al., 2015; Köhler et al., 2020), potentially playing a role in the aberrant nuclear phenotype observed in HGPS and natural aging (Scaffidi & Misteli 2006).

In this review, we give a brief recap on chromatin and its epigenetic modifications, and the NE. Then, we summarize the literature covering the epigenetics, cell biology, and biophysical aspects of both HGPS and natural aging, here used as models where interactions between NE and chromatin, in the context of nuclear mechanics, have been studied mostly. Finally, we propose possible crosstalk between chromatin epigenetics changes, in terms of DNA methylation, and NE composition in both HGPS and natural aging.

Chromatin and DNA methylation

The fundamental unit of chromatin is the nucleosome consisting of a 147 bp segment of genomic DNA tightly wrapped 1.65 times around a core of eight positively charged histone proteins, namely two H2A–H2B dimers and an H3–H4 tetramer (Luger et al., 1997). Neighboring nucleosomes are connected by linker DNA segments (20–80 bp) which can be bound to histone H1 to stabilize

each nucleosome and contribute to chromatin compaction (Bednar et al., 1998). Nucleosomes connected by linker DNA create the first level of DNA packaging, however, to fit within the nucleus chromatin undergoes higher order folding whose physical state is still a subject of intense research. It has become clear, though, that chromatin is made up of rather irregular and variable nucleosome organization (for reviews, see Gilbert, 2019; Maeshima et al., 2021). Chromosome conformation capture (3C)/Hi-C and super-resolution imaging have shown that in the nucleus the genome is organized in chromatin domains: Topologically Associating Domains (TADs) and cohesin-mediated loop domains (Bintu et al., 2018; Dekker, 2008; Rao et al., 2017) able to interact between long distances forming two kinds of spatial compartments (Lieberman-Aiden et al., 2009). Compartment A is a gene-dense region characterized by open and transcriptionally active chromatin (euchromatin) that is mostly found in the interior of the nucleus, while compartment B is gene-poor and heterochromatic regions preferentially associated with the nuclear lamina and nucleolus (reviewed in Zheng & Xie, 2019). This organization mirrors the different locations of heterochromatin and euchromatin observed by electron microscopy in the cell nucleus: indeed, heterochromatin is mainly located at the nuclear periphery and around nucleoli; whereas euchromatin is found in the nuclear interior and around the nuclear pores (Croft et al., 1999; Kalverda et al., 2008; Rae & Franke, 1972). Inside the interphase nuclei, the compartments A and B belonging to an individual chromosome are located together within specific discrete territories called chromosome territories (Cremer & Cremer, 2010). Besides this idea of static compartmentalization that contributes to genome regulation and stability, recent research demonstrated that the nature of chromatin spatial organization is rather dynamic, being specific for different tissues (Parada et al., 2004) and having a role in multiple cell processes from transcription to DNA damage repair (Hauer et al., 2017; Shaban et al., 2020).

Three-dimensional chromatin structure on both a global and a local scale can be changed by epigenetic modifications resulting in the tightly packed heterochromatin or the more accessible euchromatin. Euchromatin is generally associated with high levels of histones' acetylation, especially at histones H3 and H4 correlating with transcriptional activity (Bernstein et al., 2007). As opposed to euchromatin, heterochromatin is characterized by histones' deacetylation and by the methylation of histone H3 on lysine 9 (H3K9) and 27 (H3K27), performed by the SUV39H family of SET domain proteins and PRC2 (Polycomb Repressive Complex 2) respectively (Müller et al., 2002; Rea et al., 2000), and of histone H4 on lysine 20 (H4K20) performed by Suv4-20 h enzymes (Schotta et al., 2004). These epigenetic modifications trigger the closed and transcriptionally inactive heterochromatin (Bannister & Kouzarides, 2011). Specifically, methylation on H3K9 is the marker recognizing the so-called constitutive heterochromatin, that is repressed chromatin; whereas methylation on H3K27 is present on the facultative heterochromatin indicating chromatin that, in specific moments and cell contexts, can be transcriptionally active (Bannister & Kouzarides, 2011).

Heterochromatin is also characterized by cytosine methylation in the CpG dinucleotides (Mazzio & Soliman, 2012; Rose & Klose, 2014). In this regard, a methyl group is covalently attached to the 5' position of the cytosine by enzymes called DNA methyltransferases (DNMTs). Precisely, DNMT3A and DNMT3B are specific for de novo methylation, whereas DNMT1 is involved in the maintenance of the methylation throughout cell divisions (Mazzio & Soliman, 2012). DNA methylation is directly involved in chromatin repression by expediting nucleosome assembly through rigidification of DNA and thus facilitating chromatin packaging (Lee et al., 2015).

Heterochromatin is further enforced by the association of epigenetic readers, proteins that specifically recognize the epigenetic marks of heterochromatin. The most important one is Heterochromatin protein 1 (HP1) which can bind H3K9me3 through its N-terminal chromodomain, and interact with several proteins to spread the heterochromatin state (reviewed in Eissenberg & Elgin, 2014). It is also worth noting that proteins that can specifically bind the methylated cytosine in position 5' of the CpG via their MBD (Methyl Binding Domain), such as MeCP2 (Methyl-CpG binding protein 2) and Mbd1, concentrated at heterochromatin regions and interact with H3K9 methyltransferases (SUV39H and SETDB1) (Good et al., 2021; Rose & Klose, 2014). Intriguingly, MeCP2 co-precipitates with HP1 in RKO cells, suggesting an interaction that is not only associated with myogenic differentiation where it was first observed (Agarwal et al., 2007; Pandey et al., 2015).

Heterochromatin establishment appears to be a multistep process whose exact molecular mechanism is not yet clear in mammals. However, it is widely agreed that at least for constitutive heterochromatin the sequence of events should be initiated by SUV39H enzymes that trimethylate H3K9. H3K9me3 constitutes the docking site for HP1 that, in turn, would recruit Suv4-20H enzymes and DNMTs to methylate H4K20 and cytosines, respectively. H4K20me3 and methylated cytosines can also be recognized and bound by other proteins such as MeCP2 and Mdb1 to spread and stabilize the heterochromatinization (reviewed in Saksouk et al., 2015). It has been also recently observed that noncoding RNAs arising from the repetitive sequences within constitutive heterochromatin are involved in the recruitment and stabilization of SUV39H1 to heterochromatin (Iglesias & Moazed, 2017). Nevertheless, the scenario could not be so simple and the order of events not so strict. Indeed, it has been observed in mouse embryonic stem cells (mESCs) that the

DNA hypomethylation at pericentromeres induced by SUV39H1/2 double knockout was due to impairment of only the maintenance methylation machinery and not of the de novo methylation machinery. On the other hand, in the same cells, the triple knockout of the DNMTs resulted in the reduction of H3K9me3 and SUV39H2 at pericentromeric heterochromatin (Saksouk et al., 2014). This suggests that there is very close interdependence between H3K9me3 and DNA methylation marks on constitutive heterochromatin. It is also worth mentioning that the process of maintaining heterochromatin, which must take place at every cell cycle after or during the DNA replication phase, is different from its establishment, coming into play the maintenance DNMT1 instead of the de novo DNMTs, and another key protein UHRF1 that on one side recognizes and binds hemimethylated sites and on the other recruits DNMT1 (for a review see Rose & Klose, 2014). Moreover, MeCP2 was shown to interact in vivo, via its TRD domain (Transcription Repressor Domain) with DNMT1, which could contribute to the maintenance of DNA methylation (Kimura & Shiota, 2003). It should be also noted that the MeCP2 mutations typical of Rett syndrome – a rare genetic disorder caused by several kinds of MeCP2 mutations that affect brain development and result in severe mental and physical disabilities – in mice caused significant changes in heterochromatin structure in parallel with changes in gene transcription, suggesting an important role in heterochromatin regulation (Ito-Ishida et al., 2020).

Alterations of DNA methylation have been observed in multiple human diseases, including cancer, and natural aging (Jin & Liu, 2018; Pappalardo & Barra, 2021; Salameh et al., 2020), and they have been correlated with genome instability (Barra et al., 2012; Cilluffo et al., 2020; Costa et al., 2016; Karpf & Matsui, 2005), though the mechanisms and the direct impact on the pathologies have not been fully clarified yet.

Nuclear envelope and nuclear lamina

The NE is composed of two lipid bilayers, the Outer Nuclear Membrane (Scaffidi & Misteli, 2006) (ONM) and the already cited INM, which are separated by a 30–50 nanometer-wide perinuclear space (PNS) (Maurer & Lammerding, 2019). As such, NE properly holds chromatin in the nucleoplasm, by separating it from the cytoplasm. Exchanges between the nucleus and cytoplasm are made possible due to the presence, on the NE, of the selective filter of the nuclear pore complex (NPC) (Matsuda & Mofrad, 2022).

Nuclear lamina, a thick protein meshwork just below the INM, is composed of Lamins, type V nuclear intermediate filaments (Maurer & Lammerding, 2019). Mammalian Lamins are classified as A-type and B-type Lamins (Janin & Gache, 2018). Two major isoforms of A-type lamins exist, namely Lamin A and Lamin C, which are encoded by the gene *LMNA* via alternative splicing on exon 10. Compared to Lamin C, Lamin A shows an extra domain encoded by exons 11 and 12 of the *LMNA* gene (Fisher et al., 1986; Lin & Worman, 1993; McKeon et al., 1986). Concerning B-type lamins, they are represented by lamins B1 and B2, which are encoded by *LMNB1* and *LMNB2* genes respectively (Peter et al., 1989; Vorburger et al., 1989). Lamin B1, specifically, is bound by Lamin B Receptor (LBR) (Worman et al., 1988), a protein of the INM, which was shown to bind heterochromatin (Makatsori et al., 2004).

Lamins can interact with heterochromatin in the so-called LADs (Lamin Associated Domains), genomic regions involved in physical contact with lamins (Guelen et al., 2008). Interestingly, the group of Karen Reddy has recently shown that, by using 3D-immunoFISH on single MEFs (Mouse Embryonic Fibroblasts), the depletion of Lamin C, but neither the depletion of Lamin A nor Lamin B, causes dispersion of LADs and disruption of LADs' attachment to the NE. However, the researchers did not find the same outcomes by using the DamID-seq, a technique through which it is possible to map protein/DNA interaction sites (Greil et al., 2006), because the data generated in this way took into account entire cell populations and not the single cells (Wong et al., 2021). In general, LADs can be divided into constitutive LADs (cLADs) and into facultative LADs (fLADs). cLADs are characterized by the histone mark H3K9me2/3 and are associated with nuclear lamina; whereas fLADs display the histone mark H3K27me3, and their association with nuclear lamina is facultative. Transcriptional activity in LADs is usually repressed. In addition, cLADs make stable contact with the nuclear lamina, whereas, fLADs have variable contacts and this can result in a cell-type-specific manner of gene activation (Rullens & Kind, 2021). LADs can also contain TADs (because of TADs' interaction, as previously reported, with the nuclear lamina), which can be also found at the level of the nuclear pore (Gonzalez-Sandoval & Gasser, 2016). Importantly, alterations in TAD-TAD interactions have been found in mouse embryonic stem cells (mESCs) triple knockout (TKO) for *LMNB1*, *LMNB2*, and *LMNA* genes, despite no actual changes in the overall TAD structure, (Kim et al., 2019).

Nuclear lamina directly interacts also with SUN (Sad1p and UNC-84 homology) domain proteins of the LINC complex (Lygerou et al., 1999; Malone et al., 1999). SUN proteins, in turn, indirectly connect nuclear lamina to the cell cytoskeleton (Haque et al., 2006), by interacting with Nesprin proteins (Zhang et al., 2001).

Lamins play a fundamental role in cell functionality, and their impairment leads to pathological states and diseases known under the name of laminopathies, among which we find HGPS (Janin et al., 2017).

HGPS and natural aging

HGPS is a genetic disease where patients develop distinct features of elderly people (bone and joint abnormalities and alopecia, for instance) and cardiac issues which lead to premature death (Romero-Bueno et al., 2019). Cells from HGPS patients accumulate a truncated form of the wild-type Lamin A, called Progerin, which is generated because of the activation of a cryptic splicing site in the Lamin A gene (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003). Normally, the precursor form of Lamin A, called Prelamin A, undergoes a round of farnesylation before reaching its final and mature form, which is devoid of the farnesyl group. Instead, Progerin is unable to be proteolytically processed, and so accumulates as a stably farnesylated protein (Capell et al., 2005). Progerin accumulates to form aggregates in both HGPS patient fibroblasts and Progerin expressing cells (Danielsson et al., 2020). It has been recently demonstrated that Progerin impairs the diffusion of NE proteins. Chang et al. showed that three NE proteins, specifically Sun2, Emerin, and miniNesprin-2G (a truncated form of Nesprin 2G retaining the functional connection with cytoskeleton) (Luxton et al., 2010; Markiewicz et al., 2006; Sakaki et al., 2001), have reduced diffusional mobility in fibroblasts from both HGPS patients and aged individuals compared to normal fibroblasts (Chang et al., 2019). They also demonstrated that Progerin expression is directly responsible for these altered mobilities. Therefore, miniNesprin-2G, Sun2, and Emerin could be trapped in Progerin aggregates causing an impaired diffusion. All this could result in a decreased or lost function of the trapped proteins.

Even though natural aging is not a pathological condition, it shares several features of HGPS. Indeed, Scaffidi and Misteli showed that skin fibroblasts from aged donors (87 years old) displayed a restricted distribution of Lamin A only around the nuclear rim and not throughout the nucleoplasm, NE aberrations, such as nuclear blebs, and lower levels of the Histone H3 tri-methylated on lysine 9 (H3K9me3), compared to the young counterpart (7 years old), all characteristics recurrent in cells from HGPS individuals (Scaffidi & Misteli, 2006). Another common feature between HGPS cells and aged-donors' cells is the reduction of Lamin B1, as shown by immunofluorescence on HGPS fibroblasts by Scaffidi and Misteli and by immunohistochemistry on keratinocytes by Dreesen and colleagues (Scaffidi & Misteli, 2006; Dreesen et al., 2013).

It has also been shown that heterochromatin characteristics change in natural as well as pathological aging conditions, not only in terms of epigenetic markers but also in terms of spatial positioning, with the disappearance from the nuclear periphery, where it is linked with lamins (Chandra et al., 2015; Romero-Bueno et al., 2019). Natural aging and HGPS, thus, highlight the importance of both nuclear lamina and chromatin epigenetic marks, and this suggests crosstalk between them to maintain the nucleus and cell functioning.

The interplay between lamins and chromatin in cell nucleus mechanics

It has been recently shown that both lamins and chromatin dictate cell nucleus stiffness. In this regard, Stephens and colleagues reported that nucleus rigidity increases upon short stretching (short deformations) of the nucleus, and that is the chromatin governing this process. On the other hand, nuclear lamins are involved in nucleus rigidity regulation upon long stretching (long deformations) of the cell nucleus (Stephens et al., 2017). More specifically, treatment of nuclei of HeLa and MEF cells with micrococcal nuclease or with the chromatin decondensation inducers valproic acid (VPA) and trichostatin A (TSA) decreased nuclear spring constant (a measure of cell nucleus rigidity) upon short deformations; on the contrary, Lamin A/C knockdown decreased nuclear spring constant upon long deformations in HeLa cells. Moreover, overexpression of Lamin A in HEK 293 cells, which express low levels of Lamin A/C, increased nuclear spring constant upon long deformations (Stephens et al., 2017). The same increase strikingly happens upon Lamin B1 depletion but only in the presence of low levels of Lamin A. It is thus clear that there exists a strict dependence of nuclear rigidity on both chromatin and nuclear lamin levels. Similarly, Bustin's laboratory showed that chromatin decompaction, obtained by overexpressing the nucleosome binding protein HMGN5, leads to nuclear blebbing and reduction of nuclear elasticity in reversible swelling experiments (Furusawa et al., 2015). Interestingly, they also observed that, although mice overexpressing HMGN5 exhibit chromatin decompaction already in cardiomyocytes of newborns, altered nuclei appear only in cardiomyocytes of adults. All these strongly suggest that heterochromatin plays a role in the maintenance of nucleus rigidity and, thus, its loss weakens the nucleus in the reaction to the contractile forces over time. Moreover, MEFs derived from Lamin A null mice and overexpressing HMGN5 undergo nuclear aberrations (blebs, reduction of nuclear elasticity) synergistically (Furusawa et al., 2015), demonstrating that both lamina and heterochromatin act in parallel to maintain the mechanical properties of the nucleus. In addition, chromatin decompaction alters nucleus stiffness upon DNA damage. In fact, dos Santos and colleagues have recently demonstrated that in HeLa cells after treatment with the DNA damage inducing-agent cisplatin, a reduction of condensed chromatin happens with a concomitant reduction in nuclear stiffness, compared to the untreated cells. The

decreased nucleus stiffness seems not to be due to alterations of the actin cytoskeleton, rather it depends on ATM (ataxia-telangiectasia-mutated) kinase (dos Santos et al., 2021). In a similar way to dos Santos and colleagues, Nava et al. recently showed a connection between decreased heterochromatin and decreased nucleus stiffness, with an impact on DNA damage signaling. Specifically, the authors found that, in EPCs (skin epidermis stem/progenitor cells) monolayers, cyclic uniaxial stretch caused a reduction in H3K9me3 levels, with parallel NE wrinkling, decreased association of Nuclear Lamin to heterochromatin and reduced nuclear stiffness (not affected by actin cytoskeleton). All these events were caused by the reduction of the H3K9me3 methyltransferase SUV39H1, whose restoration, in stretched cells, resulted in increased H3K9me3 levels and nucleus stiffness, reduced NE wrinkling, and increased DNA damage, the latter showed by γH2AX staining (Nava et al., 2020).

Stephens and colleagues' data also suggested that the epigenetic modifications, by changing chromatin compaction status, play a role in nuclear stiffness as well, with heterochromatin being important for nuclear rigidity as opposed to euchromatin. Indeed, it was later reported that an increase in euchromatin levels or a decrease in heterochromatin levels causes the formation of nuclear blebs, which, in turn, are enriched in euchromatin (Stephens et al., 2018). Specifically, treatment with either VPA and TSA, which are HDAC (Histone Deacetylase) inhibitors, or with DNZep (3—Deazaneplanocin-A), an HMT (Histone Methyl Transferase) inhibitor, decreases chromatin compaction and, consequentially, nuclear stiffness, which results in the formation of nuclear blebs (Stephens et al., 2018). In addition, the authors also reported that bleb formation occurs as fast as 2 h after TSA treatment and it does not require the completion of an entire cell division (Stephens et al., 2018). This suggests that blebbing is a direct consequence of the epigenetic changes rather than the result of acquired errors during chromosome segregation. Moreover, the authors found that treatment with Cytochalasin D, a cytoskeletal actin-depolymerizing drug, blocked bleb formation (Stephens et al., 2018), which means that this phenotype needs a working cytoskeleton. Related to this aspect, Gosh and colleagues found that in murine cardiomyocytes the overall disruption of the LINC complex significantly alters the ratio of elastic modulus of the heterochromatin and the euchromatin upon nuclear deformation (Ghosh et al., 2021). This suggests that disruption of lamina-cytoskeleton interactions isolates chromatin from the NE, thus, flattening the mechanical differences between hetero- and euchromatin. Nuclear blebs are also generated upon Lamin A double knock out or in Lamin B1 deficient cells, as reported in MEFs by Lammerding and colleagues (Lammerding et al., 2006). Interestingly, increased euchromatin levels, euchromatin enrichment in newly formed blebs, and decreased heterochromatin levels were also observed in Lamin B1 null MEFs (Stephens et al., 2018). Moreover, blebs were counteracted by the presence of methylstat, an inhibitor of histone demethylases, which increased heterochromatin levels and, in turn, nuclear rigidity (Stephens et al., 2018). Methylstat treatment also counteracted nuclear abnormalities and rescued heterochromatin in both an HGPS model of HeLa cells expressing GFP-Progerin as well as in HGPS patient cells (Stephens et al., 2018). The reduction of Lamin B1 levels has been coupled not only with increased euchromatin and nuclear blebbing but also with decreased LBR levels. This was shown upon gamma ray-induced senescence in MCF7 and U2OS cells (Lukášová et al., 2017). Moreover, in these two cell lines, the only LBR depletion caused detachment of chromatin from INM and also distention of centromeres, both observed by ImmunoFISH.

Epigenetic modifications and nuclear lamina crosstalk in HGPS and natural aging

It is clear that NE aberrations are both caused by hetero-/euchromatin status alterations and loss of Lamins. This strongly suggests a close relationship between epigenetic modifications governing chromatin status and nuclear lamina. The existence of such a link has been recently demonstrated by ATAC-seq (Assays for Transposase-Accessible Chromatin) in dermal fibroblasts from HGPS patients where Lamin A-associated LADs showed both a substantially altered chromatin accessibility and DNA methylation (Köhler et al., 2020). A similar alteration in LADs was described in WI-38hTERT/GFP-RAF1-ER cells as well, upon their induction to senescence (Chandra et al., 2015), which contributes to aging and age-related pathologies (Campisi, 2013). During senescence, cells most frequently undergo the formation of DAPI-dense regions within the nucleus defined as Senescence-Associated Heterochromatic Foci (SAHFs). These SAHFs are the result of alterations in the nuclear architecture of heterochromatin that is physically reoriented from the periphery to several distinct internal areas (Chandra et al., 2012; Narita et al., 2003). SAHFs are indeed made up of a central core of highly condensed chromatin, enriched with H3K9me3, HP1, and the histone H2A variant macroH2A, and by a surrounding ring of facultative heterochromatin marked by H3K27me3 (Chandra & Narita, 2013; Paluvai et al., 2020). Chandra et al. showed that in WI-38hTERT/GFP-RAF1-ER cells the formation of SAHFs is correlated with a dramatic reduction of interactions between nuclear lamina and LADs which, once detached from the lamina, move towards the nuclear

interior probably due to the decrease in Lamin B1 typical of cell senescence (Chandra et al., 2015). Interestingly, an overall repositioning of the heterochromatin from the nuclear periphery to the nuclear interior is considered a necessary first step toward the formation of SAHFs during both replicative and induced senescence (Barra et al., 2022; Chandra et al., 2015; Kosar et al., 2011). Lamin B1 has been observed to be also reduced in aged-donor cells (Dreesen et al., 2013) and Ras-Induced Senescent IMR90 cells (Sadaie et al., 2013) creating a combination of features resembling those of HGPS cells (reduction of Lamin B1 and the INM Lap2 proteins, and nuclear blebbing). Ras-induced senescent cells also showed a reduction in both H3K9me3 foci at the level of the NE as well as the thickness of the perinuclear electron-dense heterochromatin. All of this would generate an environment prone to spatial redistribution of heterochromatin and SAHFs formation (Sadaie et al., 2013). In addition, it has been observed that during replicative senescence DNA methylation undergoes substantial changes. Genomic regions enriched by H3K27me3 are hypermethylated with a differential gene expression outcome of both up and downregulation. Instead, regions enriched by H3K9me3 and LADs are hypomethylated (Hänzelmann et al., 2015). This could suggest a role for DNA hypomethylation in the weakening of the association of heterochromatin to nuclear lamina which then eventually results in the movement of these regions (still with the H3K9me3 histone mark) to the interior of the nucleus forming the SAHFs.

How epigenetic marks and lamina interact has been under investigation. Recently, Lamin A was found in close vicinity by proximity ligation assay with SUZ12, a protein of the Polycomb Repressive Complex 2 (PRC2) that methylates lysine 9 and lysine 27 on histone H3. Interestingly, this interaction is significantly reduced in the inducible cellular model of HGPS in HeLa cells (Lionetti et al., 2020). However, in mESCs, the triple knockout of DNMTs surprisingly induced a strong reduction of components of the nuclear lamina at pericentromeric heterochromatin, mainly Lamin B1 and B2 and LBR, despite their unchanged global levels (Saksouk et al., 2014). Intriguingly, this phenotype was not generated in the SUV39H1/2 double knockout suggesting a specific role of DNA methylation in recruiting pericentromeric heterochromatin to the nuclear lamina.

In this regard, it has been shown that HP1 can directly bind LBR (Ye et al., 1997) feeding the thought that it could be the link between nuclear lamina and heterochromatin in the context of nuclear mechanics. However, recent discoveries in human U2OS cells where HP1α could be rapidly degraded through an auxin-inducible degron demolished this thought at least partially. The obtained results showed that HP1α absence made chromatin softer, decreased the short-extension rigidity of the nucleus (45% reduction of spring constant), and affected nuclear shape. Unexpectedly, this phenotype was not associated with a decrease in nuclear lamina components or H3K9me3. Instead, the authors showed that the alterations of nuclear mechanics could be rescued by histone hypermethylation and are mainly due to the ability of HP1α to crosslink chromatin fibers (Strom et al., 2021). Altogether these results demonstrated that HP1's role in nuclear mechanics is in fact independent from heterochromatin and nuclear lamina.

A link between the nuclear lamina and DNA methylation could be through MeCP2. Indeed, an interaction between LBR and MeCP2, which, as discussed above, binds and regulates methylated DNA, was shown both in vivo (HeLa cells) and in vitro (pull-down assay) (Guarda et al., 2009). Some studies also revealed that MeCP2 Rett mutations or MeCP2 knockout impacted nuclear mechanics in terms of increased nuclear size (Ito-Ishida et al., 2020), smaller nucleoli (Singleton et al., 2011), the altered shape of heterochromatin foci (Ito-Ishida et al., 2020), and increased nucleosome density and Lamin B1 enrichment at CA microsatellites within LADs in MEFs (Ibrahim et al., 2021). It is also worth mentioning that both Rett patient-derived neurons and neural stem cells from a mouse model of Rett syndrome underwent senescence accompanied by DNA damage and p53 activation (Alessio et al., 2018; Ohashi et al., 2018). Senescence triggered by impaired MeCP2 has been hence suggested as the reason why Rett patients show progressive neurological regression after birth, however, the exact mechanism is not clear yet. Moreover, in PC-3 (human prostate cancer cells) and NIH-3T3 (mouse embryonic fibroblasts) cells, MeCP2 depletion caused progeria-like nuclear aberrations with reduction of Lamin A/C, Lamin B1, and LBR (Babbio et al., 2012). Interestingly, MeCP2 depletion caused also the formation of SAHFs, reinforcing the possibility of a correlation between NE and heterochromatin, namely methylated DNA, through MeCP2. It can be thus speculated that MeCP2 depletion can have an impact on nuclear stiffness by interfering with both nuclear lamina and chromatin status – by altering DNA methylation - , and, more broadly, on nuclear mechanotransduction. From this perspective, it would be interesting to evaluate, then, MeCP2 in HGPS patients' cells or nuclear lamina in Rett patients. We do know, however, that HGPS cells have an altered DNA methylation pattern on CpG dinucleotides (in terms of both hypo- and hyper-methylation) (Heyn et al., 2013; Köhler et al., 2020), which we can only hypothesize to be due to impairment of MeCP2. In addition, alterations of MeCP2 could be the consequence of altered LBR in HGPS. Therefore, MeCP2 could work as a "platform" of mechanotransduction in order to regulate NE dynamics, whose alterations could lead not only to HGPS but could also be part of normal aging.

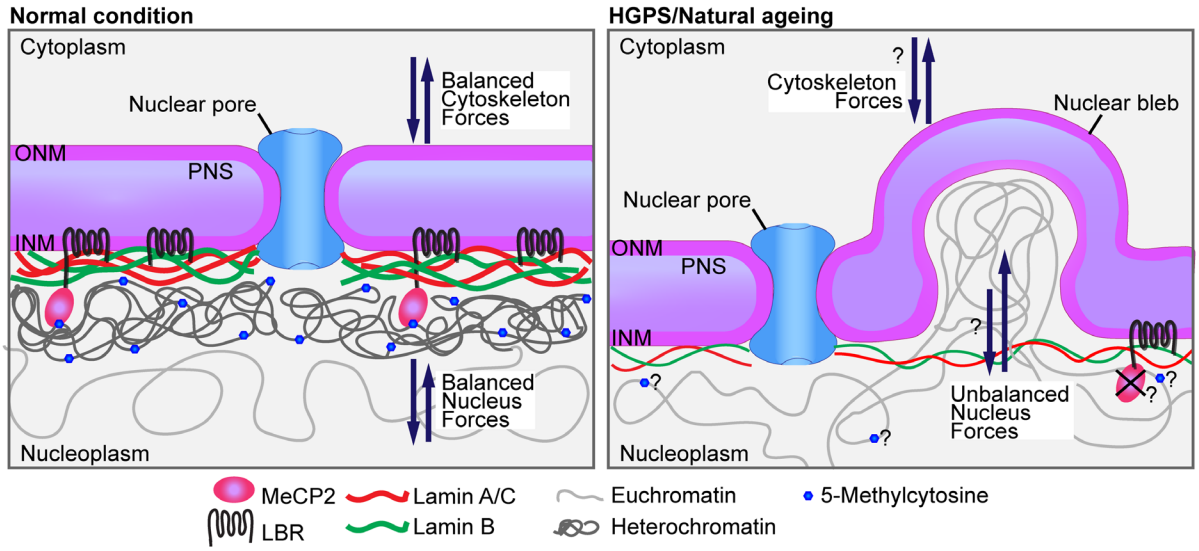

FIGURE 1 Nuclear lamina and chromatin crosstalk in normal condition and HGPS/natural aging. In normal condition, Lamin A/C and B together with heterochromatin of the nuclear periphery maintain nuclear stiffness while balancing inward/outward mechanical forces. LBR binds MeCP2 which, in turn, hooks the heterochromatin to the nuclear envelope (NE) by interacting with 5-methylcytosines. In HGPS and natural aging lamins and LBR are reduced, heterochromatin disappears from the nuclear periphery, resulting in unbalanced mechanical forces that induce nuclear blebbing. Euchromatin can enrich nuclear blebs. MeCP2 levels and localization at the NE could be affected by LBR reduction impacting on DNA methylation and thus on heterochromatin.

CONCLUSIONS AND FUTURE PERSPECTIVES

Here we reviewed some of the scientific literature with the focus on how differences in chromatin epigenetic markers as well as NE protein composition could influence both chromatin dynamics and NE mechanics. It can be easily found that both HGPS and natural aging are somehow correlated since the same epigenetic (reduction of H3K9me3 and rearrangement of cytosine methylation in CpG dinucleotides) and NE features (reduction in Lamin B1, bleb formation) characterize both the accelerated aging and the natural one. Thus, it can be speculated that both HGPS and natural aging undergo the same epigenetic variations which result in aberrant nuclear morphology acquisition which might alter nuclear mechanics and, consequentially, cellular functions. Indeed, an imbalance between hetero- and euchromatin may provoke the formation of blebbed/misshapen nuclei, with a consequent negative impact on cytoskeletal organization and force generation/sensing. Thus, by restoring this hetero-/euchromatin imbalance, there could be a way to mitigate progeroid and natural aging phenotypes. Also, it would be of great interest to investigate how the crosstalk between epigenetic marks and nuclear lamina is exerted. In this regard, we propose MeCP2 as a link between the nuclear lamina and DNA methylation (Figure 1). In a normal condition, MeCP2 would hook methylated heterochromatin to the NE by binding both LBR and 5-methylcytosine. In HGPS and natural aging, the alterations of nuclear lamina could affect MeCP2 binding to the NE and, consequently, the hooking of methylated heterochromatin, inducing the observed delocalization and eventually loss of heterochromatin. In natural aging, this scenario could also be inverted. In this case, DNA methylation loss at repetitive sequences that is typical of aging (Pappalardo & Barra, 2021), could make MeCP2 binding to NE unnecessary, and MeCP2 reduction could, in turn, induce the decrease of LBR and lamins. Research in these directions could help to find new strategies to tune and mitigate the effects of both lamina alterations and epigenetic modifications on nuclear mechanics.

AUTHOR CONTRIBUTIONS

Pietro Salvatore Carollo performed the literature research and wrote the first draft of the manuscript. Viviana Barra conceived the manuscript, commented, and critically revised the manuscript. All the authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors have no relevant financial or non-financial interests to disclose.

DATA AVAILABILITY STATEMENT

Not applicable.

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REFERENCES

Agarwal, N., Hardt, T., Brero, A., Nowak, D., Rothbauer, U., Becker, A., Leonhardt, H. & Cardoso, M.C (2007) MeCP2 interacts with HP1 and modulates its heterochromatin association during myogenic differentiation. *Nucleic Acids Resarch*, 35, 5402–5408. https://doi.org/10.1093/nar/gkm599

Aiello, NM., Maddipati, R., Norgard, RJ., Balli, D., Li, J., Yuan, S., Yamazoe, T., Black, T., Sahmoud, A., Furth, EE., Bar-Sagi, D. & Stanger, BZ. (2018) EMT subtype influences epithelial plasticity and mode of cell migration. *Developmental Cell*, 45, 681-695.e4.e4. https://doi.org/10.1016/j.devcel.2018.05.027

Alessio, N., Riccitiello, F., Squillaro, T., Capasso, S., Del Gaudio, S., Di Bernardo, G., Cipollaro, M., Melone, MA.B., Peluso, G. & Galderisi, U. (2018) Neural stem cells from a mouse model of Rett syndrome are prone to senescence, show reduced capacity to cope with genotoxic stress, and are impaired in the differentiation process. *Experimental & Molecular Medicine*, 50, 1–9. https://doi.org/10.1038/s12276-017-0005-x

Babbio, F., Castiglioni, I., Cassina, C., Gariboldi, M.B., Pistore, C., Magnani, E., Badaracco, G., Monti, E. & Bonapace, I.M. (2012) Knock-down of methyl CpG-binding protein 2 (MeCP2) causes alterations in cell proliferation and nuclear lamins expression in mammalian cells. *Bmc Cell Biology [Electronic Resource]*, 13, 19. https://doi.org/10.1186/1471-2121-13-19

Bannister, A.J. & Kouzarides, T. (2011) Regulation of chromatin by histone modifications. *Cell Research*, 21, 381–395. https://doi.org/10.1038/cr.2011.22

Barra, V., Chiavetta, R.F., Titoli, S., Provenzano, I.M., Carollo, P.S. & Di Leonardo, A. (2022) Specific irreversible cell-cycle arrest and depletion of cancer cells obtained by combining curcumin and the flavonoids quercetin and fisetin. *Genes*, 13, 1125. https://doi.org/10.3390/genes13071125

Barra, V. & Fachinetti, D. (2018) The dark side of centromeres: types, causes, and consequences of structural abnormalities implicating centromeric DNA. *Nature Communication*, 9, 4340. https://doi.org/10.1038/s41467-018-06545-y

Barra, V., Schillaci, T., Lentini, L., Costa, G. & Di Leonardo, A. (2012) Bypass of cell cycle arrest induced by transient DNMT1 post-transcriptional silencing triggers aneuploidy in human cells. *Cell Div*, 7, 2. https://doi.org/10.1186/1747-1028-7-2

Bednar, J., Horowitz, RA., Grigoryev, SA., Carruthers, LM., Hansen, JC., Koster, AJ. & Woodcock, CL. (1998). Nucleosomes, linker DNA, and linker histone form a unique structural motif that directs the higher-order folding and compaction of chromatin. *Pnas*, 95, 14173–14178. https://doi.org/10.1073/pnas.95.24.14173

Bernstein, BE., Meissner, A. & Lander, ES. (2007) The mammalian epigenome. *Cell*, 128, 669–681. https://doi.org/10.1016/j.cell.2007.01.033

Bintu, B., Mateo, LJ., Su, J.H., Sinnott-Armstrong, NA., Parker, M., Kinrot, S., Yamaya, K., Boettiger, AN. & Zhuang, X. (2018) Super-resolution chromatin tracing reveals domains and cooperative interactions in single cells. *Science*, 362, eaau1783. https://doi.org/10.1126/science.aau1783

Campisi, J. (2013). Aging, cellular senescence, and cancer. *Annual Review of Physiology*, 75, 685–705. https://doi.org/10.1146/annurev-physiol-030212-183653

Canever, H., Carollo, P.S., Fleurisson, R., Girard, P.P. & Borghi, N. (2021) Molecular tension microscopy of e-cadherin during epithelial-mesenchymal transition. In: Campbell, K. & Theveneau, E. (Eds.) *The epithelial-to mesenchymal transition: methods and protocols*. Springer US: New York, NY, pp 289–299

Capell, BC., Erdos, MR., Madigan, JP., Fiordalisi, JJ., Varga, R., Conneely, KN., Gordon, LB., Der, CJ., Cox, AD. & Collins, FS. (2005) Inhibiting farnesylation of progerin prevents the characteristic nuclear blebbing of Hutchinson-Gilford Progeria Syndrome. *Pnas*, 102, 12879–12884. https://doi.org/10.1073/pnas.0506001102

Chandra, T., Ewels, P.A., Schoenfelder, S., Furlan-Magaril, M., Wingett, S.W., Kirschner, K., Thuret, J.Y., Andrews, S., Fraser, P. & Reik, W. (2015) Global reorganization of the nuclear landscape in senescent cells. *Cell reports*, 10, 471–483. https://doi.org/10.1016/j.celrep.2014.12.055

Chandra, T., Kirschner, K., Thuret, J.Y., Pope, BD., Ryba, T., Newman, S., Ahmed, K., Samarajiwa, SA., Salama, R., Carroll, T., Stark, R., Janky, R's, Narita, M., Xue, L., Chicas, A., Nũnez, S., Janknecht, R., Hayashi-Takanaka, Y., Wilson, MD., Marshall, A., Odom, DT., Babu, M.M, Bazett-Jones, D.P., Tavaré, S., Edwards, P.A.W., Lowe, SW., Kimura, H., Gilbert, DM. & Narita, M. (2012) Independence of repressive histone marks and chromatin compaction during senescent heterochromatic layer formation. *Molecular Cell*, 47, 203–214. https://doi.org/10.1016/j.molcel.2012.06.010

Chandra, T. & Narita, M. (2013) High-order chromatin structure and the epigenome in SAHFs. *Nucleus*, 4, 23–28. https://doi.org/10.4161/nucl.23189

Chang, W., Wang, Y., Luxton, G., Östlund, C., Worman, H.J. & Gundersen, G.G. (2019) Imbalanced nucleocytoskeletal connections create common polarity defects in progeria and physiological aging. *Pnas*, 116, 3578–3583. https://doi.org/10.1073/pnas.1809683116

Cilluffo, D., Barra, V., Spatafora, S., Coronnello, C., Contino, F., Bivona, S., Feo, S. & Di Leonardo, A. (2020) Aneuploid IMR90 cells induced by depletion of pRB, DNMT1 and MAD2 show a common gene expression signature. *Genomics*, 112, 2541–2549. https://doi.org/10.1016/j.ygeno.2020.02.006

Costa, G., Barra, V., Lentini, L., Cilluffo, D. & Di Leonardo, A. (2016) DNA demethylation caused by 5-Aza-2'-deoxycytidine induces mitotic alterations and aneuploidy. *Oncotarget*, 7, 3726–3739. https://doi.org/10.18632/oncotarget.6897

Cremer, T. & Cremer, M. (2010) Chromosome territories. *Cold Spring Harbor Perspectives in Biology*, 2, a003889. https://doi.org/10.1101/cshperspect.a003889

Crisp, M., Liu, Q., Roux, K., Rattner, J.B., Shanahan, C., Burke, B., Stahl, PD. & Hodzic, D. (2006) Coupling of the nucleus and cytoplasm: role of the LINC complex. *Journal of Cell Biology*, 172, 41–53. https://doi.org/10.1083/jcb.200509124

Croft, JA., Bridger, JM., Boyle, S., Perry, P., Teague, P. & Bickmore, WA. (1999) Differences in the localization and morphology of chromosomes in the human nucleus. *Journal of Cell Biology*, 145, 1119–1131. https://doi.org/10.1083/jcb.145.6.1119

Danielsson, BE., Tieu, KV., Bathula, K., Armiger, TJ., Vellala, PS., Taylor, RE., Dahl, K.N. & Conway, DE. (2020) Lamin microaggregates lead to altered mechanotransmission in progerin-expressing cells. *Nucleus*, 11, 194–204. https://doi.org/10.1080/19491034.2020.1802906

De Sandre-Giovannoli, A., Bernard, R.L., Cau, P., Navarro, C., Amiel, J., Boccaccio, I.N., Lyonnet, S., Stewart, CL., Munnich, A., Le Merrer, M. & Levy, N., (2003) Lamin a truncation in Hutchinson-Gilford

progeria. *Lamin A Truncation in Hutchinson-Gilford Progeria. Science*, 300, 2055–2055. https://doi.org/10.1126/science.1084125

Déjardin, T., Carollo, P.S., Sipieter, F., Davidson, P.M., Seiler, C., Cuvelier, D., Cadot, B., Sykes, C., Gomes, E.R. & Borghi, N. (2020) Nesprins are mechanotransducers that discriminate epithelial-mesenchymal transition programs. *Journal of Cell Biology*, 219, e201908036. https://doi.org/10.1083/jcb.201908036

Dekker, J. (2008) Mapping in vivo chromatin interactions in yeast suggests an extended chromatin fiber with regional variation in compaction. *Journal of Biological Chemistry* 283, 34532–34540. https://doi.org/10.1074/jbc.M806479200

Dos Santos, Á., Cook, A.W., Gough, R.E., Schilling, M., Olszok, N.A., Brown, I., Wang, L., Aaron, J., Martin-Fernandez, M.L., Rehfeldt, F. & Toseland, C.P. (2021) DNA damage alters nuclear mechanics through chromatin reorganization. *Nucleic Acids Res*., 49, 340–353. https://doi.org/10.1093/nar/gkaa1202

Dreesen, O., Chojnowski, A., Ong, P.F., Zhao, T.Y., Common, JE., Lunny, D., Lane, E.B, Lee, S.J., Vardy, LA., Stewart, CL. & Colman, A. (2013). Lamin B1 fluctuations have differential effects on cellular proliferation and senescence. *Journal of Cell Biology*, 200, 605–617. https://doi.org/10.1083/jcb.201206121

Eissenberg, JC. & Elgin, SC.R. (2014) HP1a: a structural chromosomal protein regulating transcription. *Trends Genet TIG*, 30, 103–110. https://doi.org/10.1016/j.tig.2014.01.002

Engler, AJ., Sen, S., Sweeney, H.L & Discher, DE. (2006) Matrix elasticity directs stem cell lineage specification. *Cell*, 126, 677–689. https://doi.org/10.1016/j.cell.2006.06.044

Eriksson, M., Brown, W.T, Gordon, LB., Glynn, MW., Singer, J., Scott, L., Erdos, MR., Robbins, CM., Moses, TY., Berglund, P., Dutra, A., Pak, E., Durkin, S., Csoka, AB., Boehnke, M., Glover, TW. & Collins, FS. (2003) Recurrent de novo point mutations in lamin A cause Hutchinson–Gilford progeria syndrome. *Nature*, 423, 293–298. https://doi.org/10.1038/nature01629

Farge, E. (2003) Mechanical induction of twist in the drosophila foregut/stomodeal primordium. *Curr Biol CB*, 13, 1365–1377. https://doi.org/10.1016/s0960-9822(03)00576-1

Fisher, D.Z., Chaudhary, N. & Blobel, G. (1986) cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. *Pnas*, 83, 6450–6454. https://doi.org/10.1073/pnas.83.17.6450

Furusawa, T., Rochman, M., Taher, L., Dimitriadis, EK., Nagashima, K., Anderson, S. & Bustin, M. (2015) Chromatin decompaction by the nucleosomal binding protein HMGN5 impairs nuclear sturdiness. *Nature Communication*, 6, 6138. https://doi.org/10.1038/ncomms7138

Ghosh, S., Cuevas, V.C., Seelbinder, B. & Neu, CP. (2021) Image-based elastography of heterochromatin and euchromatin domains in the deforming cell nucleus. *Small Weinh Bergstr Ger*, 17, 2006109. https://doi.org/10.1002/smll.202006109

Gilbert, N. (2019). Biophysical regulation of local chromatin structure. *Current opinion in Genetics & Development*, 55, 66–75. https://doi.org/10.1016/j.gde.2019.06.001

Gonzalez-Sandoval, A. & Gasser, SM. (2016) On TADs and LADs: spatial control over gene expression. *Trends Genet TIG*, 32, 485–495. https://doi.org/10.1016/j.tig.2016.05.004

Good, KV., Vincent, JB. & Ausió, J. (2021) MeCP2: the genetic driver of Rett syndrome epigenetics. *Front Genet*, 12, 620859.

Greil, F., Moorman, C. & van Steensel, B. (2006) DamID: mapping of in vivo protein-genome interactions using tethered DNA adenine methyltransferase. *Methods in Enzymology*, 410, 342–359. https://doi.org/10.1016/S0076-6879(06)10016-6

Guarda, A., Bolognese, F., Bonapace, I.M. & Badaracco, G. (2009) Interaction between the inner nuclear membrane lamin B receptor and the heterochromatic methyl binding protein, *MeCP2. Exp Cell Res*, 315, 1895–1903. https://doi.org/10.1016/j.yexcr.2009.01.019

Guelen, L., Pagie, L., Brasset, E., Meuleman, W., Faza, MB., Talhout, W., Eussen, BH., De Klein, A., Wessels, L., De Laat, W. & Van Steensel, B. (2008) Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. *Nature*, 453, 948–951. https://doi.org/10.1038/nature06947

Hänzelmann, S., Beier, F., Gusmao, E.G., Koch, C.M., Hummel, S., Charapitsa, I., Joussen, S., Benes, V., Brümmendorf, T.H., Reid, G., Costa, I.G. & Wagner, W. (2015) Replicative senescence is associated with nuclear reorganization and with DNA methylation at specific transcription factor binding sites. *Clin Epigenetics*, 7, 19. https://doi.org/10.1186/s13148-015-0057-5

Haque, F., Lloyd, DJ., Smallwood, DT., Dent, CL., Shanahan, CM., Fry, AM., Trembath, RC. & Shackleton, S. (2006) SUN1 Interacts with nuclear lamin A and cytoplasmic Nesprins to provide a physical connection between the nuclear lamina and the cytoskeleton. *Molecular and Cellular Biology*, 26, 3738–3751. https://doi.org/10.1128/MCB.26.10.3738-3751.2006

Hauer, M.H., Seeber, A., Singh, V., Thierry, R., Sack, R., Amitai, A., Kryzhanovska, M., Eglinger, J., Holcman, D., Owen-Hughes, T. & Gasser, S.M. (2017) Histone degradation in response to DNA damage enhances chromatin dynamics and recombination rates. *Nature structural & Molecular Biology*, 24, 99–107. https://doi.org/10.1038/nsmb.3347

Heyn, H., Moran, S. & Esteller, M. (2013) Aberrant DNA methylation profiles in the premature aging disorders Hutchinson-Gilford Progeria and Werner syndrome. *Epigenetics*, 8, 28–33. https://doi.org/10.4161/epi.23366

Ibrahim, A., Papin, C., Mohideen-Abdul, K., Le Gras, S., Stoll, I., Bronner, C., Dimitrov, S., Klaholz, B.P. & Hamiche, A. (2021) MeCP2 is a microsatellite binding protein that protects CA repeats from nucleosome invasion. *Science*, 372, eabd5581. https://doi.org/10.1126/science.abd5581

Iglesias, N. & Moazed, D. (2017) Silencing repetitive DNA. *eLife*, 6, e29503. https://doi.org/10.7554/eLife.29503

Ingber, DE. (2006) Cellular mechanotransduction: putting all the pieces together again. *Faseb Journal*, 20, 811–827. https://doi.org/10.1096/fj.05-5424rev

Isermann, P. & Lammerding, J. (2013) Nuclear mechanics and mechanotransduction in health and disease. *Current Biology*, 23, R1113–R1121. https://doi.org/10.1016/j.cub.2013.11.009

Ito-Ishida, A., Baker, SA., Sillitoe, RV., Sun, Y., Zhou, J., Ono, Y., Iwakiri, J., Yuzaki, M. & Zoghbi, HY. (2020) MeCP2 levels regulate the 3D structure of heterochromatic foci in mouse neurons. *Journal of Neuroscience*, 40, 746–8766. https://doi.org/10.1523/JNEUROSCI.1281-19.2020

Janin, A., Bauer, D., Ratti, F., Millat, G. & Méjat, A. (2017) Nuclear envelopathies: a complex LINC between nuclear envelope and pathology. *Orphanet journal of rare diseases*, 12, 147. https://doi.org/10.1186/s13023-017-0698-x

Janin, A. & Gache, V. (2018) Nesprins and lamins in health and diseases of cardiac and skeletal muscles. *Front Physiol*, 9, 1277.

Janssen, A., Colmenares, SU. & Karpen, GH. (2018) Heterochromatin: guardian of the genome. *Annual Review of Cell and Developmental Biology*, 34, 265–288. https://doi.org/10.1146/annurev-cellbio-100617-062653

Jin, Z. & Liu, Y. (2018) DNA methylation in human diseases. *Genes Dis*, 5, 1–8. https://doi.org/10.1016/j.gendis.2018.01.002

Kalverda, B., Röling, MD. & Fornerod, M. (2008) Chromatin organization in relation to the nuclear periphery. *Febs Letters*, 582, 2017–2022. https://doi.org/10.1016/j.febslet.2008.04.015

Karpf, AR. & Matsui, S.I. (2005) Genetic Disruption of cytosine DNA methyltransferase enzymes induces chromosomal instability in human cancer cells. *Cancer Research*, 65, 8635–8639. https://doi.org/10.1158/0008-5472.CAN-05-1961

Kim, Y., Zheng, X. & Zheng, Y. (2019) Role of lamins in 3D genome organization and global gene expression. *Nucl Austin Tex*, 10, 33–41. https://doi.org/10.1080/19491034.2019.1578601

Kimura, H. & Shiota, K. (2003) Methyl-CpG-binding protein, MeCP2, is a target molecule for maintenance DNA methyltransferase, Dnmt1. *Journal of Biological Chemistry*, 278, 4806–4812. https://doi.org/10.1074/jbc.M209923200

Köhler, F., Bormann, F., Raddatz, G, Gutekunst, J., Corless, S., Musch, T., Lonsdorf, AS., Erhardt, S., Lyko, F. & Rodrã-Guez-Paredes, M. (2020) Epigenetic deregulation of lamina-associated domains in Hutchinson-Gilford Progeria Syndrome. *Genome Med*, 12, 46. https://doi.org/10.1186/s13073-020-00749-y

Kosar, M., Bartkova, J., Hubackova, S., Hodny, Z., Lukas, J. & Bartek, J. (2011) Senescence-associated heterochromatin foci are dispensable for cellular senescence, occur in a cell type- and insult-dependent manner and follow expression of p16ink4a. *Cell Cycle*, 10, 457–468. https://doi.org/10.4161/cc.10.3.14707

Krause, M., Te Riet, J. & Wolf, K. (2013) Probing the compressibility of tumor cell nuclei by combined atomic force–confocal microscopy. *Physical Biology*, 10, 065002. https://doi.org/10.1088/1478-3975/10/6/065002

Lammerding, J., Fong, LG., Ji, JY., Reue, K., Stewart, CL., Young, SG. & Lee, RT. (2006) Lamins A and C but not lamin B1 regulate nuclear mechanics*. *Journal of Biological Chemistry*, 281, 25768–25780. https://doi.org/10.1074/jbc.M513511200

Lee, JuY, Lee, J., Yue, H. & Lee, T.H. (2015) Dynamics of nucleosome assembly and effects of DNA methylation. *Journal of Biological Chemistry*, 290, 4291–4303. https://doi.org/10.1074/jbc.M114.619213

Lieberman-Aiden, E., Van Berkum, NL., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, BR., Sabo, PJ., Dorschner, MO., Sandstrom, R., Bernstein, B., Bender, M.A., Groudine, M., Gnirke, A., Stamatoyannopoulos, J., Mirny, LA., Lander, ES. & Dekker, J. (2009) Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326:289–293. https://doi.org/10.1126/science.1181369

Lin, F. & Worman, H.J. (1993) Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. *Journal of Biological Chemistry*, 268, 16321–16326. https://doi.org/10.1016/S0021-9258(19)85424-8

Lionetti, M.C., Bonfanti, S., Fumagalli, M.R., Budrikis, Z., Font-Clos, F., Costantini, G., Chepizhko, O., Zapperi, S. & La Porta, C.A.M. (2020) Chromatin and cytoskeletal tethering determine nuclear morphology in progerin-expressing cells. *Biophysical Journal*, 118, 2319–2332. https://doi.org/10.1016/j.bpj.2020.04.001

Luger, K., Mäder, AW., Richmond, RK., Sargent, DF. & Richmond, TJ. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*, 389, 251–260. https://doi.org/10.1038/38444

Lukášová, E., Kovarˇík, A., Bacˇíková, A., Falk, M. & Kozubek, S. (2017) Loss of lamin B receptor is necessary to induce cellular senescence. *Biochemical Journal*, 474, 281–300. https://doi.org/10.1042/BCJ20160459

Luxton, G.W.G, Gomes, ER., Folker, ES. & Vintinner, E., Gundersen, GG. (2010) Linear arrays of nuclear envelope proteins harness retrograde actin flow for nuclear movement. *Science*, 329, 956–959. https://doi.org/10.1126/science.1189072

Lygerou, Z., Christophides, G. & Seraphin, B. (1999) A novel genetic screen for snRNP assembly factors in yeast identifies a conserved protein, Sad1p, also required for Pre-mRNA splicing. *Molecular and Cellular Biology*, 19, 2008–2020. https://doi.org/10.1128/MCB.19.3.2008

Maeshima, K., Iida, S. & Tamura, S. (2021) Physical nature of chromatin in the nucleus. *Cold Spring Harbor perspectives in biology*, 13, a040675. https://doi.org/10.1101/cshperspect.a040675

Makatsori, D., Kourmouli, N., Polioudaki, H., Shultz, LD., Mclean, K., Theodoropoulos, PA., Singh, PB. & Georgatos, SD. (2004) The inner nuclear membrane protein lamin B receptor forms distinct microdomains and links epigenetically marked chromatin to the nuclear envelope*. *Journal of Biological Chemistry*, 279, 25567–25573. https://doi.org/10.1074/jbc.M313606200

Malone, C.J., Fixsen, W.D., Horvitz, H.R. & Han, M. (1999) UNC-84 localizes to the nuclear envelope and is required for nuclear migration and anchoring during C. elegans development. *Development (Cambridge, England)*, 126, 3171–3181. https://doi.org/10.1242/dev.126.14.3171

Maniotis, AJ., Chen, CS. & Ingber, DE. (1997) Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Pnas*, 94, 849–854. https://doi.org/10.1073/pnas.94.3.849

Markiewicz, E., Tilgner, K., Barker, N., Van De Wetering, M., Clevers, H., Dorobek, M., Hausmanowa-Petrusewicz, I., Ramaekers, F.C.S., Broers, J.L.V., Blankesteijn, W.M., Salpingidou, G., Wilson, R.G., Ellis, J.A. & Hutchison, C.J. (2006) The inner nuclear membrane protein emerin regulates beta-catenin activity by restricting its accumulation in the nucleus. *Embo Journal*, 25, 3275–3285. https://doi.org/10.1038/sj.emboj.7601230

Matsuda, A. & Mofrad, MR.K. (2022) On the nuclear pore complex and its emerging role in cellular mechanotransduction. *APL Bioeng*, 6, 011504. https://doi.org/10.1063/5.0080480

Maurer, M. & Lammerding, J. (2019) The driving force: nuclear mechanotransduction in cellular function, fate, and disease. *Annual Review of Biomedical Engineering*, 21, 443–468. https://doi.org/10.1146/annurev-bioeng-060418-052139

Mazzio, EA. & Soliman, K.F.A. (2012) Basic concepts of epigenetics: impact of environmental signals on gene expression. *Epigenetics*, 7, 119–130. https://doi.org/10.4161/epi.7.2.18764

Mckeon, FD., Kirschner, MW. & Caput, D. (1986) Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins. *Nature*, 319, 463–468. https://doi.org/10.1038/319463a0

Müller, J., Hart, CM., Francis, NJ., Vargas, ML., Sengupta, A., Wild, B., Miller, EL., O'connor, MB., Kingston, RE. & Simon, JA. (2002) Histone methyltransferase activity of a drosophila polycomb group repressor complex. *Cell*, 111, 197–208. https://doi.org/10.1016/S0092-8674(02)00976-5

Narita, M., Nũnez, S., Heard, E., Narita, M., Lin, AW., Hearn, SA., Spector, DL., Hannon, GJ. & Lowe, SW. (2003) Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell*, 113, 703–716. https://doi.org/10.1016/s0092-8674(03)00401-x

Nava, M.M., Miroshnikova, Y.A., Biggs, L.C., Whitefield, D.B., Metge, F., Boucas, J., Vihinen, H., Jokitalo, E., Li, X., García Arcos, J.M., Hoffmann, B., Merkel, R., Niessen, C.M., Dahl, K.N. & Wickström, S.A. (2020) Heterochromatin-driven nuclear softening protects the genome against mechanical stress-induced damage. *Cell*, 181, 800-817.e22.e22. https://doi.org/10.1016/j.cell.2020.03.052

Ohashi, M., Korsakova, E., Allen, D., Lee, P., Fu, K., Vargas, BS., Cinkornpumin, J., Salas, C., Park, JC., Germanguz, I., Langerman, J., Chronis, C., Kuoy, E., Tran, S., Xiao, X., Pellegrini, M., Plath, K. & Lowry, WE. (2018) Loss of MECP2 leads to activation of P53 and neuronal senescence. *Stem Cell Rep*, 10, 1453–1463. https://doi.org/10.1016/j.stemcr.2018.04.001

Paluvai, H., Di Giorgio, E. & Brancolini, C. (2020) The histone code of senescence. *Cells*, 9, 466. https://doi.org/10.3390/cells9020466

Pandey, S., Simmons, GE. Jr., Malyarchuk, S., Calhoun, TN. & Pruitt, K. (2015) A novel MeCP2 acetylation site regulates interaction with ATRX and HDAC1. *Genes & cancer*, 6, 408–421. https://doi.org/10.18632/genesandcancer.84

Pappalardo, X.G. & Barra, V. (2021) Losing DNA methylation at repetitive elements and breaking bad. *Epigenetics & chromatin*, 14, 25. https://doi.org/10.1186/s13072-021-00400-z

Parada, L.A., Mcqueen, P.G. & Misteli, T. (2004) Tissue-specific spatial organization of genomes. *Genome biology* 5:R44. https://doi.org/10.1186/gb-2004-5-7-r44

Peter, M., Kitten, G.T., Lehner, C.F., Vorburger, K., Bailer, S.M., Maridor, G. & Nigg, E.A. (1989) Cloning and sequencing of cDNA clones encoding chicken lamins A and B1 and comparison of the primary structures of vertebrate A- and B-type lamins. *Journal of Molecular Biology*, 208, 393–404. https://doi.org/10.1016/0022-2836(89)90504-4

Rae, PM.M. & Franke, WW. (1972) The interphase distribution of satellite DNA-containing heterochromatin in mouse nuclei. *Chromosoma*, 39, 443–456. https://doi.org/10.1007/BF00326177

Rao, SS.P., Huang, Su-C, Glenn St Hilaire, B., Engreitz, JM., Perez, EM., Kieffer-Kwon, K.R., Sanborn, AL., Johnstone, SE., Bascom, GD., Bochkov, ID., Huang, X., Shamim, MS., Shin, J., Turner, D., Ye, Z., Omer, AD., Robinson, JT., Schlick, T., Bernstein, BE., Casellas, R., Lander, ES. & Aiden, E.L. (2017) Cohesin loss eliminates all loop domains. *Cell*, 171, 305-320.e24.e24. https://doi.org/10.1016/j.cell.2017.09.026

Rea, S., Eisenhaber, F., O'carroll, D., Strahl, BD., Sun, Zu-W, Schmid, M., Opravil, S., Mechtler, K., Ponting, CP., Allis, C.D & Jenuwein, T. (2000) Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature*, 406, 593–599. https://doi.org/10.1038/35020506

Romero-Bueno, R., de la Cruz Ruiz, P., Artal-Sanz, M., Askjaer, P. & Dobrzynska, A. (2019) Nuclear organization in stress and aging. *Nuclear Organization in Stress and Aging Cells*, 8, 664. https://doi.org/10.3390/cells8070664

Rose, NR. & Klose, RJ. (2014) Understanding the relationship between DNA methylation and histone lysine methylation. *Biochim Biophys Acta BBA - Gene Regul Mech*, 1839, 1362–1372. https://doi.org/10.1016/j.bbagrm.2014.02.007

Rullens, PM.J. & Kind, J. (2021) Attach and stretch: emerging roles for genome–lamina contacts in shaping the 3D genome. *Current Opinion in Cell Biology*, 70, 51–57. https://doi.org/10.1016/j.ceb.2020.11.006

Sadaie, M., Salama, R., Carroll, T., Tomimatsu, K., Chandra, T., Young, AR.J., Narita, M., Pérez-Mancera, PA., Bennett, DC., Chong, H., Kimura, H. & Narita, M. (2013) Redistribution of the Lamin B1 genomic binding profile affects rearrangement of heterochromatic domains and SAHF formation during senescence. *Genes & development*, 27, 1800–1808. https://doi.org/10.1101/gad.217281.113

Sakaki, M., Koike, H., Takahashi, N., Sasagawa, N., Tomioka, S., Arahata, K. & Ishiura, S. (2001) Interaction between Emerin and Nuclear Lamins1. *The Journal of Biochemistry (Tokyo)*, 129, 321–327. https://doi.org/10.1093/oxfordjournals.jbchem.a002860

Saksouk, N., Barth, T.K., Ziegler-Birling, C., Olova, N., Nowak, A., Rey, E., Mateos-Langerak, J., Urbach, S., Reik, W., Torres-Padilla, M.E., Imhof, A. & Déjardin, J.R (2014) Redundant mechanisms to form silent chromatin at pericentromeric regions rely on BEND3 and DNA methylation. *Molecular Cell*, 56, 580–594. https://doi.org/10.1016/j.molcel.2014.10.001

Saksouk, N., Simboeck, E. & Déjardin, J. (2015) Constitutive heterochromatin formation and transcription in mammals. *Epigenetics & chromatin*, 8, 3. https://doi.org/10.1186/1756-8935-8-3

Salameh, Y., Bejaoui, Y. & El Hajj, N. (2020) DNA methylation biomarkers in aging and age-related diseases. *Front Genet*, 11, 171.

Scaffidi, P. & Misteli, T. (2006) Lamin A-dependent nuclear defects in human aging. *Science*, 312, 1059–1063. https://doi.org/10.1126/science.1127168

Schotta, G., Lachner, M., Sarma, K., Ebert, A., Sengupta, R., Reuter, G., Reinberg, D. & Jenuwein, T. (2004) A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes & development*, 18, 1251–1262. https://doi.org/10.1101/gad.300704

Shaban, HA., Barth, R. & Bystricky, K. (2020) Navigating the crowd: visualizing coordination between genome dynamics, structure, and transcription. *Genome biology*, 21, 278. https://doi.org/10.1186/s13059-020-02185-y

Shimamoto, Y., Tamura, S., Masumoto, H. & Maeshima, K. (2017) Nucleosome–nucleosome interactions via histone tails and linker DNA regulate nuclear rigidity. *Molecular Biology of the Cell*, 28, 1580–1589. https://doi.org/10.1091/mbc.e16-11-0783

Singleton, MK., Gonzales, ML., Leung, KN., Yasui, DH., Schroeder, DI., Dunaway, K. & Lasalle, JM. (2011) MeCP2 is required for global heterochromatic and nucleolar changes during activity-dependent neuronal maturation. *Neurobiology of Disease*, 43, 190–200. https://doi.org/10.1016/j.nbd.2011.03.011

Stephens, AD., Banigan, EJ., Adam, SA., Goldman, RD. & Marko, JF. (2017) Chromatin and lamin A determine two different mechanical response regimes of the cell nucleus. *Molecular Biology of the Cell*, 28, 1984–1996. https://doi.org/10.1091/mbc.e16-09-0653

Stephens, AD., Liu, PZ., Banigan, EJ., Almassalha, LM., Backman, V., Adam, SA., Goldman, RD. & Marko, JF. (2018) Chromatin histone modifications and rigidity affect nuclear morphology independent of lamins. *Molecular Biology of the Cell*, 29, 220–233. https://doi.org/10.1091/mbc.E17-06-0410

Strom, A.R., Biggs, R.J., Banigan, E.J., Wang, X., Chiu, K., Herman, C., Collado, J., Yue, F., Ritland Politz, J.C., Tait, L.J., Scalzo, D., Telling, A., Groudine, M., Brangwynne, C.P., Marko, J.F. & Stephens, A.D. (2021) HP1α is a chromatin crosslinker that controls nuclear and mitotic chromosome mechanics. *eLife*, 10, e63972. https://doi.org/10.7554/eLife.63972

Vorburger, K., Lehner, C.F., Kitten, G.T., Eppenberger, H.M. & Nigg, E.A. (1989) A second higher vertebrate B-type lamin: cDNA sequence determination and in vitro processing of chicken lamin B2. *Journal of Molecular Biology*, 208, 405–415. https://doi.org/10.1016/0022-2836(89)90505-6

Wong, X., Hoskins, VE., Melendez-Perez, AJ., Harr, JC., Gordon, M. & Reddy, KL. (2021) Lamin C is required to establish genome organization after mitosis. *Genome biology*, 22, 305. https://doi.org/10.1186/s13059-021-02516-7

Worman, H.J., Yuan, J., Blobel, G. & Georgatos, S.D. (1988) A lamin B receptor in the nuclear envelope. *Pnas*, 85, 8531–8534. https://doi.org/10.1073/pnas.85.22.8531

Ye, Q., Callebaut, I., Pezhman, A., Courvalin, J.C. & Worman, HJ. (1997) Domain-specific interactions of human HP1-type chromodomain proteins and inner nuclear membrane protein LBR*. *Journal of Biological Chemistry*, 272, 14983–14989. https://doi.org/10.1074/jbc.272.23.14983

Zhang, Q., Skepper, JN., Yang, F., Davies, JD., Hegyi, L., Roberts, RG., Weissberg, PL., Ellis, JA. & Shanahan, CM. (2001) Nesprins: a novel family of spectrin-repeat-containing proteins that localize to the nuclear membrane in multiple tissues. *Journal of Cell Science*, 114, 4485–4498. https://doi.org/10.1242/jcs.114.24.4485

Zheng, H. & Xie, W. (2019) The role of 3D genome organization in development and cell differentiation. *Nature Reviews Molecular Cell Biology*, 20, 535–550. https://doi.org/10.1038/s41580-019-0132-4

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