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Review

Deciphering aging at three-dimensional genomic resolution

Zunpeng Liu [a,b,c,d,e], Juan Carlos Izpisua Belmonte [h], Weiqi Zhang [e,i,j,**], Jing Qu [b,c,d,e,***], Guang-Hui Liu [a,c,d,e,f,g,*]

[a] State Key Laboratory of Membrane Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China
[b] State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China
[c] Institute for Stem Cell and Regeneration, Chinese Academy of Sciences, Beijing, 100101, China
[d] Beijing Institute for Stem Cell and Regenerative Medicine, Beijing, 100101, China
[e] University of Chinese Academy of Sciences, Beijing, 100049, China
[f] Advanced Innovation Center for Human Brain Protection, and National Clinical Research Center for Geriatric Disorders, Xuanwu Hospital, Capital Medical University, Beijing, 100053, China
[g] Aging Translational Medicine Center, International Center for Aging and Cancer, Xuanwu Hospital, Capital Medical University, Beijing, 100053, China
[h] Gene Expression Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA
[i] CAS Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, 100101, China
[j] China National Center for Bioinformation, Beijing, 100101, China

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ABSTRACT

Aging is characterized by progressive functional declines at the organismal, organic, and cellular levels and increased susceptibility to aging-related diseases. Epigenetic alteration is a hallmark of aging, senescent cells show epigenomic changes at multiple scales, such as 3D genome reorganization, alterations of histone modifications and chromatin accessibility, and DNA hypomethylation. Chromosome conformation capture (3C)-based technologies have enabled the generation of key information on genomic reorganizations during senescence. A comprehensive understanding of epigenomic alterations during aging will yield important insights into the underlying epigenetic mechanism for aging regulation, the identification of aging-related biomarkers, and the development of potential aging intervention targets.

Recent advances in various high-throughput sequencing methodologies, such as high-throughput capture sequencing (Hi-C), DNA adenine methyltransferase identification (DamID), genome architecture mapping (GAM), and split-pool recognition of interactions by tag extension (SPRITE), and high-resolution imaging approaches, such as fluorescence *in situ* hybridization (FISH), have provided key principles regarding the 3D genomic organization (Kempfer and Pombo, 2020; Misteli, 2020; van Steensel and Belmont, 2017). It is well-established that eukaryotic genomes exhibit complex, refined, and highly dynamic hierarchical 3D organizations at multiple scales (Gibcus and Dekker, 2013). Throughout the whole genome, each interphase chromosome is organized into distinct nuclear areas termed as "chromosome territories" (Wang et al., 2021). At the megabase pair (Mbp) scale, chromosomes are partitioned into active A compartments and repressive B compartments. A compartments are actively transcribed gene-rich regions enriched for active chromatin marks, such as H3K4me3, H3K4me1, and H3K27ac, and with typically

Abbreviations: CAEA, convergent alteration of the epigenomic landscape during aging; CTCF, CCCTC-binding factor; DamID, DNA adenine methyltransferase identification; E-P loop, enhancer-promoter chromatin loop; ERVK, endogenous retrovirus-K; GAM, genome architecture mapping; HGPS, Hutchinson-Gilford progeria syndrome; hMSC, human mesenchymal stromal/stem cell; HUVEC, human umbilical vein endothelial cell; LAD, lamina-associated domain; LINE, long interspersed nuclear element; MEF, mouse embryonic fibroblast; NL, nuclear lamina; OIS, oncogene-induced senescence; PSG, pregnancy-specific beta-1 glycoprotein; RS, replicative senescence; SAE, senescence-activated enhancer; SAHD, senescence-associated heterochromatin domain; SAHF, senescence-associated heterochromatin foci; SASP, senescence-associated secretory phenotype; SINE, short interspersed nuclear element; SIPS, stress-induced premature senescence; SPRITE, split-pool recognition of interactions by tag extension; TAD, topologically associating domain; WS, Werner syndrome.

* Corresponding author. State Key Laboratory of Membrane Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China.
** Corresponding author. CAS Key Laboratory of Genomic and Precision Medicine, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, 100101, China.
*** Corresponding author. State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China.
E-mail addresses: zhangwq@big.ac.cn (W. Zhang), qujing@ioz.ac.cn (J. Qu), ghliu@ioz.ac.cn (G.-H. Liu).

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high chromatin accessibility (Bonev and Cavalli, 2016). In contrast, B compartments are transcriptionally repressed gene-poor regions featured by high A/T sequence density, decoration of heterochromatin marks, such as H3K9me2, H3K9me3, and H4K20me3, and condensed chromatin (Kalukula et al., 2022; Meuleman et al., 2013). Moreover, A and B compartments show distinct nuclear radial positioning; B compartments are tethered to the nuclear lamina and form lamina-associated domains (LADs), whereas A compartments tend to localize at the nuclear interior and form inter-LADs (iLADs) (Kempfer and Pombo, 2020; Misteli, 2020; van Steensel and Belmont, 2017). Subsequent studies have proposed that chromatin compartments contain topologically associating domains (TADs) at the sub-Mbp scale. The boundaries of TADs are enriched for CCCTC-binding factor (CTCF) binding sites, highly transcribed genes, and short interspersed nuclear elements (SINEs) (Rowley and Corces, 2018). Chromatin loops frequently form within or at the boundaries of TADs by a proposed loop-extrusion mode (Dekker and Mirny, 2016; Ong and Corces, 2014; Sanborn Adrian et al., 2015). Advances in 3D genome research methodologies have enhanced our understanding on the reorganization of higher-order chromatin architecture during senescence. Below, we summarize recent works regarding chromatin structure dynamics during senescence and discuss the relationship between the higher-order chromatin structure reorganization at each scale level and the regulation of gene expression.

At the genome-wide scale, increased long-range contacts are observed in replicative senescence (RS) or oncogene-induced senescence (OIS) of human fibroblasts and premature senescence (the pathogenic mutation of Hutchinson-Gilford progeria syndrome [HGPS] and Werner syndrome [WS] induced senescence) of human mesenchymal stromal/stem cells (hMSCs) (Liu et al., 2022; Sati et al., 2020). In OIS, genomic regions that gain long-range interactions, defined as the senescence-associated heterochromatin domains (SAHDs), are enriched at B compartments and are associated with the formation of senescence-associated heterochromatin foci (SAHFs) (Sati et al., 2020). SAHFs are aging-related chromatin structures and frequently present in acute senescence systems, i.e., OIS, but not in other senescent types, such as replicative senescence and premature senescence (Chandra et al., 2015; Chiang et al., 2019; Liu et al., 2022; Sadaie et al., 2013; Sati et al., 2020; Zhang et al., 2021) (Fig. 1). Enhanced chromatin interactions between B compartments are also found in replicative senescence of hMSCs and stress-induced premature senescence (SIPS) of human fibroblasts (Liu et al., 2022; Zhang et al., 2021), but increased B–B compartment interactions in these cases are associated with the loosening status of heterochromatin rather than the formation of compacted SAHFs (Liu et al., 2022; Zhang et al., 2021). Furthermore, replicatively senescent human fibroblasts preferentially gain A-B interactions (Sati et al., 2020) and, in concordance, interactions between A and B compartments are also preferentially increased in deep senescent (DS) progeroid hMSCs (Liu et al., 2022). These results demonstrate a decrease of genome compartmentalization during senescence (Fig. 1). To characterize the unconstrained and less-ordered rewiring of higher-order chromatin structure, Liu et al. implemented the entropy theory to measure the disorder of 3D genome during aging (Liu et al., 2022). They found that the Shannon's information entropy was significantly increased in B compartments in replicative senescence, but not in deep senescent progeroid cells (Liu et al., 2022). However, the whole-genome entropy was drastically increased in these deep senescent progeroid cells (Liu et al., 2022). Thus, these data suggest that the epigenomic chaos originates from the loosening of the repressive B compartments, and then this epigenetic disorder spreads to the whole genome. Consistently, the globally increased epigenetic entropy quantified by methylation stochasticity was reported in the senescent fibroblasts (Jenkinson et al., 2017; Seale et al., 2022). The principles of epigenome remodeling in aging suggest that the survival of cells lies in the ability to stay out of increased entropy, whereas, the senescent cells lost this capacity (Tuyéras et al., 2021).

Although mounting evidence supports that the chromatin is still partitioned into compartments A and B in senescent cells, compartment switch has been reported in multiple cellular senescence contexts, including replicatively senescent hMSCs and fibroblasts and prematurely senescent hMSCs (Criscione et al., 2016; Liu et al., 2022). The percentage of genomes undergoing A/B compartment switch varies in different studies, which may be due to differences in senescent cell types and degrees of cellular senescence in these studies (Criscione et al., 2016; Liu et al., 2022). Compartments that switch during senescence show typical (epi)genetic signatures. Facultative heterochromatin regions, which are decorated with H3K27me3, tend to switch from B compartments to A compartments in replicatively senescent hMSCs and fibroblasts, prematurely senescent hMSCs, and senescent fibroblasts induced by bleomycin treatment (Criscione et al., 2016; Liu et al., 2022; Zhang et al., 2021). Intriguingly, a recent study shows that peri-telomeric regions are predominantly enriched for B to A compartment switches, which provide a potential epigenetic explanation for telomere erosion during aging (Liu et al., 2022). Genomic regions that switch from the A compartment in proliferating cells to the B compartment in senescent cells are associated with several categories, including low signal or quiescent, Polycomb repressed, Repeats or ZNF, and weak transcription, enhancer or promoter regions (Criscione et al., 2016; Liu et al., 2022; Zhang et al., 2021). The dramatic repositioning of LADs at the nuclear periphery is tightly correlated with compartment switches in aged stem cells. Genomic regions that underwent LAD-to-iLAD switches showed the higher tendency of compartment transitions from repressive (B) to active (A) ones (Liu et al., 2022). In addition, the A/B compartment and LAD/iLAD switches show distinctive sequence features. Genomic regions with typical A compartment/iLAD sequence features (i.e. relatively lower A/T content) undergo B to A compartment switches and detach from the nuclear lamina (NL) (Liu et al., 2022). On the other hand, genomic regions with typical B compartment/LAD sequence features (i.e. relatively higher A/T content) gain association with the nuclear lamina and undergo A to B compartment switches (Liu et al., 2022). These results suggest that the convergent alteration driven by sequence feature and the phase separation of chromatin may also contribute to the reorganization of higher-order chromatin structures during senescence.

Compartment switches are linked to transcriptomic changes during senescence. In senescent fibroblasts, B to A compartment switches are associated with the silence of key cell cycle regulators, such as *MCM6*, whereas A to B compartment switches are associated with the activation of senescence-promoting genes, such as the senescence-associated secretory phenotype (SASP)-related gene *EGF* (Criscione et al., 2016). Compartment switches are also coupled with changes of the enhancer activity in replicative senescence of mouse embryonic fibroblasts (MEFs), in which activated senescence-activated enhancers (SAEs) are positively correlated with the transcription of SASP genes (Guan et al., 2020). This study demonstrates a coordinated relationship between the 3D genome reorganization and the enhancer activity in modulating the SASP gene expression (Guan et al., 2020) (Fig. 1).

The overall 3D genomic organization of senescent cells retains typical A and B compartments (Chandra et al., 2015; Liu et al., 2022). However, the DamID approach, a robust method to decipher genomic regions associated with the nuclear lamina, has revealed decreased DamID signals in LAD regions and increased DamID signals in iLAD regions, indicating the decompartmentalization, in senescent hMSCs (Liu et al., 2022). The global redistribution of chromatin positioning in the nucleus is associated with a smoothened epigenomic status, which is referred to as “Convergent Alteration of the Epigenomic landscape during Aging (CAEA)” (Liu et al., 2022). These epigenome changes include the de-repression of heterochromatin and the weakened euchromatin (Liu et al., 2022). The former is associated with decreased heterochromatin marks (H3K9me3 and H4K20me3), DNA hypomethylation, and increased chromatin accessibilities in LAD regions (Chandra et al., 2015; De Cecco et al., 2013; Liu et al., 2022; Sadaie et al., 2013), whereas the latter is associated with decreased active chromatin marks and chromatin accessibility (De Cecco et al., 2013; Liu et al., 2022).

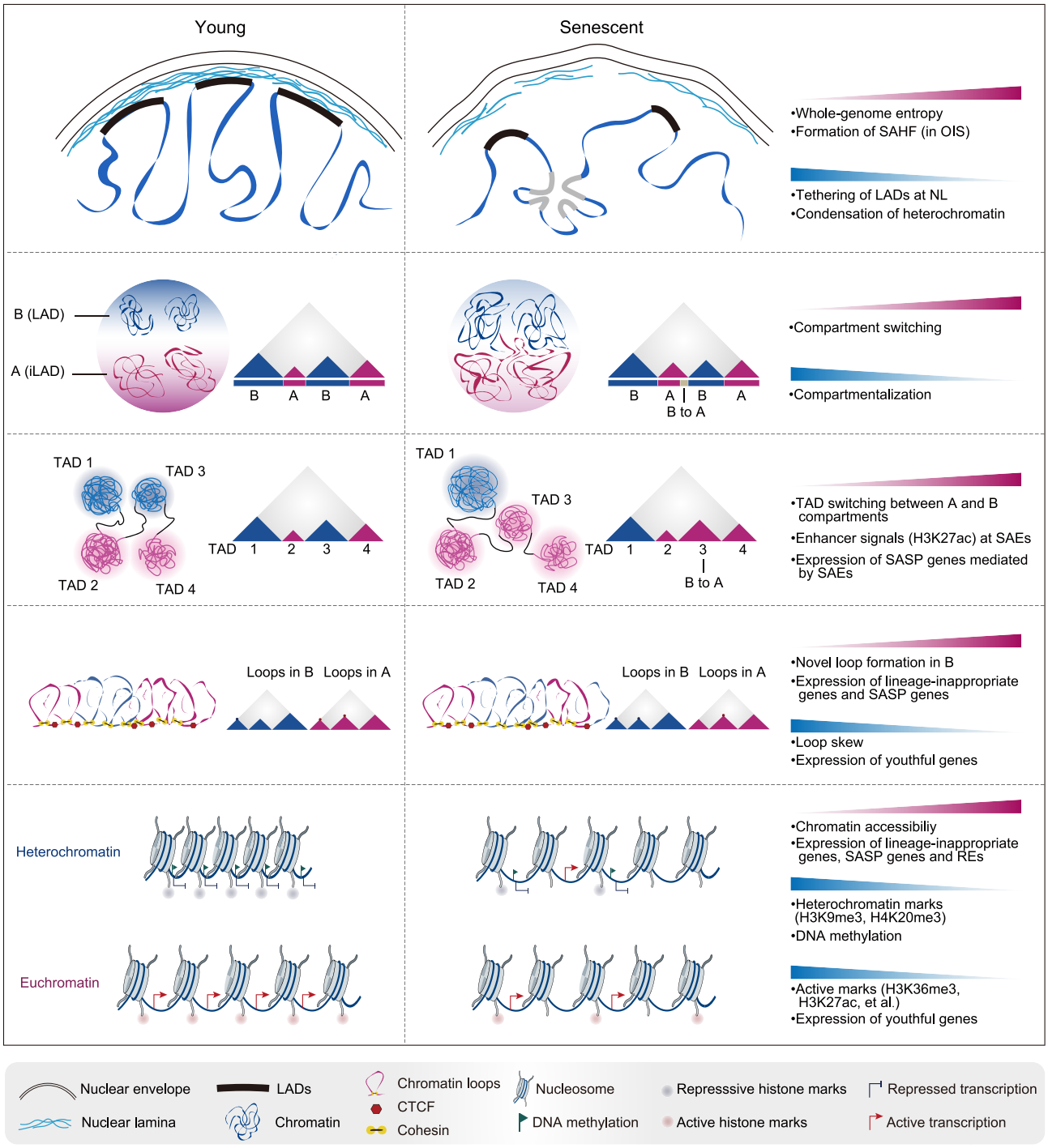

Fig. 1. 3D genome reorganization during senescence. Schematic diagram showing the epigenomic alterations and the association with transcription changes during cellular senescence. Senescent cells undergo multiple higher-order chromatin changes, including the increased whole-genome epigenome entropy, the radial repositioning of chromatin, and compartment switching. The detachment of heterochromatin from nuclear lamina is associated with the loss of repressive histone marks (H3K9me3 and H4K20me3), DNA hypomethylation, increased chromatin accessibility, and active expression of lineage-inappropriate genes, SASP genes, and repetitive elements (REs). In the active chromatin regions, the decrease of active histone marks and E-P loop interaction strength is associated with the repression of youthful genes, such as cell cycle regulators.

The smoothened chromatin status and the reduced distinction between heterochromatin and euchromatin in senescent cells have also been reported to be associated with senescence-related transcription regulation, and the de-repression of heterochromatin is associated with the leakage expression of cell fate restricted genes (Fig. 1). In replicatively senescent and prematurely senescent HGPS and WS hMSCs, the compromised heterochromatin strength is associated with the activation of development-associated genes silenced by the heterochromatin (e.g. pregnancy-specific beta-1 glycoprotein (*PSG*) genes that are normally specifically expressed in the syncytiotrophoblast (STB) of the placenta during pregnancy) (Liu et al., 2022). Cardiomyocytes carrying pathogenic *LMNA* variants show disrupted nuclear morphology and peripheral chromatin, which is correlated with the aberrant expression of non-myocyte lineage genes (Shah et al., 2021). Consistently, the decompaction of heterochromatin in senescent fibroblasts is associated with the misexpression of lineage-inappropriate genes, including *LCE2*

genes that are highly expressed in keratinocytes of the epidermis and the inflammatory cytokine gene *NLRP3* which is preferentially expressed in macrophages (Tomimatsu et al., 2022). In addition, altered chromatin states at heterochromatin are also linked to the aberrant expression of repetitive elements enriched in heterochromatin regions, including long interspersed nuclear element 1 (LINE1) and endogenous retrovirus-K (ERVK) retrotransposons and satellite elements (Bi et al., 2020; Liang et al., 2022; Liu et al., 2022; Sadaie et al., 2013; Zhang et al., 2021). The increased levels of LINE1s or ERVKs cDNAs in the cytoplasm during reverse transcription are sensed by cytosolic DNA-sensing factor, cyclic GMP-AMP synthase (cGAS), and stimulator of interferon genes (STING), thus further triggering the downstream type I interferon and SASP-associated inflammatory response pathways (Bi et al., 2020; De Cecco et al., 2019; Liang et al., 2022; Liu et al., 2021; Van Meter et al., 2014). The elevated retrotransposition events for LINE1s and ERVKs during aging may further contribute to increased genomic instabilities. The weakened euchromatin strength in senescent cells is associated with the silence of youthful genes, such as cell cycle regulating genes (Liu et al., 2022). Thus, the loss of epigenomic stiffness during aging is associated with the loss of transcriptional precision, including the mis-expression of lineage-inappropriate and aging-promotion genes and specific repetitive elements, as well as the silence of youthful genes (Fig. 1).

High-resolution genome-wide chromatin contact mapping reveals that compartments A and B can respectively be further classified into smaller subcompartments A1 and A2 and subcompartments B1, B2 , and B3, with each subcompartment associated with a specific chromatin state (Rao et al., 2014; Xiong and Ma, 2019). Both A1 and A2 subcompartments are enriched with active chromatin states but A1 subcompartments are more active and with relatively higher enrichment of active marks than A2 subcompartments. Subcompartments B1 are enriched at facultative heterochromatin regions with the decoration of H3K27me3. B2 and B3 subcompartments are both enriched at heterochromatin/LAD regions, but B3 subcompartments are less repressed (Liu et al., 2022). Chromatin architecture changes during senescence at the sub-compartment level are first reported in replicatively and prematurely senescent hMSCs, up to ~20% of the genome of those cells undergo subcompartment switches, which greatly exceeds the genomic coverages of A/B compartment switches (Liu et al., 2022). Three classes of sub-compartment switches occur during senescence, 1) weakened heterochromatin (B2-to-B1 and B2-to-B3 transitions), 2) weakened euchromatin (A1-to-A2 transitions), 3) the transitions from the repressed compartment to the active compartment (B1-to-A2 and B3-to-A2) (Liu et al., 2022). These changes further demonstrate an overall weakened epigenetic compartment during aging.

Chromatin compartments can be further segregated into submegabase-sized TAD structures. TADs are self-interacting structural blocks that are the fundamental and functional units of 3D chromatin organization (Dixon et al., 2012). The global TAD structures are maintained in cellular senescence but a subset of TADs switches between A and B compartments, these TADs show intermediate TAD boundary strength as compared to TADs localize at the interior of A or B compartments (Liu et al., 2022). The switch of TADs between A and B compartments is associated with the transcription change of specific genes (Criscione et al., 2016). Detailed analyses of TAD changes during senescence have been conducted in human umbilical vein endothelial cells (HUVECs), human fibroblasts, and hMSCs (Zirkel et al., 2018) and identified unchanged TADs, TADs with shifted boundaries (≥80 kilobase pair), fusions, and separations. The study shows that TAD structure changes are most dramatic in HUVECs, with 43% TADs fusing into larger ones and 24% TADs shifting their boundaries, and the remodeling of TAD structures is partially associated with the depletion of HMGB2 binding at TAD boundaries (Zirkel et al., 2018).

In young cells, chromatin loops located in the A compartments show relatively higher chromatin interaction strength than those in B compartments, indicating a high "Loop skew" between A and B compartments (Liu et al., 2022). However, the interaction strength of loops increases in the B compartments and decreases in the A compartments in senescent hMSCs, resulting in the decreased "Loop skew" between A and B compartments (Liu et al., 2022). The reorganization of spatial chromatin architecture at the loop level combined with the large-scale chromatin repositioning and compromised compartmentalization conjointly prove that the senescent epigenome exhibits loss of chromatin stiffness, increased laxity and epigenomic entropy, and the attenuated epigenetic polarity (De Cecco et al., 2013; Liu et al., 2022). Consistent with this study, increased loop interactions at heterochromatin regions are also found in deep senescent human diploid fibroblasts (Zhang et al., 2021). Novel loops formed in heterochromatin are associated with the loss of H3K9me3 marks and increased accessibility at CTCF binding sites (Zhang et al., 2021). The chromatin interactions between enhancers and promoters play a key role in the transcription regulation (Kubo et al., 2021; Smallwood and Ren, 2013). The alterations of enhancer-promoter chromatin loop (E-P loop) interactions and their relevance in transcriptional changes during senescence have been reported in recent studies (Liu et al., 2022; Olan et al., 2020). Differential E-P loop interactions are positively correlated with the transcriptional changes of significantly differentially expressed genes (DEGs) in oncogenic HRAS-G12V-induced senescence of human diploid fibroblasts (Olan et al., 2020). Genes upregulated in association with enhanced E-P loop interactions are extensively enriched in inflammatory response pathways whereas genes downregulated in association with decreased E-P loop interactions are enriched for cell cycle terms (Liu et al., 2022; Olan et al., 2020). Specifically, the formation of novel loops at the *IL1* locus (harboring two proximal SASP component genes *IL1A* and *IL1B*) is reported in HRAS-G12V-induced senescence (Olan et al., 2020). The *de novo* binding of cohesions (RAD21 and SMC3) at the *IL1* genomic locus is associated with the novel EP-loop formation and the transcriptional activation of canonical SASP gene *IL1B* in OIS (Olan et al., 2020). Consistently, another study conducted in replicatively senescent and prematurely senescent hMSCs finds that downregulated genes with decreased E-P loop strength are associated with cell cycle and metabolic pathways, whereas upregulated genes with increased E-P loop strength are involved in various developmental processes and the extracellular matrix (ECM) organization (Liu et al., 2022). Consistent with this study, the upregulation of the ECM gene *COL22A1* in deep senescence is reported to be associated with the novel loop formation and increased chromatin accessibility at the loop interaction pairs within heterochromatin (Zhang et al., 2021). These data suggest that the two canonical hallmarks of senescence (the SASP and proliferative arrest) and the leakage expression of lineage-inappropriate genes might be altered by the rewiring of the E-P loop network (Fig. 1).

Concluding remarks and perspectives

The advance of Hi-C methodologies and high-resolution imaging techniques has improved our understanding of chromatin structure and 3D genome organization during aging. The pictures of higher-order chromatin structure during senescence have been reported in recent studies (Chiang et al., 2019; Criscione et al., 2016; Guan et al., 2020; Liu et al., 2022; McCord et al., 2013; Sati et al., 2020; Zirkel et al., 2018). Although those epigenomic studies are conducted in distinct cell types with the combined analyses of Hi-C and various epigenetic datasets, the predominantly epigenomic changes are uniformly reported at the interface between nuclear lamina and heterochromatin (Chiang et al., 2019; Liu et al., 2022; Sati et al., 2020). Morphologically, senescent cells typically have nuclear envelope defects and loss of nuclear-lamina-associated proteins and heterochromatin proteins, especially Lamin B1, LAP2 and HP1 (Booth and Brunet, 2016; Liu et al., 2022; López-Otín et al., 2013; Sadaie et al., 2013; Zhang et al., 2015). The loss of structural basis (i.e., nuclear lamina and heterochromatin proteins) is associated with the decreased anchoring effect of heterochromatin, thus linking to the detachment of LADs from nuclear lamina (Bi et al., 2020;

Deng et al., 2019; Hu et al., 2020; Liu et al., 2022; Sadaie et al., 2013). The disassociation of peripheral heterochromatin from the nuclear lamina results in two distinct patterns of heterochromatin changes, i.e., the formation of condensed SAHFs in OIS (Chiang et al., 2019; Sati et al., 2020) and the depression of heterochromatin in most senescent systems (Liu et al., 2022; Sati et al., 2020; Zhang et al., 2021). Although a polymer model provides a full-phase illustration of lamina-mediated chromatin reorganization in OIS and premature senescence (Chiang et al., 2019), mechanisms underlying two distinct consequences of the detached heterochromatin during senescence remain elusive. Further studies investigating underlying mechanisms for nuclear lamina and heterochromatin proteins in regulating the heterochromatin stability during aging can help the development of novel anti-aging interventions and strategies by reinforcing the heterochromatin/epigenome stability.

In addition to the nuclear lamina, other membrane-less subnuclear organelles and structures, such as nuclear speckles and nucleolus, are critical for the maintenance of higher-order chromatin organization and transcription regulation (Chen et al., 2018). The gene-enriched and highly transcribed regions tend to organize around nuclear speckles to form speckle associating domains (SPADs), which extensively overlap with A1-subcomaprtments. In contrast, centromere-proximal, gene-poor, and transcriptionally inactive genomic regions organize around the nucleolus to form nucleolus-associated chromatin domain (NADs) (Ahanger et al., 2021; Bersaglieri et al., 2022; Chen et al., 2018). However, key functions of nucleolar and nuclear speckle in maintaining epigenomic homeostasis and transcription regulation remain unclear and need further investigation.

The large-scale higher-order structure reorganization has been widely investigated, while many critical scientific questions regarding intermediate structures remain unanswered. Senescence-associated 3D genome reorganizations at inter-, intra- and sub-TAD levels require further exploring. Studies with higher resolution Hi-C datasets and imaging strategies will provide additional knowledge for those intermediate structure changes (Chen et al., 2019; Gonzalez-Sandoval and Gasser, 2016; Kempfer and Pombo, 2020; Rowley and Corces, 2018; Zhang et al., 2019). CTCF and cohesins are both key structure proteins for maintaining TAD and chromatin loop stability (Chen et al., 2019; Dixon et al., 2012; Kubo et al., 2021) and have been shown to be reduced during aging in multiple systems (Hou et al., 2021). Although a few studies have reported the alteration of binding strengths for CTCF and cohesions at specific genomic loci during senescence and its association with transcriptional changes (i.e., SASP gene *IL1* and cell cycle repressors $p15^{INK4b}$ and $p16^{INK4a}$ et al.) (Hirosue et al., 2012; Hou et al., 2021; Olan et al., 2020; Zhang et al., 2021), the detailed regulatory functions of CTCF, cohesins and the mediator proteins remain largely unknown. The genetic manipulation of CTCF and cohesions followed by phenotypic and systematic epigenomic analysis will provide more understanding on roles of CTCF and cohesins in epigenome maintenance and gene expression regulation.

Most studies on aging-associated 3D genome organization are conducted in mitotic cells, of which cell cycle arrest and elevated DNA replication stress and DNA damage response are hallmarks of cellular senescence (Flach et al., 2014; Hernandez-Segura et al., 2018; Schumacher et al., 2021). Previous studies show that DNA replication timing is key for the establishment of epigenome (Ma and Duan, 2019; Marchal et al., 2019). Specifically, late-replicating regions co-localize with LAD regions and display typical DNA hypomethylation during aging (Marchal et al., 2019). However, little is known about the relationship between cell cycle arrest, DNA replication timing changes, the block of the replication forks, the formation of DNA damage/fragile sites, and the (epi)genome reorganizations during aging. Further studies at these interfaces may help to identify the intrinsic drivers for cell cycle arrest during aging, and also understand the underlying mechanisms of DNA replication precision for maintaining chromosome conformation/(epi)genome integrity.

Importantly, the current principle for senescence-associated reorganization of 3D genome is limited in mitotic cells (Chandra et al., 2015; Criscione et al., 2016; Liu et al., 2022; Sati et al., 2020; Zhang et al., 2021). Moreover, the aging-associated alterations of higher-order chromatin structures for physiologically aged tissues and cell types remain undetermined. In addition, aging is a process of increasing heterogeneity, the genome reorganization and chromatin state shifts may exhibit cell-to-cell variability during aging. Elucidation of detailed higher-order chromatin changes and their interplay with other layers of epigenomic marks using single-cell multi-omics technologies or improved higher-resolution imaging approaches is instrumental for understanding how the physiological aging and aging-associated diseases are regulated and, in the longer term, for identifying potential intervention targets and developing therapeutic intervention strategies.

Disclosure

The authors declare no conflict of interest.

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