# Chapter 5
# Senescence and the Genome

**Joanna M. Bridger and Helen A. Foster**

**Abstract** Cellular senescence is commonly initiated in response to replicative or cell stress pathways. Senescent cells remain in a state of permanent cell cycle arrest, and although being metabolically active, they exhibit distinct senescence phenotypes. Though cellular senescence may be beneficial in tumour suppression and wound healing, it is commonly associated with age-related diseases. There are various mechanisms and drivers that contribute to ageing, but it is becoming increasingly apparent that processes related to chromatin and the epigenome are also important. Indeed, three of the nine hallmarks of ageing are genome specific including genomic instability, epigenetic alterations and telomere attrition. With the advent of new technologies like DNA adenine methyltransferase identification and chromosome conformation capture, the features and complexity of the ageing genome are being revealed. This chapter will address key characteristics of interphase nuclei during cellular senescence including the spatio-temporal organisation of chromosomes, chromatin remodelling and epigenome changes.

## The Senescence Phenotype

The term "cellular senescence" was originally coined by Hayflick in 1965 (Hayflick 1965). It was described as an important mechanism to suppress tumorigenicity. Senescence can be categorised into four types as shown in Fig. 5.1: (1) replicative senescence (RS) as a result of telomere dysfunction or shortening; (2) genotoxic stress-induced senescence due to endogenous stress, e.g. oxidative stress and severe or irreparable DNA damage; (3) oncogene-induced senescence (OIS) via the activation of aberrant signalling pathways caused by different mechanisms including

J. M. Bridger
Centre for Genome Engineering and Maintenance, Department of Life Sciences, Division of Biosciences, College of Health, Medicine and Life Sciences, Brunel University London, Uxbridge, UK

H. A. Foster (✉)
Department of Clinical, Pharmaceutical and Biological Science, School of Life and Medical Sciences, University of Hertfordshire, Hatfield, UK
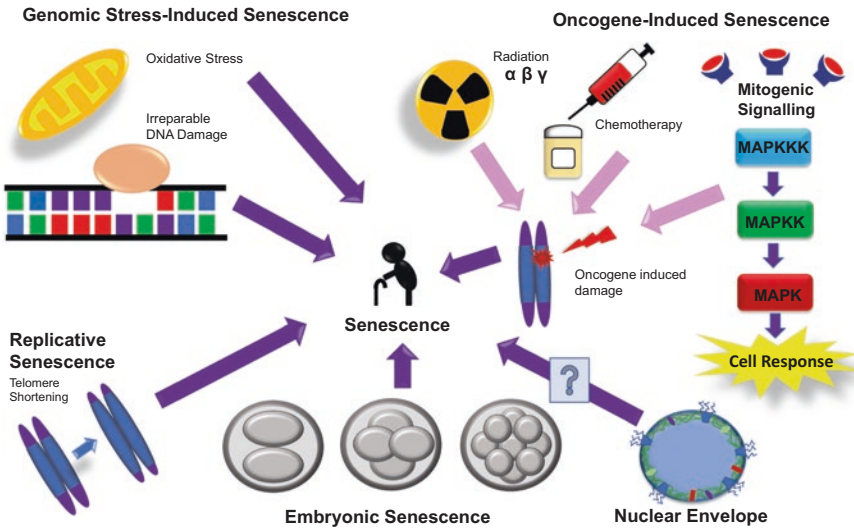e-mail: h.foster2@herts.ac.uk

**Fig. 5.1 The four main categories of senescence**. Genomic stress-induced senescence can be induced via products from cellular metabolism, e.g. reactive oxygen species (ROS) produced by mitochondria or DNA damage due to errors in DNA replication, recombination or repair mechanisms. Activation of aberrant signalling pathways can lead to oncogene-induced senescence and may result from mitogenic signalling or genotoxic agents such as chemical mutagens and radioactivity. Embryonic senescence is important for developmentally regulated growth and patterning. Replicative senescence can lead to irreversible cell cycle arrest by telomere shortening or dysfunction

natural endogenous processes such as mitogenic signalling or oxidative respiration, physical or chemical insults encountered during life or therapeutic treatment such as irradiation or chemotherapy; and (4) embryonic-senescence which occurs in a developmentally regulated manner (Coppé et al. 2010; Munoz-Espin et al. 2013; Storer et al. 2013; Graziano and Gonzalo 2017). The characteristics of senescence may vary depending on the mechanism by which it was induced; for instance, a senescence-associated secretory phenotype (SASP) that secretes proinflammatory mediators is present with some forms of senescence, but not others (Coppé et al. 2010). Regardless of the type of senescence, they each share the characteristic arrest in cell proliferation (Coppé et al. 2010). This chapter will concentrate on how the genome and its behaviour are altered during senescence.

## Organisation of Chromatin and the Epigenome During Ageing

DNA contains genetic information that, when expressed, ultimately codes for the synthesis of a range of proteins vital for the correct functioning of cells, tissues and the whole organism. The genome, when housed in cell nuclei, needs to be organised

correctly so that this information can be safely conveyed during proliferation and cell division to daughter cells, be protected from damage and allow genes to be expressed or repressed depending on the protein requirements of the cell and differentiated tissue. The nucleosome is an octamer of histone proteins composed of two copies of histones H2A, H2B, H3 and H4. There are approximately 30 million nucleosomes within the genome, and DNA wraps around these nucleosome complexes to form chromatin (Xu and Liu 2019). Epigenetics involves heritable changes that alter the expression of genes but do not change the DNA sequence. These modifications can act directly on the DNA by adding methyl groups to cytosine; through post-translational modifications to histones including acetylation, methylation, phosphorylation, sumoylation or ubiquitination; or via non-coding RNAs such as microRNA (miRNA), Piwi-interacting RNA (piRNA) and small interfering RNA (siRNA) (Dupont et al. 2009; Wei et al. 2017). Deregulation of epigenetic mechanisms has been highlighted in disease aetiology and ageing. Epigenetic clocks predict the chronological age of individuals by studying the methylation status of cytosines in specific GC-rich regions of the genome, known as CpG islands (Horvath 2013). Mathematical algorithms are employed to determine DNA methylation levels (5-methylcytosine or 5mC) from sets of CpG islands to estimate the age of the DNA source (Horvath and Raj 2018). CpG islands are commonly found near promoter regions of the genome, are ≥0.5 kb long with a GC content of ≥55% and are generally unmethylated (Jeziorska et al. 2017). Global DNA hypomethylation, with hypermethylation of specific loci, is associated with physiological ageing (Gensous et al. 2017). Changes in DNA methylation have been demonstrated in a number of age-related diseases such as cancer (Xie et al. 2019), Parkinson's disease (Miranda-Morales et al. 2017; Navarro-Sánchez et al. 2018) and Alzheimer's disease (Levine et al. 2015) as well in cells derived from Hutchinson-Gilford progeria syndrome (HGPS) patients (Ehrlich 2019). However, epigenetic changes during ageing are complex. Although region-specific hypermethylation may be determined at specific CpG islands and gene loci, ageing is also associated with global hypomethylation across the genome (Gensous et al. 2017) and loss of heterochromatin (Goldman et al. 2004; Chandra et al. 2015).

The degree of chromatin compaction can vary in cells, with euchromatin being less compact and open in structure and heterochromatin being more condensed. Generally, euchromatin is rich in CpG islands, has a high GC content, is gene-dense and is associated with short interspersed elements (SINEs) and transcriptional activity (Medstrand et al. 2002; Elbarbary et al. 2016; Vanrobays 2017). Conversely, heterochromatin is AT-rich and gene-poor, associated with long interspersed elements (LINEs), and is inaccessible to transcription factors (Vanrobays 2017; Medstrand et al. 2002; Elbarbary et al. 2016). Epigenetically, histones in heterochromatin generally have methylated H3K9 and H3K27, whilst euchromatin has both acetylation and methylation of H3K4 and H3K36 (Ahringer and Gasser 2018). Heterochromatin can be further subdivided into constitutive heterochromatin and facultative heterochromatin. Constitutive heterochromatin is not transcribed, contains highly repetitive sequences and is H3K9 methylated to maintain a stable condensed state important for chromosome structure such as in centromeres and

telomeres (Ahringer and Gasser 2018). Facultative heterochromatin is reversible and may adopt both open or compact conformations according to (1) spatial parameters, e.g. changes in nuclear localisation due to factors such as signalling; (2) temporal changes, e.g. within the cell cycle or development; or (3) heritable factors, e.g. chromosome X inactivation (Trojer and Reinberg 2007). Thus, euchromatin has the potential to be decondensed and express genes in certain tissues. Euchromatic and heterochromatic domains are established during embryogenesis and development to generate tissue-specific gene expression patterns (Villeponteau 1997). Commonly within interphase nuclei, heterochromatin is concentrated at the nuclear periphery, nucleoli, centromeres and telomeres (Goldman et al. 2002), whilst euchromatin is positioned within the nuclear interior (Romero-Bueno et al. 2019). However, ageing is associated with substantial changes in heterochromatin distribution and epigenetic modifications.

During ageing, altered histone modifications and the redistribution of hetero-chromatin is thought to be associated with changes in global gene expression and genomic instability. Whole-genome bisulfite sequencing (WGBS) and CpG DNA methylation microarrays have been used to examine the epigenetic profiles of samples derived from a newborn and centenarian (103-year-old) (Heyn et al. 2012). Overall, the centenarian sample had a lower DNA methylation content, with the most hypomethylated sequences in CpG-poor promoters and tissue-specific genes (Heyn et al. 2012). Interestingly, the methylation status in middle-aged adults showed an intermediate level of global DNA methylation, suggesting an accumulative change with advancing age (Heyn et al. 2012). This is not unique as loss of heterochromatin has also been linked to an ageing phenotype in model organisms including *Caenorhabditis elegans* and *Drosophila* (Haithcock et al. 2005; Larson et al. 2012; Maleszewska et al. 2016). Modifications to histones are made through histone-modifying enzymes including histone methyltransferases, histone demethylases, histone deacetylases and histone acetylases (Black et al. 2012). Therefore, changes in expression or activity of these enzymes may have a profound influence on the epigenetic landscape of the genome. This is observed in *Arabidopsis thaliana* whereby reduced transcription of methyltransferases and increased transcription of demethylases are associated with hypomethylation in ageing (Ogneva et al. 2016). Furthermore, mutations in a H3K4 methyltransferase in *C. elegans* and yeast have been shown to reduce longevity, whilst reduced levels of H3K36 demethylase increases lifespan (Sen et al. 2015; Ni et al. 2012).

Epigenetic changes during ageing and loss of heterochromatin also contribute to the derepression of previously silenced genes at those loci (Sturm et al. 2015). This can result in the activation and potential remobilisation of transposable elements (TEs) throughout the genome (Sturm et al. 2015). Given that nearly half of the human genome consists of TEs, this could lead to genomic instability if a TE were to relocate into a coding or regulatory sequence within the genome (Mills et al. 2006; de Koning et al. 2011; Sturm et al. 2015). Ultimately, the resulting DNA damage and instability may result in age-related diseases such as cancer (O'Donnell and Burns 2010), and there are data to demonstrate this mobility, enhancing senescence in humans (Baillie et al. 2011; De Cecco et al. 2013; Keyes 2013).

Nucleosome density has been shown to alter during ageing and is associated with a loss of histones (Hu et al. 2014; Song and Johnson 2018). Nucleosome density naturally varies across the genome with transcriptionally active regions having a lower density and more open chromatin and transcriptionally inactive regions being densely populated with nucleosomes (Boeger et al. 2003; Sidler et al. 2017). Loss of nucleosomes in yeast leads to an increase in transcriptional activity from previously repressed promoters and corresponds with extensive chromosomal alterations and elevation of DNA strand breaks (Hu et al. 2014). Changes in nucleosome density could be due to two mechanisms: (1) alterations in the activity of histone chaperones and (2) reduction in histone biogenesis within the cell (Booth and Brunet 2016). There is evidence that nucleosome assembly may be regulated by the histone chaperone ASF1 in both a DNA synthesis-dependent and DNA synthesis-independent manner along with other histone chaperones, chromatin assembly factor 1 (CAF-1) and histone repression A factor (HIRA) (Galvani et al. 2008). In yeast, loss of function of ASF1 may lead to aberrant heterochromatin formation and genomic instability (Tanae et al. 2012). Indeed, ASF1 expression decreases with increasing age in human cells (O'Sullivan et al. 2010). Here, the synthesis of histones in fibroblasts derived from an old individual was half that compared to those derived from a child (O'Sullivan et al. 2010). Histone biosynthesis was also altered in replicative senescent IMR90 and WI38 cells, leading to downregulation of the synthesis of histones H3 and H4 and post-translational modifications (O'Sullivan et al. 2010; Song and Johnson 2018). Thus, nucleosome density combined with changes in epigenetic post-translational modifications could be an important factor in the loss of heterochromatin observed in ageing.

Conversely, there are regions of the genome that become associated with heterochromatin during ageing (Tsurumi and Li 2012). Chromatin may be organised within senescence-associated heterochromatin foci (SAHF) (Morris et al. 2019; Lenain et al. 2017; Braig et al. 2005; Michaloglou et al. 2005; Haugstetter et al. 2010). SAHF share epigenetic features and characteristics commonly found in heterochromatin including late replicating DNA domains (Shah et al. 2013); epigenetic markers H3K9me3 and H3K27me3 (Chandra et al. 2012; Chandra et al. 2015; Chandra and Narita 2013); HP1 α, β and γ (Boumendil et al. 2019); heterochromatic proteins; histone variant macroH2A; and high-mobility group A (HMGA) proteins (Morris et al. 2019). SAHF structure encompasses a chromatin core that is compacted and enriched in H3K9m3 (a marker of constitutive heterochromatin) and an outer ring of chromatin containing H3K27me3 (a marker of facultative heterochromatin), which is protein rich but more relaxed (Lenain et al. 2017; Chandra et al. 2012; Sadaie et al. 2013). Over 90% of SAHF are commonly observed in cells that have undergone OIS (Chandra et al. 2015) with only a small proportion seen in replicative senescence in cultures (Chandra et al. 2015; Boumendil et al. 2019). SAHFs are not present in HGPS or senescent mouse cells, and it is unclear if they occur in vivo (Lazzerini Denchi et al. 2005; Shumaker et al. 2006; Scaffidi and Misteli 2006; Swanson et al. 2013). The formation of SAHF represses the expression of genes that are important for proliferation and the cell cycle such as cyclin A, proliferating nuclear antigen (PCNA), E2F target genes (Aird and

Zhang 2013) and cyclin D1 (Zhang et al. 2007; Park et al. 2018) and thus leads to senescence. Evidence suggests that SAHF may result from an increase in nuclear pore density during OIS, with the nucleoporin TPR having a vital role in inducing the formation of SAHF and their maintenance (Boumendil et al. 2019).

Epigenetic modifications and heterochromatin distribution are altered in premature ageing syndromes. The majority of premature ageing syndromes are caused by either mutations leading to alterations in the nuclear lamina and matrix proteins or via defects in DNA repair systems (Musich and Zou 2009; Tiwari and Wilson 3rd 2019). Hutchinson-Gilford progeria syndrome (HGPS) is a premature ageing disease caused by a mutated lamin A protein. Here, there is a reduction in H3K9me3 and HP1 and loss of peripheral heterochromatin (Shumaker et al. 2006; Scaffidi and Misteli 2006). Werner syndrome (WS) is another progeroid syndrome that similarly has a loss of H3K9me3. WS is caused by mutations within the Werner helicase (WRN). Interestingly, WRN has been shown to associate with the methyltransferase SUV39H1 and HP1α and thus may be important in regulating heterochromatin during ageing (Zhang et al. 2015; Wang et al. 2016). Mesenchymal stem cells with an induced WRN deficiency show altered heterochromatin distribution and global loss of associated epigenetic methylation of histone H3 (Zhang et al. 2015; Shumaker et al. 2006). Loss of peripheral heterochromatin adjacent to the nuclear envelope (NE) (Goldman et al. 2004; Zhang et al. 2015) and a reduction in H3K9me3 and H3K27me3 levels but increase in H4K27me3 have been shown in HGPS cultured cells (Shumaker et al. 2006; Scaffidi and Misteli 2006).

## Nuclear Lamina and Nucleoskeleton

The nuclear lamina is located adjacent to the inner nuclear membrane (INM) and is composed of type V intermediate filaments proteins—lamins and lamina-associated proteins. Lamins are subdivided into the B-type lamins which are constitutively expressed within mammalian cells and A-type lamins that are developmentally regulated in differentiated cells. The nuclear lamina interacts with INM proteins, nuclear pore complexes and chromatin. It has a number of important roles including organising chromatin, involvement in DNA replication and gene expression and to support structurally the nucleus and its processes (Cau et al. 2014). The peripheral nuclear lamina is interconnected and part of a larger structural protein network known as the nuclear matrix (NM) (Cau et al. 2014) or nucleoskeleton. This structure is believed to be a filamentous meshwork of proteins (e.g. lamins A and C), DNA and RNA localised throughout the nucleoplasm that are resistant to high-salt treatment and nucleases during experiments. Similarly, the matrix structure is important for the structural integrity of nuclei and also supports gene expression, chromatin organisation, DNA replication and repair (Chattopadhyay and Pavithra 2007; Wilson and Coverley 2017; Bridger et al. 2014; Mehta et al. 2007; Elcock and Bridger 2008; Godwin et al. 2021). The NM interacts with chromatin typically via specialised AT-rich DNA sequences called scaffold/matrix attachment regions (S/

MARs) (Barboro et al. 2012) and helps maintain the compartmentalisation of the nucleus and higher-order chromatin organisation important for the spatio-temporal dynamics of the cell.

Laminopathies include progeroid syndromes linked to A-type lamin mutations. These are typically characterised as having nuclear envelope deformities (Cau et al. 2014), with blebbing, herniations, invaginations and altered nuclear shape. These are caused by mutations that influence the post-translational processing of proteins, ultimately leading to defective protein function. For instance, in HGPS, there is a cryptic splice site that leads to a truncated form of lamin A which is permanently bound to a farnesyl moiety, termed "progerin" (Gilbert and Swift 2019). The build-up of progerin at the INM is toxic, leading to altered nuclear envelope integrity and perturbed chromatin organisation (Chandra et al. 2015; Stephens et al. 2018; Bikkul et al. 2018). Although progerin is primarily associated with HGPS, it has been suggested that a progerin-dependent mechanism may lead to natural ageing (Scaffidi and Misteli 2006; McClintock et al. 2007; Ashapkin et al. 2019). Evidence acquired by reverse transcription polymerase chain reaction (RT-PCR) has shown that fibroblasts obtained from naturally aged individuals expressed progerin mRNA, albeit at a low frequency of less than 50-fold (Scaffidi and Misteli 2006). Progerin has also been detected in cell lines derived from skin biopsies that had undergone prolonged cell culture, particularly in cells derived from older individuals (McClintock et al. 2007). However, it should be noted that the levels were very low. Senescence is frequently accompanied with profound changes to the INM organisation and accompanying processes.

The nuclear lamina interacts with the genome directly through lamina-associated domains (LADs) and via lamin-binding partners. DNA adenine methyltransferase identification (DamID) technology has been used to extensively map LADs throughout the nucleus. This technique is used to identify binding sites between DNA and chromatin-binding proteins. For instance, combining a nuclear lamin protein (e.g. lamin B1) to a bacterial DNA adenine methyltransferase (Dam) will highlight areas of DNA that have been in contact with the nuclear lamins as they will undergo adenine methylation. As adenine methylation does not naturally occur in eukaryotes, it acts as a detectable marker. LADs are of fundamental importance in anchoring transcriptionally silent heterochromatin to the nuclear lamina and maintaining the three-dimensional spatial arrangement of chromosomes (van Steensel and Belmont 2017; Romero-Bueno et al. 2019). However, lamins can be found throughout the nucleoplasm (Bridger et al. 1993) and not just at the nuclear envelope, and thus, this should be taken into consideration. LADs are also heterogeneous between cell types (Peric-Hupkes et al. 2010; Meuleman et al. 2013) but are associated with lamin B1 and lamin B1 receptor (LBR), which anchor heterochromatin to the nuclear lamina (Lukasova et al. 2018). However, during cellular senescence, LADs become extensively redistributed (Lochs et al. 2019). Normally, after DNA replication, DNA methyltransferase DNMT1 restores the histone methylation pattern; however, this appears to fail during senescence (Lochs et al. 2019) leading to hypomethylation. This hypomethylation, combined with the loss of lamin B1, leads to heterochromatin dissociating from the nuclear lamina

(Lochs et al. 2019) and away from the nuclear periphery. This LAD rearrangement may also be associated with the accumulation of SAHF, which relocates heterochromatin to the nuclear interior (Lenain et al. 2017; Chandra et al. 2015).

## Nucleolus

The nucleolus is also important for spatio-temporal regulation of the genome and is formed from chromosomes containing active nucleolar organiser regions (NORs) and other non-acrocentric but gene-rich chromosomes (van Koningsbruggen et al. 2010; Nemeth et al. 2010). Nucleoli are important in ribosome biogenesis and are initiated from the transcription of ribosomal RNA (rRNA) genes found in high copy number and arranged in tandem repeats within NORs (Bersaglieri and Santoro 2019). Genomic regions that are localised in close proximity to the nucleolus are termed nucleolus-associated domains (NADs) (Nemeth et al. 2010). Genome-wide mapping has demonstrated that NADs derived from HeLa, IMR90 and HT1080 human cell lines have a low gene density, low transcriptional levels, late-replicating loci and heterochromatin enriched with repressive histone modifications H4K20me3, H3K27me3 and H3K9me3 (van Koningsbruggen et al. 2010; Nemeth et al. 2010; Dillinger et al. 2017). During senescence, nucleoli may fuse and are associated with an increased size (Mehta et al. 2007). H3K9me3 modified heterochromatin localised at the nucleolus, is remodelled and is coupled with an observed dissociation of centromeric and pericentromeric satellite regions away from the nucleolus (Dillinger et al. 2017). Interestingly, mapping of NADs using Hi-C in senescent cells remains similar to that seen in proliferating cell lines although there are changes in sub-NADs association with nucleoli (subdomains smaller than 100 kb), which appear to correspond to transcriptional changes (Dillinger et al. 2017; Mehta et al. 2010).

## Centromeres and Telomeres

Centromeres are heterochromatic regions and have satellite II and α-satellite repeat sequences that are normally constitutively repressed (De Cecco et al. 2013). However, in replicative senescent cells, the pericentric satellite has been shown to distend, and chromatin is reorganised becoming more accessible and hypomethylated (De Cecco et al. 2013; Cruickshanks et al. 2013). This centromere distension has been termed "senescence-associated distension of satellites" (SADS) and is associated with epigenetic modifications associated with early senescence (Criscione et al. 2016). Silencing of pericentric satellite DNA is helped and maintained by SIRT6, a histone deacetylase, which removes H3K18 acetylation in normal proliferative cells (Nagai et al. 2015; Tasselli et al. 2016). It is possible that SIRT6 depletion could lead to senescence (Tasselli et al. 2016; Nagai et al. 2015). Indeed, SIRT6 is an early factor sequestered to double-strand breaks, so prolonged

recruitment to irreversibly damaged DNA associated with ageing may lead to depletion of SIRT6 at pericentric satellite DNA leading to the unravelling and SADS phenotype (Toiber et al. 2013; Nagai et al. 2015; Tasselli et al. 2016). SADS occurs as a common feature of senescence, irrespective of how senescence is induced and whether the p16 or p21 pathways are activated (Swanson et al. 2013). Unlike SAHF, SADS are found both during normal senescence and in progeria (Swanson et al. 2013).

Telomeres, and their associated shelterin protein complex, are located at the ends of linear chromosomes and have a protective role in preventing genome instability by shielding exposed ends of DNA. During replication, DNA polymerases are unable to completely replicate the telomere region of the lagging strand leading to shortening due to the progressive loss of telomere repeats. This has been termed the "end-replication problem." Consequently, the length of the telomeres shortens with each cell division leading to attrition. This has been extensively reported in ageing studies and is particularly pronounced within in vitro primary cells leading to a finite number of cell divisions or "replicative senescence" due to the shortened telomere lengths. The resulting exposure of the chromosome ends leads to the activation of DNA repair mechanisms and a persistent DNA damage response (DDR) (Victorelli and Passos 2017). Nevertheless, telomere dysfunction can occur irrespective of length with telomeric DNA damage being associated with an increase in senescence markers such as p16 (Victorelli and Passos 2017; Birch et al. 2015). Indeed, in postmitotic cardiomyocytes, there is an increase in DNA damage foci associated with telomeres during ageing (Anderson et al. 2019). Telomeres interact with a telomere repeat-binding factors 1 and 2 (TRF1 and TRF2, respectively) to form t-loops. TRF1 is thought to prevent fusion of telomere ends and regulate telomere length (van Steensel et al. 1998; Celli and de Lange 2005), whilst TRF2 forestalls the DNA damage response (Karlseder et al. 2004).

The positioning of telomeres in interphase nuclei appears to vary between species, cell type and disease status (Weierich et al. 2003; Chuang et al. 2004; Arnoult et al. 2010; Gilson et al. 2013). Nevertheless, positioning is non-random and integral to genomic stability (Chuang et al. 2004). There is evidence that telomeres are closely associated with the nucleoskeleton and A-type lamins (Ottaviani et al. 2009; de Lange 1992) in addition to reports that the telomeres of acrocentric chromosomes localise to perinucleolar regions (Ramirez and Surralles 2008). Loss of TRF2 has been linked to an increased DNA damage response and senescence (van Steensel et al. 1998; Okamoto et al. 2013). TRF2 may interact with lamin A/C, which are important proteins within the INM and nuclear matrix (Wood et al. 2014). In HGPS, there is a reduction in TRF2 (Wood et al. 2014) and apparent telomere loss. Interestingly, studies using human telomerase reverse transcriptase (hTERT) to evade replicative senescence on proliferating fibroblasts and HGPS cells have shown dramatic genome reorganisation with the mislocalisation of whole chromosomes 18 in the control cells and chromosome 18 and X in HGPS cells (Bikkul et al. 2019). Differences in telomere organisation have also been demonstrated in terminally differentiated cells or quiescent cells in culture that have been contact inhibited (Nagele et al. 2001). Here, interphase nuclei exhibit close telomeric associations

within quiescent, non-cycling cells compared with proliferating cells (Nagele et al. 2001). Clustering of telomeres has also been demonstrated in mouse embryonic fibroblasts, as well as partial association with centromeric clusters and promyelocytic leukaemia bodies (PML) (Molenaar et al. 2003; Weierich et al. 2003; Uhlirova et al. 2010).

Epigenetic changes also accompany telomere maintenance during ageing (Uhlirova et al. 2010). Levels of H3K9me3, H4K20me3 and HP1 protein have been shown to decrease in HGPS (Scaffidi and Misteli 2006). Treatment of embryonic mouse fibroblast with the histone deacetylase inhibitor Trichostatin A (TSA) led to the repositioning of telomeres to the nuclear interior and centromeres towards the nuclear periphery (Uhlirova et al. 2010). This has been observed in another system where telomere and centromeres were often polarised to opposite ends of the chromosome territories (Amrichova et al. 2003). This may have further ramifications as genes located in close proximity to telomeric heterochromatin are often silenced due to the "telomeric position effect" (TPE) (Baur et al. 2001; Ning et al. 2003). There is evidence of expression changes in telomeric genes during senescence (Ning et al. 2003) with increased expression of the 16q telomeric genes MGC3101 and CPNE7 in senescence and GAS11 and CDK10 in both senescent/quiescent cells (Ning et al. 2003). Thus, change in the epigenetic status of constitutive heterochromatin could lead to senescent-specific expression patterns.

## Chromosomes and Chromosome Territories

In interphase nuclei, whole chromosomes occupy specific non-random locations within the nuclear space called chromosome territories (CTs), occupying similar locations between cell types and in vitro compared to ex vivo (Foster et al. 2012). In proliferating human fibroblasts, CTs are functionally compartmentalised with gene-rich chromosomes occupying a central position within the nucleus and are generally characterised as having higher levels of gene expression, open chromatin conformations and early replication timing (Croft et al. 1999; Cremer and Cremer 2001; Foster and Bridger 2005; Bridger et al. 2014). Conversely, gene-poor chromosomes are commonly associated with the nuclear periphery or nucleoli and are synonymous with heterochromatin, repression of gene expression, repressive histone modifications and late replication timing (Chiang et al. 2018; Croft et al. 1999). These functionally different compartments have been shown to be of importance during ageing. There is evidence that whole chromosome territories can occupy different nuclear locations during senescence (Bridger et al. 2000; Mehta et al. 2007). For instance, human chromosome 18 has been shown to occupy a peripheral position within proliferating fibroblast nuclei; however, upon replicative senescence, chromosome 18 was shown to be repositioned away from the nuclear periphery (Bridger et al. 2000). Therefore, CTs appear to be repositioned from a gene-density radial distribution in proliferating fibroblasts to a size-correlated radial

position within senescent fibroblasts whereby small chromosomes are positioned within the nuclear interior and large chromosomes are localised at the nuclear periphery (Mehta et al. 2007). Altered nuclear positioning of whole chromosomes 13 and 18 from the nuclear periphery towards the interior has also been shown in cells with A-type lamins (Meaburn et al. 2005; Meaburn et al. 2007). Indeed, genome reorganisation is more finely observed with changes in the Topologically Associated Domain (TADs) compartment re-positioning in replicative senescent cells (Criscione et al. 2016; Sun et al. 2018).

## Advances in Technologies

New technologies including CRISPR-multicolour (Ma et al. 2015) and CRISPRainbow (Ma et al. 2016) enable the study of higher-order chromatin and nuclear architecture organisation. Here, a live-cell system that utilises super-resolution microscopy is used to track genomic loci that are labelled by different coloured fluorescent-tagged dCas9-sgRNAs (Ma et al. 2016). Changes in transcriptional activity upon a specific stimulus can also be investigated by tracking the dynamics of a promoter and its interaction with cis-/trans-acting regulatory elements (Lau and Suh 2017). New advances in interrogating Hi-C data from senescent cells are permitting chromosome territory postions to be extrapolated from these data sets (Das et al. 2020).

## Summary

The complexity of the ageing genome and structural organisation of the nucleus during senescence are becoming increasingly apparent, especially with advances in microscopy and global analyses such as super-resolution microscopy and chromosome conformation capture. Generally, the ageing epigenome is characterised by global hypomethylation and loss of heterochromatin; however, on the contrary, some regions of the genome are packaged into heterochromatin, e.g. SAHF. Satellite sequences may also be altered in senescence with distension of centromeres, or SADS, and shortening or dysfunction of telomeres. Characteristic structural changes to the nucleus include an increased size in senescent cells, reorganisation of LADs and sub-NADs and nuclear envelope deformities associated with mutations in lamin and lamin-associated proteins. Together, these can lead to large-scale reorganisation of the genome with repositioning of whole chromosome territories. Overall, these fundamental changes to the epigenome lead to alterations in global gene expression and genomic instability associated with ageing (Fig. 5.2).
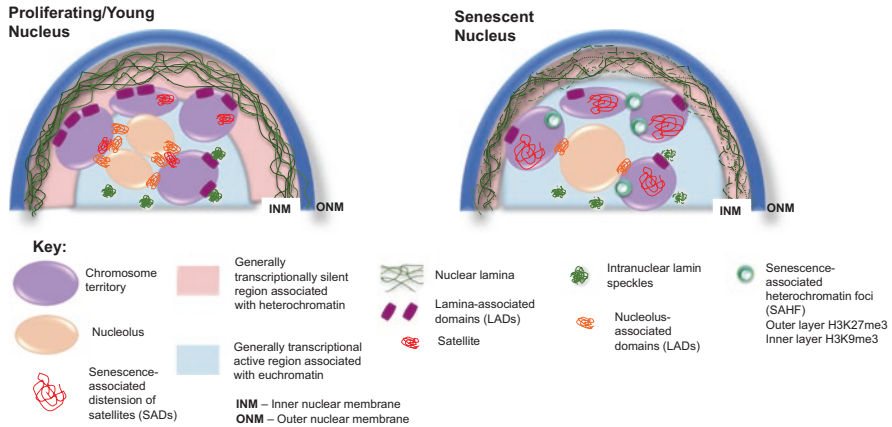
**Fig. 5.2 Differences in nuclear structure organisation within proliferating/young cells and senescent cells**. Senescence is associated with chromatin remodelling, loss of peripheral nuclear heterochromatin and an increase in hypomethylation. In addition, satellite DNA becomes unravelled to form SADs, and LADs and NADs are redistributed in senescent cells. Nucleoli may fuse and often have an increased size. SAHF formation is apparent in some senescence cells with a chromatin core enriched in H3K9me3 surrounded by an outer rim rich in H3K27me3. The nuclear lamina contains A- and B-type lamins and lamina-associated proteins that play a role in organising the genome. Within senescent and progeroid cells, the INM organisation is altered and often associated with nuclear envelope deformities

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