Chapter 8
Small Changes, Big Effects: Chromatin Goes Aging

Asmitha Lazarus, Kushal Kr. Banerjee, and Ullas Kolthur-Seetharam*

Abstract Aging is a complex trait and is influenced by multiple factors that are both intrinsic and extrinsic to the organism (Kirkwood et al. 2000; Knight 2000). Efforts to understanding the mechanisms that extend or shorten lifespan have been made since the early twentieth century. Aging is characteristically associated with a progressive decline in the overall fitness of the organism. Several studies have provided valuable information about the molecular events that accompany this process and include accumulation of nuclear and mitochondrial mutations, shortened and dysfunctional telomeres, oxidative damage of protein/DNA, senescence and apoptosis (Muller 2009). Clinical studies and work on model organisms have shown that there is an increased susceptibility to conditions such as neurological disorders, diabetes, cardiovascular diseases, degenerative syndromes and even cancers, with age (Arvanitakis et al. 2006; Lee and Kim 2006; Rodriguez and Fraga 2010).

Investigations into aging mechanisms in unicellular systems, like yeast and *in vitro* cell culture models, have identified several pathways involved in this process. In cells aging is typically associated with a senescent phenotype. Cells are known to have a limited proliferative capacity (Hayflick limit) (Hayflick 1965) and senescence can be defined as a state in which cells cease to proliferate after a finite number of divisions (Adams 2009). Some of the well-known triggers that induce senescence include DNA damage, telomere shortening and redox stress (Rodier and Campisi 2011). From literature, it is evident that in most of these cases, factors/pathways which bring about cell cycle arrest are activated and include p53/p21, and p16/RB

*Asmitha Lazarus and Kushal Kr. Banerjee have contributed equally

A. Lazarus • K.K. Banerjee • U. Kolthur-Seetharam (✉)
B-306, Department of Biological Sciences, Tata Institute of Fundamental Research, Dr. Homi Bhabha Road, Colaba, Mumbai 400 005, India
e-mail: ullas@tifr.res.in

T.K. Kundu (ed.), *Epigenetics: Development and Disease*, Subcellular Biochemistry 61,
DOI 10.1007/978-94-007-4525-4_8,

pathways (Ben-Porath and Weinberg 2005). However, the cellular/molecular signatures that characterize senescence are typically scored by induction of senescence associated β-galactosidase activity, marks of cell cycle arrest, changes in cellular morphology and/or organization, secretion of numerous proteins including cytokines and chemokines, and DNA damage (Rodier and Campisi 2011). The quest to decipher the molecular events that induce or bring about cellular senescence have unraveled the role of chromatin as an important component of this response (Misteli 2010).

The DNA in every eukaryotic cell exists as a complex with specialized proteins called histones that form chromatin. Chromatin plays a central role in processes that range from gene expression to chromosome dynamics during the cell cycle. Chromatin can be broadly categorized into two types, namely, euchromatin and heterochromatin (Bassett et al. 2009). Euchromatin appears decondensed cytologically and is mostly transcriptionally active. Heterochromatin, on the other hand, is highly compact and mostly contains transcriptionally silenced genes (Bassett et al. 2009; Frenster et al. 1963). The building block of chromatin is a nucleosome that consists of 147 base pairs of DNA wrapped around a protein octamer containing two molecules of each canonical histone H2A, H2B, H3 and H4, and is separated from one another by 10–60 base pairs of linker DNA (Luger et al. 1997). Histones contain a typical histone-fold domain, which is required to form the octamer, and their N-termini protrude out of the nucleosomes (Luger et al. 1997). Most of the residues on these histone tails are subject to posttranslational modifications (Jenuwein and Allis 2001). Some of the most prevalent modifications of histones are phosphorylation, acetylation, methylation, ubiquitination, sumoylation, ADP-ribosylation and biotinylation (Jenuwein and Allis 2001; Margueron et al. 2005). Recent reviews have illustrated biophysical and physiological consequences of such modifications on chromatin structure and function (Li and Reinberg 2011). In addition to histone and non-histone proteins that bind to DNA, modification of DNA (Cytosine methylation in eukaryotes) is also an important component of chromatin (Li and Reinberg 2011). It is interesting to note that there is a dynamic interplay between histone and DNA modifications that determine chromatin structure/function (Bonasio et al. 2010; Li and Reinberg 2011).

As mentioned earlier, the most obvious associations of DNA with aging are increased DNA damage (or reduced repair) (Seviour and Lin 2010) and telomere shortening (Kenyon and Gerson 2007). Increasing evidence in literature indicates that chromatin plays a major role in affecting both these processes (Shin et al. 2011a). In addition to these, the ability of chromatin to affect gene expression patterns in a cell has huge consequences on the ability to maintain homeostasis. Therefore, given the central role of chromatin in affecting various cellular processes, intuitively one would expect it to be a crucial component of cellular aging. In this chapter, we review the recent progress on the role of chromatin in aging. Specifically, we highlight the chromatin changes that have been associated with cellular and/or organismal aging. Importantly, we also highlight the role of histone modifiers in affecting lifespan.

8.1 Chromatin and Aging

8.1.1 DNA Methylation

DNA methylation, one of the most well studied epigenetic marks, involves the methylation of cytosines in CpG dinucleotides and is catalyzed by enzymes termed as DNA methyl transferases (DNMTs: DNMT1, DNMT3a, DNMT3b) (Jurkowska et al. 2011). It is well established that DNA methylation constitutes mechanisms required for both short-term and long-term effects on gene expression (Bonasio et al. 2010; Li and Reinberg 2011). Specifically, alterations in methylation of CpGs at upstream regulatory elements are known to modulate transcription of genes (Li and Reinberg 2011). Due to its ability to control both global and locus specific chromatin functions, DNA methylation is known to play a critical role in cellular physiology. It is important to note that key biological processes such as development, differentiation and cell death are affected by DNA methylation (De Carvalho et al. 2010; Geiman and Muegge 2010; Gibney and Nolan 2010). Its role in aging and/or senescence has been addressed in the recent past, and it is apparent that DNA methylation is one of the key factors involved in cellular and/or organismal aging (Feser and Tyler 2011; Fraga and Esteller 2007; Sedivy et al. 2008; Dimauro and David 2009). Figure 8.1 illustrates the changes in DNA methylation during aging.

An important role for DNA methylation in aging was first evidenced in replicative senescence of primary fibroblasts from mice, hamsters and humans. The study showed that in these cells, levels of 5-methylcytosine markedly declined during senescence (Wilson and Jones 1983). A follow up study demonstrated that accelerated 5-methylcytosine loss (by 5-azacytidine treatment) shortened the *in vitro* lifespan of human diploid fibroblasts (Fairweather et al. 1987). This phenomenon was reconfirmed by various *in vivo* and *in vitro* studies wherein it was observed that DNA methylation levels fell during aging, both at certain specific loci and at a genome wide level (Fairweather et al. 1987; Christensen et al. 2009; Fuke et al. 2004; Singhal et al. 1987). Interestingly, recent reports have indicated that this age-associated decline in total genomic DNA methylation occurs mostly at repetitive DNA sequences (Koch et al. 2011; Romanov and Vanyushin 1981; Singhal et al. 1987; Wilson et al. 1987). These observations have led to the speculation that the decrease in DNA methylation affects constitutive heterochromatin (DePinho 2000). Specifically, it has been suggested that with age de-heterochromatinization of repetitive regions could lead to deleterious recombinations which may cause increased incidences of age-associated diseases such as cancer (DePinho 2000). Further, the importance of DNA methylation in aging is supported by observations that show age-dependent decrease in the expression of the DNA methyltransferase (DNMT1) (see below) (Casillas et al. 2003; Lopatina et al. 2002). Supporting that the gradual loss of DNA methylation could function as a "counting hypothesis" for senescence (Hoal-van Helden and van Helden 1989; Wilson and Jones 1983), CpG methylation was shown to decrease with increased population doublings of normal cells in

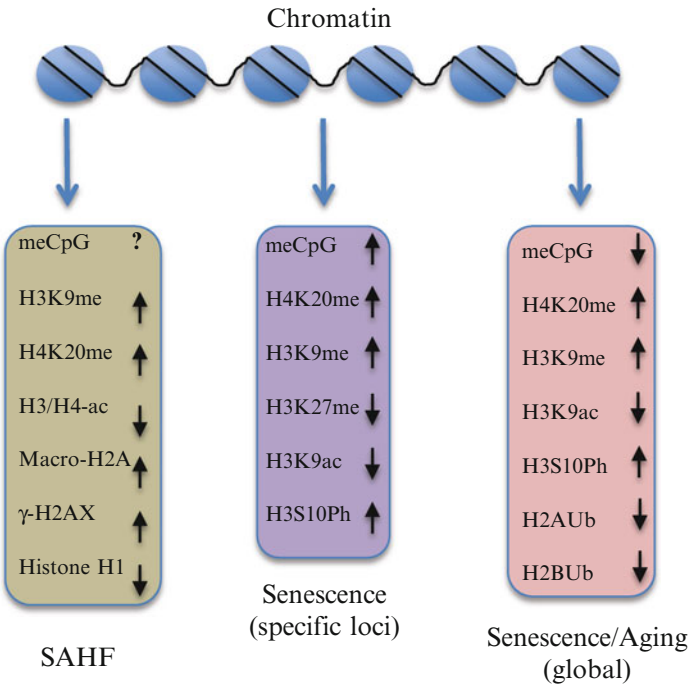

Fig. 8.1 Epigenetic changes during aging. Alterations in epigenetic marks in cultured cells and/or tissues during aging affects chromatin both globally and at specific loci. In general, histone modifications associated with heterochromatin seem to accumulate during aging. The association between DNA methylation and aging is context dependent and often determined by global and locus specific changes

culture (Fairweather et al. 1987; Wilson and Jones 1983) and during organismal aging (Hornsby et al. 1992; Singhal et al. 1987).

Contrary to observations of a decrease in global DNA methylation during aging specific loci and promoter regions of key cell cycle regulatory genes have been shown to be hypermethylated (Fig. 8.1). For example, the Estrogen receptor gene (Issa et al. 1994), *INK4A/ARF/INK4b* locus (which codes for p16, p14 and p15 proteins respectively) (Koch et al. 2011), ribosomal RNA genes (Swisshelm et al. 1990; Oakes et al. 2003) and multiple tumor suppressor or tumour-associated genes like APC and E-cadherin (Bornman et al. 2001; Waki et al. 2003) accumulate DNA methylation during aging. Interestingly, in another study it was observed that DNA methylation levels were maintained in long-term culture of mesenchymal stromal cells (MSE) and MSEs from young and old donors. However, they exhibited differential DNA methylation patterns at specific loci, like in the homeobox genes and genes involved in cell differentiation (Bork et al. 2010). It has been hypothesized that this locus specific hypermethylation in the background of a global reduction of

methyl-CpGs could be due to an increase in Dnmt3b expression that has been observed in senescent cells (Casillas et al. 2003; So et al. 2006).

Observations from some studies suggest that hypermethylation depends upon the prevalent density of methyl-cytosines at specific loci with sparsely methylated regions more amenable to hypermethylation (Song et al. 2002; Stirzaker et al. 2004). These observations have led to a 'seeds of methylation' hypothesis based on increasing CpG methylation levels (Rakyan et al. 2010). A recent genome-scale study addressed dynamic changes in the epigenome in normal human aging (Rakyan et al. 2010). This report identified aging-associated differentially methylated regions (aDMRs) that gain methylation with age in different tissues, thus suggesting that aDMR signature is a multi-tissue phenomenon. Further, it was also demonstrated that aging associated DNA hypermethylation occurs predominantly at bivalent chromatin/promoters (Rakyan et al. 2010). These studies point out an interesting aspect of locus specific methylation contributing to aging. In this scenario one would expect an inherent bias in methylation rates at loci that would ultimately (or cumulatively) result in a senescent phenotype.

8.1.2 Histone Modifications

As mentioned in the introduction, histone modifications are one of the most central elements that affect chromatin structure and function. The most common and well-studied histone modifications that are known to impact chromatin are acetylation of lysines, methylation of lysines and arginines, phosphorylation of serine and threonine, and ubiquitination of lysines (Jenuwein and Allis 2001; Margueron et al. 2005). It is evident from literature that interfering with these modifications affects both global and locus specific chromatin, and as a consequence impinges on various cellular processes (Murr 2010). Some recent reviews provide exhaustive information about histone modifications and their role in chromatin structure and function. Figure 8.1 illustrates the histone modifications, which are associated with 'open or closed' chromatin and their associations with aging.

Specific histone modifications undergo distinct changes in profile during aging (Fig. 8.1). The levels of histone H4 lysine-20 tri-methylation (H4K20Me3), a mark of constitutive heterochromatin and which is enriched in differentiated cells, have been found to increase in senescent cells. This has been speculated to cause the accumulation of heterochromatic structures in senescent human fibroblasts (Narita et al. 2003). The total abundance of histone H4K20Me3 has also been reported to increase with age in rat liver and kidney (Kouzarides 2007; Sarg et al. 2002), supporting the notion that heterochromatin may accumulate with tissue aging, at least at some sites. In another study, Bracken et al. observed a loss of histone H3 lysine-27 tri-methylation (H3K27Me3), a mark associated with silent chromatin, at the *INK4b* and *INK4a–ARF* loci in senescent human diploid lung embryonic fibroblast cell line (Bracken et al. 2007). This decrease in H3K27Me3 was accompanied by a decrease in EZH2, the histone methyltransferase responsible for this modification

(Bracken et al. 2007). Several groups have studied changes in histone H3 modifications with age in rat liver. They found that histone H3 lysine-9 acetylation (H3K9Ac) decreased and histone H3 Serine-10 phosphorylation (H3S10Ph) increased with age significantly (Braig et al. 2005; O'Sullivan et al. 2010; Kawakami et al. 2009). These independent observations both in cells in culture and in aged animals clearly establish a positive correlation between heterochromatic marks and aging (Fig. 8.1).

Mono-ubiquitination of histones H2A and H2B is known to alter chromatin dynamics and regulate gene expression. While H2A ubiquitination leads to silencing, ubiquitination of H2B has been implicated in active transcription. Interestingly, these modifications have been associated with aging. The link between histone ubiquitination and aging was first demonstrated by a study which showed that the proportion of ubiquitinated histones was about 30% higher in old mice than in young ones (Morimoto et al. 1993). However, reduced expressions of H2B ubiquitin ligases RNF20/Bre1 have been associated with senescence/aging phenotypes. In yeast, absence of Bre1 results in reduced lifespan during chronological aging due to enhanced apoptotic cell death (Walter et al. 2010). Similarly, depletion of RNF20 has been shown to induce cellular senescence in glioma cells (Gao et al. 2011). Like H2B, ubiquitination of histone H2A has also been implicated in aging. Downregulation of BMI1, a component of the polycomb repressive complex (PRC), which ubiquitinates histone H2A (Cao et al. 2005) has been shown to result in derepression of growth inhibitory genes and putative tumor suppressors. As a consequence these cells display premature senescence and apoptosis (Bommi et al. 2010). Although, these studies suggest that histone ubiquitination is involved in aging, whether these effects are mediated through alterations in global chromatin architecture or transcription of specific genes is still not clear.

8.1.3 Senescence Associated Heterochromatic Foci (SAHF)

Cells grown in culture have provided valuable insights into aging mechanisms. In this regard, most of our understanding of the role of chromatin on aging has come from studies on senescing cells. Not surprisingly, alterations of chromatin structure are associated with the irreversible state of senescent cells (Braig and Schmitt 2006; Narita et al. 2003). Many senescent human cells, when stained with the DNA staining dye 4′, 6-diamidino-2-phenylindole (DAPI), show visible punctuate DNA foci known as senescence associated heterochromatic foci (SAHF), a new type of facultative heterochromatin (Narita 2007; Narita et al. 2003). RNA-FISH and *in situ* labeling of nascent RNAs demonstrate that SAHF contain transcriptionally inactive chromatin (Funayama et al. 2006; Narita 2007). In addition to being transcriptionally inactive, these loci are also enriched with typical proteins that are found in heterochromatin. For example, SAHFs in general contain heterochromatin protein-1 (HP1), repressive histone modifications like H3K9 methylation and hypoacetylated histones (Narita et al. 2006). However, these SAHFs do not show some usual marks of condensed chromatin, like the phosphorylation of histone H3 at Serine-10 or

Serine-28, marks of mitotic chromatin or of histone H2B at Serine-14, a mark of apoptotic chromatin (Funayama et al. 2006; Peterson and Laniel 2004).

It is important to note that in addition to changes in histone modifications, histone chaperones, and alterations in chromatin composition have also been implicated in senescence. For example, studies have shown that the formation of SAHF during cellular senescence depends on histone H3 chaperones, ASF1 (anti-silencing function 1) (Zhang et al. 2005) and HIRA (histone cell cycle regulation defective homologue A) (Ye et al. 2007). Interestingly, these loci are also known to contain variants of histones which have been otherwise associated with silenced chromatin. SAHFs are enriched with macro-H2A (histone H2A variant) that is mainly required for inactivation of X-chromosome (Costanzi and Pehrson 1998; Funayama et al. 2006; Zhang et al. 2005). The role of histone variants in the formation of SAHF is also supported by findings that report an increase in γ-H2AX in early neoplastic lesions that contain senescent cells *in vivo* and also in aging tissues. This is thought to contribute to senescence and proliferation arrest of damaged cells (Bartkova et al. 2006; Herbig et al. 2006). Chromatin compaction can also be altered by the recruitment of factors that are known to replace histone HI and bind to linker DNA. In this regard, the finding which shows that in SAHFs there is a decrease in linker histone H1 occupancy and increased levels of chromatin-bound high mobility group-A proteins (HMGA) becomes relevant (Funayama et al. 2006; Narita et al. 2006). The exact molecular mechanisms of SAHF formation are not very clear, but independent studies have demonstrated that the ectopic expression of either HMGA1 or HMGA2 induces SAHF formation and other senescence phenotypes in normal human fibroblasts. It was also observed that knockdown of HMGA proteins by RNAi prevents SAHF formation, thus indicating that HMGA are essential components for SAHF formation (Funayama et al. 2006; Narita et al. 2006). It has been speculated that the DNA-bending properties of HMG family proteins may help induce SAHF formation by binding and bending linker DNA (Hock et al. 2007; Paull et al. 1993).

Formation of heterochromatin is often facilitated by enzymatic activities that are known to repress transcription. Notably, histone deacetylases and histone methyl transferases that add 'repressive chromatin marks' play essential roles in heterochromatin formation. The Sin3 multiprotein complex is a repressor complex recruited by several sequence specific transcription factors. The repressor activity of the Sin3 complex is brought about by the Sin3A/Sin3B-associated HDAC1 and HDAC2 proteins. A study by Grandinetti et al. has demonstrated that Sin3B-null fibroblasts are resistant to replicative and oncogene-induced senescence (Grandinetti et al. 2009). They also showed that over-expression of Sin3B triggers senescence and the formation of SAHF. However, the role of histone deacetylation in inducing SAHF seems to be HDAC specific. While Sin3 complex via HDAC activity aids in the formation of SAHFs, a study by Huang et al. has suggested that Sirt1, a NAD^+-dependent deacetylase (described below), antagonizes cellular senescence in human diploid fibroblasts (Huang et al. 2008). Their experiments demonstrated that over-expressing Sirt1 led to a reduction of senescence associated biomarkers, which included the formation of SAHFs (Huang et al. 2008).

Although, SAHFs seem to bring about a global change in chromatin architecture, it is not clear if SAHF formation contributes to senescence. In support of SAHF contributing to senescence, evidence show that SAHF formation contributes to stable proliferative arrest by repressing transcription of E2F target genes that are required for G1 to S phase transition (Narita et al. 2003). Chromatin immunoprecipitation analyses have demonstrated that the promoters of E2F target genes become heterochromatic in senescent cells but not in proliferating or quiescent cells. In addition, overexpression of E2F-1 was not able to derepress these genes indicating heterochromatinization mediated transcriptional silencing (Narita et al. 2003). Interestingly, SAHF-dependent silencing of E2F genes requires the retinoblastoma (Rb) protein at these gene promoters (Narita et al. 2003). Further, studies have shown that Rb associates with HP1 and the histone methyltransferase Suv39H1 to facilitate senescence. Specifically, Rb family members have been shown to interact with HDAC1, DNA methyltransferase and polycomb proteins among other transcriptional co-repressors to repress the activity of E2F1 (Trimarchi and Lees 2002; Narita et al. 2003). Prohibitin, a protein implicated in cell cycle control and antiproliferative activities, is found in SAHF and colocalizes with HP1 (Rastogi et al. 2006). This finding suggests that SAHFs might actively contribute to senescence. In this study prohibitin, Suv39H1 and HP1 were detected on E2F target promoters during senescence, and a deletion of prohibitin led to a loss of senescent phenotype (Rastogi et al. 2006).

Although, there is a lot of evidence to suggest that SAHF formation is important for induction of senescence, formation of heterochromatin itself seems to be the most important feature of senescence. A recent study has shown an increase in the abundance of heterochromatin proteins and marks in senescence but without the formation of SAHF (Kosar et al. 2011). Hence, local heterochromatinization, but not global SAHF, may induce senescence-associated proliferation arrest by mediating the silencing of proliferation genes.

8.1.4 microRNAs, Epigenetics and Aging

MicroRNAs are ~22 bases long RNAs, which bind to the 3′UTR of target mRNAs and regulate gene expression post-transcriptionally by translational inhibition or mRNA degradation (He and Hannon 2004). Due to their ability to target multiple mRNAs, they are now considered as major factors that affect cellular physiology (He and Hannon 2004; Sayed and Abdellatif 2011). Originally appreciated for their role in cancer and development, microRNAs have also been shown to be involved in regulating factors or pathways, which impinge on aging. In the recent past, studies have identified many microRNAs that target ‘aging’ factors and several reviews have highlighted these reports (Bates et al. 2009; Gorospe and Abdelmohsen 2011; Grillari and Grillari-Voglauer 2010). Rather than detailing microRNAs and their targets that have been implicated in aging, we specifically highlight studies that have addressed altered expression of microRNAs during aging.

Intriguingly, global microarray profiling studies suggest that more microRNAs are upregulated rather than downregulated during aging (Li et al. 2011; Maes et al. 2008; Zhang et al. 2010). It is important to note that upregulation of some of these microRNAs have been implicated in regulating the expression of genes, which are known to affect organismal physiology. For example, miR-669c and miR-709 (up-regulated at 18 months with a maximum expression at 33 months), and miR-93 and miR-214 (up-regulated around 33 months) have been shown to target genes associated with detoxification and regenerative capacity of the liver, functions that slowly decline in aged liver (Maes et al. 2008). In another study, Bates et al. profiled microRNAs regulated in the liver of Ames dwarf mice, which display a delayed onset of aging (Steuerwald et al. 2010). They found that miR-27a is upregulated in these dwarf mice at an early age. Their results also suggest that miR-27a regulates two key metabolic proteins ornithine decarboxylase and spermidine synthase. Based on these observations the authors have speculated that miR-dependent regulation of metabolic pathways such as glutathione metabolism, urea cycle, and polyamine biosynthesis maybe important for health span and longevity in these mice (Bates et al. 2010). However, studies which link microRNAs with DNA repair or cell proliferation pathways have raised the possibility that age related alterations in microRNA expressions maybe relevant in mediating the aging process (Chen et al. 2010).

The link between microRNAs and aging has been further strengthened by a study in which reducing the activity of *C. elegans* linage 4 (*lin-4*) microRNA shortened lifespan and its overexpression led to a longevity phenotype (Boehm and Slack 2005). Another study that looked at senescence in normal human keratinocytes (NHK) found microRNAs miR-137 and miR-668 to be upregulated during replicative senescence (Shin et al. 2011b). Interestingly, induction of senescence by ectopic over-expression of miR-137 and miR-668 was associated with an increase in senescence associated (SA) β-galactosidase activity, p53 and p16INK4A levels. Further, expressions of these microRNAs were also observed to be elevated during organismal aging of normal human oral epithelia (Shin et al. 2011b).

Although, it is increasingly becoming apparent that microRNAs play a vital role in regulating aging, very little is known about epigenetic changes that mediate the expression of such key microRNAs. Recent studies have clearly shown that microRNA expression is regulated by epigenetic marks (Liang et al. 2009). Importantly, their promoters have been shown to exhibit differential DNA methylation and histone modifications, that are reminiscent of modifications on protein coding genes (Lee et al. 2011; Saito and Jones 2006). Lee et al. have demonstrated that inhibition of HDACs triggers cellular senescence by inducing the expression of miR-23a, miR-26a and miR-30a. Interestingly, these microRNAs target and downregulate HMGA2 expression that has been associated with induction of senescence (Lee et al. 2011).

Further studies aimed at profiling microRNAs during aging, and in specific tissues, will aid in appreciating the regulation of pathways that mediate lifespans of organisms. Importantly, investigating the mechanisms that control mircoRNA expression, specifically histone deacetylases and DNA methyltransferases

(which have been associated with aging, see below), will highlight the importance of posttranscriptional control of 'aging genes'. In addition, such insights will provide a holistic picture of changes in gene regulation, mediated by chromatin modifiers, in affecting organismal longevity.

8.2 Role of Chromatin Modifiers in Aging

The previous section highlights the importance of chromatin associated changes in aging and cellular senescence. Although, it is clear that these changes are strong correlates of aging, whether they are causal factors or mere consequences of aging remains unclear (Dimauro and David 2009). Also, aging/senescence dependent changes that the enzymes which affect these modifications themselves undergo are less appreciated. As reviewed elsewhere, post-translational modifications of histones are catalyzed by specific enzymatic machineries (Bannister and Kouzarides 2011). Histone acetylation is affected by opposing activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Legube and Trouche 2003). Separate families of enzymes are known to methylate and demethylate lysine/arginine residues in histones (Yoshimi and Kurokawa 2011). Interestingly, unlike these modifications histone phosphorylation and dephosphorylation are brought about by a diverse set of enzymes (Hans and Dimitrov 2001). In this section, we have attempted to review the studies which have given us insights into the role of histone modifiers during aging. Specifically, we will look at the important classes of chromatin modifiers: DNMTs, Histone acetyltransferases Histone deacetylases and Sirtuins (Table 8.1).

Table 8.1 List of chromatin modifiers and their association with aging or senescence

Chromatin modifier	Modification site	Modification	Alteration with age	Role in aging
DNMT1	CpG dinucleotide	Methylation	Decrease	Anti-senescent
DNMT3a	CpG dinucleotide	Methylation	Increase	Locus specific effects
DNMT3b	CpG dinucleotide	Methylation	Increase	Locus specific effects
Mof (HAT)	H4K16	Acetylation	Increase	Pro-senescent
Sas2 (HAT)	H4K16	Acetylation	Increase	Pro-senescent
CBP (HAT)	H3K9, H3K27, H3K56	Acetylation	Decrease	Anti-senescent
P300 (HAT)	H3K9, H3K27, H3K56	Acetylation	Decrease	Anti-senescent
HDAC1	H3K9, H3K56, H4K16	Deacetylation	Increase	Pro-senescent
SIRT1	H3K9, H4K16	Deacetylation	Decrease	Anti-senescent
SIRT6	H3K9	Deacetylation	?	Anti-senescent
EZH2 (HMT)	H3K27	Methylation	Decrease	Pro-senescence

The table illustrates the roles of DNMTs, HMTases, HATs, HDACs and Sirtuins, and depicts changes in their expression during aging

8.2.1 DNA Methyl Transferases (DNMTs)

DNA methyltransferases (DNMTs) catalyze the addition of a methyl group to DNA on cytosines, typically in CpG dinucleotides. DNA methylation has been associated with gene silencing and robust regulation of transcription. DNMT mediated DNA methylation brings about chromatin silencing by inducing the formation of heterochromatin through recruitment of specific proteins like Methyl CpG binding proteins, MeCP2 (Kimura and Shiota 2003) and MBD (Fujita et al. 2003; Villa et al. 2006). It is interesting to note that DNMTs have been shown to be in complex with histone methyl transferases and histone deacetylases. In mammals there are three DNA methyltransferases, namely, Dnmt1, Dnmt3a and Dnmt3b. Dnmt1 is considered as a maintenance methylase since it methylates newly replicated DNA using hemimethylated DNA as a substrate. Dnmt3a and 3b mediate *de novo* methylation, that is, they can methylate previously unmethylated DNA.

The role of DNMTs in aging has been addressed in the recent past because of their ability to affect chromatin/epigenetic modifications. In addition, previous reports have also correlated changes in DNA methylation during aging. Several studies have thrown light upon changes in DNMT levels and activity that may have crucial roles in cellular senescence (Lopatina et al. 2003; Vogt et al. 1998). Consistent with previous observations of a decrease in global DNA methylation in senescence, studies have shown that the levels and activity of the maintenance methylase Dnmt1 decrease in aging fibroblast cells. However, an increase in Dnmt-3a and -3b activity was observed which raises the possibility of a compensatory role for these DNMTs (Casillas et al. 2003; Lopatina et al. 2003). Although, it was long observed that promoter hypermethylation of cell cycle inhibitory genes, $p16^{INK4A}$ and $p21^{CIP1/WAF1}$ tipped the balance between senescence and oncogenesis, a recent report shows the involvement of DNMTs, and their findings have provided further support to the hypothesis that DNMTs are important for inducing senescence. The authors of this study observed that upon inhibition of DNMT1 and DNMT3b in human umbilical cord blood-derived multipotent stem cells (hUCB-MSCs), p16 and p21 expression increased and activated senescence in these cells (So et al. 2011). Not surprisingly, several studies have shown that DNMTs are overexpressed in cancer cell lines resulting in silencing of the expression of p16 (So et al. 2011; Yang et al. 2001).

Calorie/Dietary restriction (CR/DR) is one of the interventions that has been commonly used to understand the molecular factors involved in aging. Although, as previously mentioned it is unclear whether DNMTs play a deterministic role in aging, studies have indicated that Dnmt3a levels in the mouse hippocampus change when the animals are subjected to dietary restriction (Chouliaras et al. 2011a, b). This study also sheds light on the possibility that one of the major mechanisms by which CR/DR mediates organismal aging is by modulating DNA methylation status by regulating expression levels/enzymatic activities of individual DNMTs. However, it is still unclear if DNMTs can by themselves induce senescence or whether other factors that initiate or require chromatin changes affect their expression and/or activities during aging. Nevertheless, these findings clearly indicate that the changes

in DNMT expressions can lead to alterations in chromatin structure during aging by influencing both global and gene specific methyl-CpG levels and/or distribution. In spite of these reports that indicate strong associations between DNMT expression/activities, DNA methylation patterns and aging, it is surprising to find that there are very few attempts to map the changes in DNMT localizations on a genome wide scale. Further, it would be interesting to similarly analyze genome-wide alterations in DNA methylation profiles in various model systems that are known to extend their lifespans in response to dietary interventions.

8.2.2 Histone Acetyl Transferases and Histone Deacetylases

Besides DNA methylation, histone modifications have become the most important determinants of chromatin structure/function involved in various cellular outputs. Specifically, histone acetylation has been one of the hallmarks of active gene transcription and often influences other modifications of histones such as methylation. As previously mentioned, histone acetylation-deacetylation reactions are catalyzed by histone acetyl transferases and histone deacetylases, respectively. These enzymes have been implicated in aging from studies that have attempted to decipher the changes observed in histone modifications during aging and/or map the genetic factors involved in aging. Although, very little is known about the role of HATs, HDACs have been well addressed with regards to their involvement in cellular or organismal aging.

HATs: An important HAT that has been linked to aging is Mof, which mediates acetylation at the H4 lysine 16 (H4K16) residue. Mof has been shown to be important for the maintenance of genome stability and its depletion leads to delayed γ-H2AX foci formation in response to DNA damage and abrogated DNA damage repair. Mof has also been shown to be an important regulator of DNA damage because of its ability to bind to 53BP1 (Krishnan et al. 2011). Mof associates with the nuclear matrix and is a key component of the pre-lamin A complex. In a very recent study, Vaidehi Krishnan et al. have shown that in mice that lack the zinc metalloproteinase (Zmpste 24), Mof localization at the nuclear matrix decreases (Krishnan et al. 2011). This effect has been linked to the accumulation of unprocessed pre-lamin A, which is associated with progeroid symptoms. Incidentally, depletion of Mof has also been shown to exacerbate the senescent phenotype of cell lines that lack Zmpste 24. In support of this, overexpression of Mof has been associated with hyperacetylation of H4K16 and a delay in cellular senescence (Hajji et al. 2010). Another HAT that seems to be an important mediator of aging is Sas2. Studies in *S. cerevisiae* have shown that Sas2 inactivation leads to delayed senescence due to activation of homologous recombination (HR) machinery at telomeric regions, thus delaying senescence by preventing telomere loss (Kozak et al. 2010).

Several studies show that the HATs p300 and CBP are important regulators of senescence (Bandyopadhyay et al. 2002; He et al. 2011; Pedeux et al. 2005; Prieur

et al. 2011). The study by Prieur et al. showed that p300 is an important regulator of chromatin dependent mediator of senescence and that this mechanism is independent of p53, p21 and p16 (Prieur et al. 2011).

HDACs: There are four classes of HDACs and specifically, Sirtuins that belong to Class-III HDACs are distinct in their activity because of their dependence on NAD^+. We have described the role of sirtuins in aging in a separate section below. Among the other HDACs, members that belong to Class I have been so far implicated for their potential roles in aging. Several studies have indicated that inhibition of HDAC activity leads to induction of a senescent phenotype. June Munro et al. in their study show that administration of HDAC inhibitors sodium butyrate and trichostatin A (TSA) induces senescence in human fibroblasts. These cells exhibit typical senescent phenotype such as β-galactosidase staining, in addition to an elevation in cyclin-Cdk inhibitors, p21and p16 (Munro et al. 2004). It is interesting to note that HDAC antagonists are potent inhibitors of cancer cell proliferation or tumorigenesis. Suberoylanilide hydroxamic acid (SAHA), is an HDAC inhibitor which is used as an anti-tumor drug. SAHA has been shown to induce polyploidy in human colon cancer cell line HCT116 and human breast cancer cell lines, MCF-7, MDA-MB- 231, and MBA-MD-468, which activates senescence in these cells (Xu et al. 2005). Results that corroborate these findings also show that senescence is accompanied by a decrease in HDAC expression. Contradictory observations regarding the role of HDAC activity and aging have also been made. A recent study showed that HDAC1 overexpression inhibited cell proliferation and induced premature senescence in cervical cancer cells through a pathway that involved the deacetylase Sp1, protein phosphatase A PP2A and retinoblastoma protein Rb (Chuang and Hung 2011).

Contrary to what has been observed in cells in culture, organismal studies have indicated that a reduced/absence of HDAC expression/activity leads to lifespan extension. Rpd3 is an HDAC found in all organisms. A study by Rogina et al. showed that reduction in Rpd3 levels in *Drosophila* renders them a longer lifespan. Moreover, these mutants fail to increase their lifespan any further in response to CR/DR (Rogina and Helfand 2004). It has to be noted that the molecular mechanisms of HDAC-dependent changes in aging and lifespan are not well understood. Interestingly, administering TSA to flies also leads to an extension in lifespan, which is accompanied by an increase in Hsp22 protein levels (Tao et al. 2004; Zhao et al. 2005). However, the global changes in histone acetylation and chromatin architecture that would be associated with an absence or inhibition of HDACs have not been addressed, yet. It is unclear if these results depict physiological differences which are elicited by HDAC inhibition at the cellular and organismal levels. It is also likely that different family members which belong to HDACs have varied roles and involve altered substrate specificities. It has to be noted that HDACs are known to deacetylate and affect non-histone proteins as well (Thevenet et al. 2004; Gregoire et al. 2007). Further analysis of individual HDAC proteins may identify their individual functions in mechanisms that induce senescence.

8.2.3 Sirtuins

Sir2 is the founding member of an evolutionarily conserved family of proteins that was first identified in *S. cerevisiae*. Sir2 has been classified as a Class III HDAC and it has been shown to depend on NAD^+ as a co-substrate for its deacetylase activity (Ghosh et al. 2010; Imai and Guarente 2010; Zhang and Kraus 2010). The role of Sir2 in regulation of chromatin has been attributed to its ability to deacetylate specific residues in histones H3 (lysine-9) and H4 (lysine-16). Sir2 activity is required to silence chromatin at sub-telomeric DNA, mating-type and ribosomal DNA (rDNA) loci (Fig. 8.2) (Ha and Huh 2011; Kaeberlein et al. 1999; Rusche et al. 2003). Further studies in yeast established Sir2 as a key link between chromatin regulation and aging. In *S. cerevisiae*, it is known that recombination at rDNA regions leads to the formation of extra chromosomal rDNA circles, which reduce replicative lifespan (Sinclair and Guarente 1997). The ability of Sir2 to mediate silencing at rDNA is considered paramount for its role as a negative regulator of aging in yeast. Additionally, a study by Dang et al. shows that in replicatively aged yeast cells, a decline in Sir2 levels correlates with an increase in acetylation of H4K16 (Dang et al. 2009).

Studies aimed at deciphering an "anti-aging" function of Sir2 in worms and flies corroborated the findings in yeast. In *C. elegans*, Sir2 is known to extend lifespan and interact with insulin IGF signaling. Similar reports in *D. melanogaster* have shown that the Sir2 ortholog mediates CR/DR dependent lifespan extension (Rogina and Helfand 2004). However, the molecular mechanisms, which are affected by Sir2 in these organisms that regulate lifespan extensions, are still unclear. The chromatin regulatory function of Sir2 orthologs in other organisms (flies and mammals) has also been addressed. From these studies it becomes evident that the role of Sir2 and its orthologs in regulating chromatin-mediated changes is evolutionarily conserved. Sir2 has been identified as a regulator of heterochromatin formation in flies and affects position effect variegation (PEV). Results indicate that the role of Sir2 in PEV is independent of its ability to extend lifespan (Frankel and Rogina 2005; Newman et al. 2002). However, whether its role in mediating locus specific chromatin changes is linked to its role in lifespan extension is still unclear.

In mammals, SIRT1, the homolog of ySir2 has been shown to be a major regulator of chromatin structure and gene expression. SIRT1 is a well-established regulator of transcription by deacetylating a host of transcription factors and co-regulators (Table 8.2) (Brooks and Gu 2008; Deng 2009). It should be noted that some of these transcription factors, such as NF-kB, FOXO and p53, have been implicated in organismal aging. However, the role of SIRT1 in mammalian aging has been difficult to address as most SIRT1 null mice die due to developmental defects. Mammalian SIRT1 regulates chromatin dynamics by mediating deacetylation of H3 lysine 9 (H3K9) and H4 lysine 16 (H4K16). In addition to contributing to histone acetylation changes, SIRT1 has also been shown to impinge on other mediators of chromatin. Importantly, SIRT1 is known to cross-talk with DNMTs (O'Hagan et al. 2008) and histone methyltransferases (Vaquero et al. 2004). SIRT1 has been shown to bind and

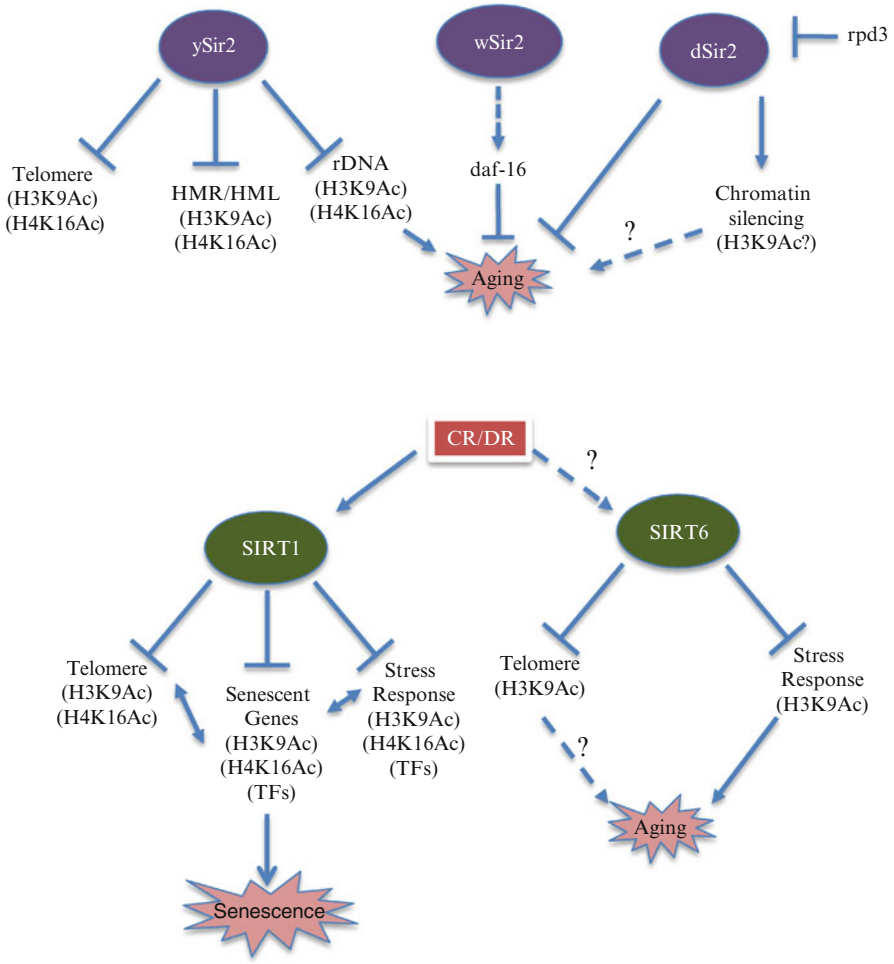

Fig. 8.2 The role of Sirtuins in aging across species. Sir2 and its homologues (including Sirt1 and Sirt6 in mammals) are key players in cellular/organismal aging. Studies in yeast, flies and mammals show that Sir2, Sirt1 and Sirt6 are NAD+-dependent deacetylases and affect chromatin by deacetylating histones (H3K9 or H4K16) as illustrated. Except in yeast, the link between sirtuins and aging is not limited to its role in affecting chromatin since they are known to regulate other pathways/factors. In worms and flies where Sir2 is now known to extend lifespan (and in response to calorie/dietary restriction) the chromatin angle in mediating this effect is still unclear. SIRT1 and SIRT6 are important in regulating the expression of a host of genes that mediate senescence, in addition to their roles at the telomere

deacetylate SUV39H1 which brings about H3K9 trimethylation (Vaquero et al. 2004). It is speculated that SIRT1 mediated histone deacetylation renders the site open for methylation. Additionally, a loss of SIRT1 is associated with a reduction in H3K9me3 levels and a concomitant impairment of heterochromatin protein-1 (HP1)

Table 8.2 Sirt1 deacetylation targets which have been implicated in aging/senescence

Interactor	Biological function
p53	Tumor suppressor and cell cycle regulator
p73	Tumor suppressor and cell cycle regulator
Ezh2	Histone methyl transferase that maintains transcriptionally repressed state
E2F1	A transcription factor important for G1/S transition
PCAF	An acetyltransferase that inhibits apoptosis
RelA/p65	A transcription factor that regulates transcription of NFkB target genes
FOXOs	A family of transcription factors that regulates cell cycle and stress response
SUV39H1	A histone methyl transferase that is important for the maintenance of heterochromatin state

recruitment. Together, these have been proposed to affect heterochromatin formation (Vaquero et al. 2007).

Independent studies have shown that SIRT1 plays a crucial role in cellular senescence. The study by Langley et al. was the first study which showed that SIRT1 negatively regulates cellular aging in mammalian cells (Langley et al. 2002). The authors showed that SIRT1 binds, deacetylates and inhibits p53 transactivation activity leading to its anti-senescent property. Subsequent studies identified that the anti-senescence effects of SIRT1 was a common feature of multiple cell types including human diploid fibroblasts (Huang et al. 2008), human umbilical vein endothelial cell line (Ota et al. 2007) and several cancer cell lines like breast cancer MCF-7, lung cancer H1299 and prostate cancer cells (Jung-Hynes et al. 2009; Ota et al. 2006). Importantly, SIRT1 is known to specifically repress genes involved in cell cycle arrest such as p16 (Huang et al. 2008, p. 21; Rathbone et al. 2008; Yuan et al. 2011, p. 27; Ota et al. 2006).

It is interesting to note that SIRT1 activity and/or levels have been proposed to decrease during aging in cells and mice (Yamakuchi et al. 2008). However, a clear picture that links the chromatin functions of SIRT1 and its role in cellular senescence is still not available. In support of such a role, reports that indicate chromatin relocalization of SIRT1 during aging imply a possible chromatin dependent effect of SIRT1 in aging/senescence (Oberdoerffer et al. 2008)**.** This finding is reminiscent of a similar phenomenon in yeast where the Sir2 redistribution on the genome has been observed in aging yeast cells (Gotta et al. 1997). It is clear that SIRT1 is important for the maintenance of telomeric chromatin in mammalian cell lines (Palacios et al. 2010). However, it is still not known if the functions of SIRT1 at the telomere are important for its role in cellular senescence. Further investigations are required to appreciate the link between Sir2/SIRT1 dependent global and/or locus specific chromatin changes and aging.

SIRT6 another important mammalian sirtuin has been clearly shown to play a major role in aging (Fig. 8.2). Mice deficient for SIRT6 exhibited progeroid symptoms (Kawahara et al. 2011) and results suggest that its ability to regulate DNA damage repair pathways were key to its role in aging (Mostoslavsky et al. 2006). Subsequently, SIRT6 was shown to deacetylate histone H3 at lysine 9 residue (Michishita et al. 2008), which incidentally is also targeted by SIRT1 (Vaquero et al. 2004). Reports that elucidated the ability of SIRT6 to regulate NF-kB dependent transcription showed that its role in aging is mostly determined by its ability to regulate inflammatory responses (Kawahara et al. 2011). It has been suggested that a dynamic relocalization of Sirt6 on chromatin is important for its ability to regulate organismal aging by controlling the expression of essential aging related genes, many of which are NF-kB targets (Kawahara et al. 2011). Further, SIRT6 has been shown to prevent telomere dysfunction in human cells by deacetylating H3K9 at telomeric loci, although, such an effect has not been observed in SIRT6 null mice (Michishita et al. 2008). Put together, it is evident that the functions of SIRT6 in mediating stress responses and at the telomere might have a bearing on aging and is probably dependent on its ability to deacetylate H3K9 residue. It is interesting to note that although both SIRT1 and SIRT6 have been implicated in similar pathways and at telomere functions, it is still unclear if they bring about a coordinated response to regulate aging.

8.3 Progeroid Syndromes and Chromatin

Progeroid syndromes are characterized by symptoms that mimic aging. Two of the most well studied clinical progeroid conditions are Werner syndrome and Hutchinson-Gilford progeria syndrome (HGPS). Werner's is a progeroid syndrome caused by mutations in the *WRN* gene, which encodes a member of the RecQ family of helicases. Intriguingly, some features of this disorder are also present in laminopathies caused by mutant *LMNA* encoding nuclear lamins A/C that causes HGPS. Recent studies suggest that epigenetic modifications in these progeroid genes lead to malignant transformation (Shumaker et al. 2006).

In HGPS, Lamin A gene is mutated resulting in a cryptic splice site in exon-11 causing 150 nucleotide deletion (LAΔ50). It was interesting to find that HGPS was associated with global changes in nuclear and chromatin architecture. Specifically, HGPS fibroblasts exhibit a loss of nuclear peripheral heterochromatin (Dechat et al. 2008), the severity of which depends on the accumulation of the abnormal LAΔ50 protein (Goldman et al. 2004). In addition, in cells derived from older HPGS patients, several heterochromatin marks, such as mono- and tri-methylated H3K9, show a dramatic decrease. A loss of the H3K27 tri-methyl mark was also observed in these cells and was correlated with a nine- to ten-fold decrease in the histone methyltransferase EZH2 expression. Another study, which looked at late-passage HGPS cells, observed an up-regulation of H4K20 tri-methylation (Shumaker et al. 2006). It is important to note that H4K20 tri-methylation has been shown to be

elevated in livers of older rats (Sarg et al. 2002) and in SAHFs in cultured cells (described above). The molecular mechanisms that link lamin A to heterochromatin formation are still not very clear. However, studies suggest that retinoblastoma protein (Rb) binds directly to type-A lamins (Ozaki et al. 1994; Johnson et al. 2004). Based on independent observations that Rb regulates histone methylation at H3K27, H3K9 and H4K20 residues, it has been speculated that Rb could be one of the factors that links aberrant histone methylation in HGPS (Blais et al. 2007).

Werner syndrome (WS) provides another example of a gene involved in aging and with tumor suppressor properties. As a result of the mutation in the *WRN* gene, cells from patients with WS show high genomic instability, especially at repetitive loci. But it is still not clear if WRN affects global chromatin that would eventually lead to aging. However, the role of chromatin in tipping the balance between senescence and cell proliferation becomes apparent from studies, which show that *WRN* is frequently repressed by CpG island hypermethylation in many human cancers (Agrelo et al. 2005).

8.4 Conclusion

Epigenetic marks, which are long lasting and inheritable, play a central role in mediating the outputs from the genome, in response to both extrinsic and intrinsic cues, and therefore, known to affect various biological processes. Hence, it is not surprising to find that chromatin is a major player in mediating cellular responses to aging. Although, all the critical chromatin components like DNA methylation, histone modifications and histone variants have been shown to be involved in this process, the mechanistic details that elicit these changes are less understood. Specifically, it will be interesting to address the cross-talk between classical aging pathways/mechanisms and chromatin signaling. It will be important to address if reversal of any of these chromatin changes would affect the aging process. In this regard, more work needs to be done on the role of chromatin modifiers, which mediate both global and locus specific effects on chromatin structure/function. Studies on proteins like HDACs and Sirtuins have indicated their involvement in the aging process. However, more insights into mechanistic details describing the chromatin effects are needed. Another important aspect that needs to be addressed is the apparent gaps in appreciating the roles of chromatin and chromatin modifiers in cellular and organismal aging.

Since aging is a complex biological process involving multiple factors, interventions aimed at one or more specific pathways/factors (to delay aging) are likely to give limited benefits. Targeting cellular components that would integrate the cues and the responses might turn out to be more beneficial. In this context, interfering with chromatin changes and/or chromatin modifiers that affect aging might become therapeutically relevant. This is crucial since small chemical modulators and/or dietary manipulations have shown promising results with regards to their ability to "delay the aging process" and are often mediated through some of these factors.

References

Adams PD (2009) Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. Mol Cell 36:2–14

Agrelo R, Setien F, Espada J, Artiga MJ, Rodriguez M, Perez-Rosado A, Sanchez-Aguilera A, Fraga MF, Piris MA, Esteller M (2005) Inactivation of the lamin A/C gene by CpG island promoter hypermethylation in hematologic malignancies, and its association with poor survival in nodal diffuse large B-cell lymphoma. J Clin Oncol 23:3940–3947

Arvanitakis Z, Wilson RS, Bennett DA (2006) Diabetes mellitus, dementia, and cognitive function in older persons. J Nutr Health Aging 10:287–291

Bandyopadhyay D, Okan NA, Bales E, Nascimento L, Cole PA, Medrano EE (2002) Down-regulation of p300/CBP histone acetyltransferase activates a senescence checkpoint in human melanocytes. Cancer Res 62:6231–6239

Bannister AJ, Kouzarides T (2011) Regulation of chromatin by histone modifications. Cell Res 21:381–395

Bartkova J, Rezaei N, Liontos M, Karakaidos P, Kletsas D, Issaeva N, Vassiliou LV, Kolettas E, Niforou K, Zoumpourlis VC, Takaoka M, Nakagawa H, Tort F, Fugger K, Johansson F, Sehested M, Andersen CL, Dyrskjot L, Orntoft T, Lukas J, Kittas C, Helleday T, Halazonetis TD, Bartek J, Gorgoulis VG (2006) Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature 444:633–637

Bassett A, Cooper S, Wu C, Travers A (2009) The folding and unfolding of eukaryotic chromatin. Curr Opin Genet Dev 19:159–165

Bates DJ, Liang R, Li N, Wang E (2009) The impact of noncoding RNA on the biochemical and molecular mechanisms of aging. Biochim Biophys Acta 1790:970–979

Bates DJ, Li N, Liang R, Sarojini H, An J, Masternak MM, Bartke A, Wang E (2010) MicroRNA regulation in Ames dwarf mouse liver may contribute to delayed aging. Aging Cell 9:1–18

Ben-Porath I, Weinberg RA (2005) The signals and pathways activating cellular senescence. Int J Biochem Cell Biol 37:961–976

Blais A, van Oevelen CJ, Margueron R, Acosta-Alvear D, Dynlacht BD (2007) Retinoblastoma tumor suppressor protein-dependent methylation of histone H3 lysine 27 is associated with irreversible cell cycle exit. J Cell Biol 179:1399–1412

Boehm M, Slack F (2005) A developmental timing microRNA and its target regulate life span in C. elegans. Science 310:1954–1957

Bommi PV, Dimri M, Sahasrabuddhe AA, Khandekar J, Dimri GP (2010) The polycomb group protein BMI1 is a transcriptional target of HDAC inhibitors. Cell Cycle 9:2663–2673

Bonasio R, Tu S, Reinberg D (2010) Molecular signals of epigenetic states. Science 330:612–616

Bork S, Pfister S, Witt H, Horn P, Korn B, Ho AD, Wagner W (2010) DNA methylation pattern changes upon long-term culture and aging of human mesenchymal stromal cells. Aging Cell 9:54–63

Bornman DM, Mathew S, Alsruhe J, Herman JG, Gabrielson E (2001) Methylation of the E-cadherin gene in bladder neoplasia and in normal urothelial epithelium from elderly individuals. Am J Pathol 159:831–835

Bracken AP, Kleine-Kohlbrecher D, Dietrich N, Pasini D, Gargiulo G, Beekman C, Theilgaard-Monch K, Minucci S, Porse BT, Marine JC, Hansen KH, Helin K (2007) The Polycomb group proteins bind throughout the INK4A-ARF locus and are disassociated in senescent cells. Genes Dev 21:525–530

Braig M, Schmitt CA (2006) Oncogene-induced senescence: putting the brakes on tumor development. Cancer Res 66:2881–2884

Braig M, Lee S, Loddenkemper C, Rudolph C, Peters AH, Schlegelberger B, Stein H, Dorken B, Jenuwein T, Schmitt CA (2005) Oncogene-induced senescence as an initial barrier in lymphoma development. Nature 436:660–665

Brooks CL, Gu W (2008) p53 Activation: a case against Sir. Cancer Cell 13:377–378

Cao R, Tsukada Y, Zhang Y (2005) Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing. Mol Cell 20:845–854
Casillas MA Jr, Lopatina N, Andrews LG, Tollefsbol TO (2003) Transcriptional control of the DNA methyltransferases is altered in aging and neoplastically-transformed human fibroblasts. Mol Cell Biochem 252:33–43
Chen LH, Chiou GY, Chen YW, Li HY, Chiou SH (2010) MicroRNA and aging: a novel modulator in regulating the aging network. Ageing Res Rev 9(Suppl 1):S59–S66
Chouliaras L, van den Hove DL, Kenis G, Dela Cruz J, Lemmens MA, van Os J, Steinbusch HW, Schmitz C, Rutten BP (2011a) Caloric restriction attenuates age-related changes of DNA methyltransferase 3a in mouse hippocampus. Brain Behav Immun 25:616–623
Chouliaras L, van den Hove DL, Kenis G, Keitel S, Hof PR, van Os J, Steinbusch HW, Schmitz C, Rutten BP (2011b) Prevention of age-related changes in hippocampal levels of 5-methylcytidine by caloric restriction. Neurobiol Aging 33(8):1672–1681
Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR, Wiemels JL, Nelson HH, Karagas MR, Padbury JF, Bueno R, Sugarbaker DJ, Yeh RF, Wiencke JK, Kelsey KT (2009) Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. PLoS Genet 5:e1000602
Chuang JY, Hung JJ (2011) Overexpression of HDAC1 induces cellular senescence by Sp1/PP2A/pRb pathway. Biochem Biophys Res Commun 407:587–592
Costanzi C, Pehrson JR (1998) Histone macroH2A1 is concentrated in the inactive X chromosome of female mammals. Nature 393:599–601
Dang W, Steffen KK, Perry R, Dorsey JA, Johnson FB, Shilatifard A, Kaeberlein M, Kennedy BK, Berger SL (2009) Histone H4 lysine 16 acetylation regulates cellular lifespan. Nature 459:802–807
De Carvalho DD, You JS, Jones PA (2010) DNA methylation and cellular reprogramming. Trends Cell Biol 20:609–617
Dechat T, Pfleghaar K, Sengupta K, Shimi T, Shumaker DK, Solimando L, Goldman RD (2008) Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. Genes Dev 22:832–853
Deng CX (2009) SIRT1, is it a tumor promoter or tumor suppressor? Int J Biol Sci 5:147–152
DePinho RA (2000) The age of cancer. Nature 408:248–254
Dimauro T, David G (2009) Chromatin modifications: the driving force of senescence and aging? Aging (Albany NY) 1:182–190
Fairweather DS, Fox M, Margison GP (1987) The in vitro lifespan of MRC-5 cells is shortened by 5-azacytidine-induced demethylation. Exp Cell Res 168:153–159
Feser J, Tyler J (2011) Chromatin structure as a mediator of aging. FEBS Lett 585:2041–2048
Fraga MF, Esteller M (2007) Epigenetics and aging: the targets and the marks. Trends Genet 23:413–418
Frankel S, Rogina B (2005) Drosophila longevity is not affected by heterochromatin-mediated gene silencing. Aging Cell 4:53–56
Frenster JH, Allfrey VG, Mirsky AE (1963) Repressed and active chromatin isolated from interphase lymphocytes. Proc Natl Acad Sci U S A 50:1026–1032
Fujita N, Watanabe S, Ichimura T, Ohkuma Y, Chiba T, Saya H, Nakao M (2003) MCAF mediates MBD1-dependent transcriptional repression. Mol Cell Biol 23:2834–2843
Fuke C, Shimabukuro M, Petronis A, Sugimoto J, Oda T, Miura K, Miyazaki T, Ogura C, Okazaki Y, Jinno Y (2004) Age related changes in 5-methylcytosine content in human peripheral leukocytes and placentas: an HPLC-based study. Ann Hum Genet 68:196–204
Funayama R, Saito M, Tanobe H, Ishikawa F (2006) Loss of linker histone H1 in cellular senescence. J Cell Biol 175:869–880
Gao Z, Xu MS, Barnett TL, Xu CW (2011) Resveratrol induces cellular senescence with attenuated mono-ubiquitination of histone H2B in glioma cells. Biochem Biophys Res Commun 407:271–276

Geiman TM, Muegge K (2010) DNA methylation in early development. Mol Reprod Dev 77:105–113
Ghosh S, George S, Roy U, Ramachandran D, Kolthur-Seetharam U (2010) NAD: a master regulator of transcription. Biochim Biophys Acta 1799:681–693
Gibney ER, Nolan CM (2010) Epigenetics and gene expression. Heredity 105:4–13
Goldman RD, Shumaker DK, Erdos MR, Eriksson M, Goldman AE, Gordon LB, Gruenbaum Y, Khuon S, Mendez M, Varga R, Collins FS (2004) Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. Proc Natl Acad Sci U S A 101:8963–8968
Gorospe M, Abdelmohsen K (2011) MicroRegulators come of age in senescence. Trends Genet 27:233–241
Gotta M, Strahl-Bolsinger S, Renauld H, Laroche T, Kennedy BK, Grunstein M, Gasser SM (1997) Localization of Sir2p: the nucleolus as a compartment for silent information regulators. EMBO J 16:3243–3255
Grandinetti KB, Jelinic P, DiMauro T, Pellegrino J, Fernandez Rodriguez R, Finnerty PM, Ruoff R, Bardeesy N, Logan SK, David G (2009) Sin3B expression is required for cellular senescence and is up-regulated upon oncogenic stress. Cancer Res 69:6430–6437
Gregoire S, Xiao L, Nie J, Zhang X, Xu M, Li J, Wong J, Seto E, Yang XJ (2007) Histone deacetylase 3 interacts with and deacetylates myocyte enhancer factor 2. Mol Cell Biol 27:1280–1295
Grillari J, Grillari-Voglauer R (2010) Novel modulators of senescence, aging, and longevity: small non-coding RNAs enter the stage. Exp Gerontol 45:302–311
Ha CW, Huh WK (2011) The implication of Sir2 in replicative aging and senescence in *Saccharomyces cerevisiae*. Aging (Albany NY) 3:319–324
Hajji N, Wallenborg K, Vlachos P, Fullgrabe J, Hermanson O, Joseph B (2010) Opposing effects of hMOF and SIRT1 on H4K16 acetylation and the sensitivity to the topoisomerase II inhibitor etoposide. Oncogene 29:2192–2204
Hans F, Dimitrov S (2001) Histone H3 phosphorylation and cell division. Oncogene 20:3021–3027
Hayflick L (1965) The limited in vitro lifetime of human diploid cell strains. Exp Cell Res 37:614–636
He L, Hannon GJ (2004) MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 5:522–531
He H, Yu FX, Sun C, Luo Y (2011) CBP/p300 and SIRT1 are involved in transcriptional regulation of S-phase specific histone genes. PLoS One 6:e22088
Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM (2006) Cellular senescence in aging primates. Science 311:1257
Hoal-van Helden EG, van Helden PD (1989) Age-related methylation changes in DNA may reflect the proliferative potential of organs. Mutat Res 219:263–266
Hock R, Furusawa T, Ueda T, Bustin M (2007) HMG chromosomal proteins in development and disease. Trends Cell Biol 17:72–79
Hornsby PJ, Yang L, Gunter LE (1992) Demethylation of satellite I DNA during senescence of bovine adrenocortical cells in culture. Mutat Res 275:13–19
Huang J, Gan Q, Han L, Li J, Zhang H, Sun Y, Zhang Z, Tong T (2008) SIRT1 overexpression antagonizes cellular senescence with activated ERK/S6k1 signaling in human diploid fibroblasts. PLoS One 3:e1710
Imai S, Guarente L (2010) Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases. Trends Pharmacol Sci 31:212–220
Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE, Baylin SB (1994) Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. Nat Genet 7:536–540
Jenuwein T, Allis CD (2001) Translating the histone code. Science 293:1074–1080

Johnson BR, Nitta RT, Frock RL, Mounkes L, Barbie DA, Stewart CL, Harlow E, Kennedy BK (2004) A-type lamins regulate retinoblastoma protein function by promoting subnuclear localization and preventing proteasomal degradation. Proc Natl Acad Sci U S A 101:9677–9682

Jung-Hynes B, Nihal M, Zhong W, Ahmad N (2009) Role of sirtuin histone deacetylase SIRT1 in prostate cancer. A target for prostate cancer management via its inhibition? J Biol Chem 284:3823–3832

Jurkowska RZ, Jurkowski TP, Jeltsch A (2011) Structure and function of mammalian DNA methyltransferases. Chembiochem 12:206–222

Kaeberlein M, McVey M, Guarente L (1999) The SIR2/3/4 complex and SIR2 alone promote longevity in *Saccharomyces cerevisiae* by two different mechanisms. Genes Dev 13: 2570–2580

Kawahara TL, Rapicavoli NA, Wu AR, Qu K, Quake SR, Chang HY (2011) Dynamic chromatin localization of Sirt6 shapes stress- and aging-related transcriptional networks. PLoS Genet 7:e1002153

Kawakami K, Nakamura A, Ishigami A, Goto S, Takahashi R (2009) Age-related difference of site-specific histone modifications in rat liver. Biogerontology 10:415–421

Kenyon J, Gerson SL (2007) The role of DNA damage repair in aging of adult stem cells. Nucleic Acids Res 35:7557–7565

Kimura H, Shiota K (2003) Methyl-CpG-binding protein, MeCP2, is a target molecule for maintenance DNA methyltransferase, Dnmt1. J Biol Chem 278:4806–4812

Kirkwood TL, Kapahi P, Shanley DP (2000) Evolution, stress, and longevity. J Anat 197(Pt 4): 587–590

Knight JA (2000) The biochemistry of aging. Adv Clin Chem 35:1–62

Koch CM, Suschek CV, Lin Q, Bork S, Goergens M, Joussen S, Pallua N, Ho AD, Zenke M, Wagner W (2011) Specific age-associated DNA methylation changes in human dermal fibroblasts. PLoS One 6:e16679

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 p16(ink4a). Cell Cycle 10:457–468

Kouzarides T (2007) Chromatin modifications and their function. Cell 128:693–705

Kozak ML, Chavez A, Dang W, Berger SL, Ashok A, Guo X, Johnson FB (2010) Inactivation of the Sas2 histone acetyltransferase delays senescence driven by telomere dysfunction. EMBO J 29:158–170

Krishnan V, Chow MZ, Wang Z, Zhang L, Liu B, Liu X, Zhou Z (2011) Histone H4 lysine 16 hypoacetylation is associated with defective DNA repair and premature senescence in Zmpste24-deficient mice. Proc Natl Acad Sci U S A 108:12325–12330

Langley E, Pearson M, Faretta M, Bauer UM, Frye RA, Minucci S, Pelicci PG, Kouzarides T (2002) Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. EMBO J 21:2383–2396

Lee ST, Kim M (2006) Aging and neurodegeneration. Molecular mechanisms of neuronal loss in Huntington's disease. Mech Ageing Dev 127:432–435

Lee S, Jung JW, Park SB, Roh K, Lee SY, Kim JH, Kang SK, Kang KS (2011) Histone deacetylase regulates high mobility group A2-targeting microRNAs in human cord blood-derived multipotent stem cell aging. Cell Mol Life Sci 68:325–336

Legube G, Trouche D (2003) Regulating histone acetyltransferases and deacetylases. EMBO Rep 4:944–947

Li G, Reinberg D (2011) Chromatin higher-order structures and gene regulation. Curr Opin Genet Dev 21:175–186

Li N, Bates DJ, An J, Terry DA, Wang E (2011) Up-regulation of key microRNAs, and inverse down-regulation of their predicted oxidative phosphorylation target genes, during aging in mouse brain. Neurobiol Aging 32:944–955

Liang R, Bates DJ, Wang E (2009) Epigenetic control of microRNA expression and aging. Curr Genomics 10:184–193

Lopatina N, Haskell JF, Andrews LG, Poole JC, Saldanha S, Tollefsbol T (2002) Differential maintenance and de novo methylating activity by three DNA methyltransferases in aging and immortalized fibroblasts. J Cell Biochem 84:324–334

Lopatina NG, Poole JC, Saldanha SN, Hansen NJ, Key JS, Pita MA, Andrews LG, Tollefsbol TO (2003) Control mechanisms in the regulation of telomerase reverse transcriptase expression in differentiating human teratocarcinoma cells. Biochem Biophys Res Commun 306:650–659

Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature 389:251–260

Maes OC, An J, Sarojini H, Wang E (2008) Murine microRNAs implicated in liver functions and aging process. Mech Ageing Dev 129:534–541

Margueron R, Trojer P, Reinberg D (2005) The key to development: interpreting the histone code? Curr Opin Genet Dev 15:163–176

Michishita E, McCord RA, Berber E, Kioi M, Padilla-Nash H, Damian M, Cheung P, Kusumoto R, Kawahara TL, Barrett JC, Chang HY, Bohr VA, Ried T, Gozani O, Chua KF (2008) SIRT6 is a histone H3 lysine 9 deacetylase that modulates telomeric chromatin. Nature 452:492–496

Misteli T (2010) Higher-order genome organization in human disease. Cold Spring Harb Perspect Biol 2:a000794

Morimoto S, Komatsu S, Takahashi R, Matsuo M, Goto S (1993) Age-related change in the amount of ubiquitinated histones in the mouse brain. Arch Gerontol Geriatr 16:217–224

Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, Liu P, Mostoslavsky G, Franco S, Murphy MM, Mills KD, Patel P, Hsu JT, Hong AL, Ford E, Cheng HL, Kennedy C, Nunez N, Bronson R, Frendewey D, Auerbach W, Valenzuela D, Karow M, Hottiger MO, Hursting S, Barrett JC, Guarente L, Mulligan R, Demple B, Yancopoulos GD, Alt FW (2006) Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 124:315–329

Muller M (2009) Cellular senescence: molecular mechanisms, in vivo significance, and redox considerations. Antioxid Redox Signal 11:59–98

Munro J, Barr NI, Ireland H, Morrison V, Parkinson EK (2004) Histone deacetylase inhibitors induce a senescence-like state in human cells by a p16-dependent mechanism that is independent of a mitotic clock. Exp Cell Res 295:525–538

Murr R (2010) Interplay between different epigenetic modifications and mechanisms. Adv Genet 70:101–141

Narita M (2007) Cellular senescence and chromatin organisation. Br J Cancer 96:686–691

Narita M, Nunez S, Heard E, 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

Narita M, Krizhanovsky V, Nunez S, Chicas A, Hearn SA, Myers MP, Lowe SW (2006) A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. Cell 126:503–514

Newman BL, Lundblad JR, Chen Y, Smolik SM (2002) A Drosophila homologue of Sir2 modifies position-effect variegation but does not affect life span. Genetics 162:1675–1685

O'Hagan HM, Mohammad HP, Baylin SB (2008) Double strand breaks can initiate gene silencing and SIRT1-dependent onset of DNA methylation in an exogenous promoter CpG island. PLoS Genet 4:e1000155

O'Sullivan RJ, Kubicek S, Schreiber SL, Karlseder J (2010) Reduced histone biosynthesis and chromatin changes arising from a damage signal at telomeres. Nat Struct Mol Biol 17:1218–1225

Oakes CC, Smiraglia DJ, Plass C, Trasler JM, Robaire B (2003) Aging results in hypermethylation of ribosomal DNA in sperm and liver of male rats. Proc Natl Acad Sci U S A 100:1775–1780

Oberdoerffer P, Michan S, McVay M, Mostoslavsky R, Vann J, Park SK, Hartlerode A, Stegmuller J, Hafner A, Loerch P, Wright SM, Mills KD, Bonni A, Yankner BA, Scully R, Prolla TA, Alt FW, Sinclair DA (2008) SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. Cell 135:907–918

Ota H, Tokunaga E, Chang K, Hikasa M, Iijima K, Eto M, Kozaki K, Akishita M, Ouchi Y, Kaneki M (2006) Sirt1 inhibitor, Sirtinol, induces senescence-like growth arrest with attenuated Ras-MAPK signaling in human cancer cells. Oncogene 25:176–185

Ota H, Akishita M, Eto M, Iijima K, Kaneki M, Ouchi Y (2007) Sirt1 modulates premature senescence-like phenotype in human endothelial cells. J Mol Cell Cardiol 43:571–579

Ozaki T, Saijo M, Murakami K, Enomoto H, Taya Y, Sakiyama S (1994) Complex formation between lamin A and the retinoblastoma gene product: identification of the domain on lamin A required for its interaction. Oncogene 9:2649–2653

Palacios JA, Herranz D, De Bonis ML, Velasco S, Serrano M, Blasco MA (2010) SIRT1 contributes to telomere maintenance and augments global homologous recombination. J Cell Biol 191: 1299–1313

Paull TT, Haykinson MJ, Johnson RC (1993) The nonspecific DNA-binding and -bending proteins HMG1 and HMG2 promote the assembly of complex nucleoprotein structures. Genes Dev 7:1521–1534

Pedeux R, Sengupta S, Shen JC, Demidov ON, Saito S, Onogi H, Kumamoto K, Wincovitch S, Garfield SH, McMenamin M, Nagashima M, Grossman SR, Appella E, Harris CC (2005) ING2 regulates the onset of replicative senescence by induction of p300-dependent p53 acetylation. Mol Cell Biol 25:6639–6648

Peterson CL, Laniel MA (2004) Histones and histone modifications. Curr Biol 14:R546–R551

Prieur A, Besnard E, Babled A, Lemaitre JM (2011) p53 and p16(INK4A) independent induction of senescence by chromatin-dependent alteration of S-phase progression. Nat Commun 2:473

Rakyan VK, Down TA, Maslau S, Andrew T, Yang TP, Beyan H, Whittaker P, McCann OT, Finer S, Valdes AM, Leslie RD, Deloukas P, Spector TD (2010) Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. Genome Res 20:434–439

Rastogi S, Joshi B, Dasgupta P, Morris M, Wright K, Chellappan S (2006) Prohibitin facilitates cellular senescence by recruiting specific corepressors to inhibit E2F target genes. Mol Cell Biol 26:4161–4171

Rathbone CR, Booth FW, Lees SJ (2008) FoxO3a preferentially induces p27Kip1 expression while impairing muscle precursor cell-cycle progression. Muscle Nerve 37:84–89

Rodier F, Campisi J (2011) Four faces of cellular senescence. J Cell Biol 192:547–556

Rodriguez RM, Fraga MF (2010) Aging and cancer: are sirtuins the link? Future Oncol 6:905–915

Rogina B, Helfand SL (2004) Sir2 mediates longevity in the fly through a pathway related to calorie restriction. Proc Natl Acad Sci U S A 101:15998–16003

Romanov GA, Vanyushin BF (1981) Methylation of reiterated sequences in mammalian DNAs. Effects of the tissue type, age, malignancy and hormonal induction. Biochim Biophys Acta 653:204–218

Rusche LN, Kirchmaier AL, Rine J (2003) The establishment, inheritance, and function of silenced chromatin in *Saccharomyces cerevisiae*. Annu Rev Biochem 72:481–516

Saito Y, Jones PA (2006) Epigenetic activation of tumor suppressor microRNAs in human cancer cells. Cell Cycle 5:2220–2222

Sarg B, Koutzamani E, Helliger W, Rundquist I, Lindner HH (2002) Postsynthetic trimethylation of histone H4 at lysine 20 in mammalian tissues is associated with aging. J Biol Chem 277:39195–39201

Sayed D, Abdellatif M (2011) MicroRNAs in development and disease. Physiol Rev 91:827–887

Sedivy JM, Banumathy G, Adams PD (2008) Aging by epigenetics–a consequence of chromatin damage? Exp Cell Res 314:1909–1917

Seviour EG, Lin SY (2010) The DNA damage response: balancing the scale between cancer and ageing. Aging (Albany NY) 2:900–907

Shin DM, Kucia M, Ratajczak MZ (2011a) Nuclear and chromatin reorganization during cell senescence and aging – a mini-review. Gerontology 57:76–84

Shin KH, Pucar A, Kim RH, Bae SD, Chen W, Kang MK, Park NH (2011b) Identification of senescence-inducing microRNAs in normal human keratinocytes. Int J Oncol 39:1205–1211

Shumaker DK, Dechat T, Kohlmaier A, Adam SA, Bozovsky MR, Erdos MR, Eriksson M, Goldman AE, Khuon S, Collins FS, Jenuwein T, Goldman RD (2006) Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. Proc Natl Acad Sci U S A 103:8703–8708

Sinclair DA, Guarente L (1997) Extrachromosomal rDNA circles–a cause of aging in yeast. Cell 91:1033–1042

Singhal RP, Mays-Hoopes LL, Eichhorn GL (1987) DNA methylation in aging of mice. Mech Ageing Dev 41:199–210

So K, Tamura G, Honda T, Homma N, Waki T, Togawa N, Nishizuka S, Motoyama T (2006) Multiple tumor suppressor genes are increasingly methylated with age in non-neoplastic gastric epithelia. Cancer Sci 97:1155–1158

So AY, Jung JW, Lee S, Kim HS, Kang KS (2011) DNA methyltransferase controls stem cell aging by regulating BMI1 and EZH2 through microRNAs. PLoS One 6:e19503

Song JZ, Stirzaker C, Harrison J, Melki JR, Clark SJ (2002) Hypermethylation trigger of the glutathione-S-transferase gene (GSTP1) in prostate cancer cells. Oncogene 21:1048–1061

Steuerwald NM, Parsons JC, Bennett K, Bates TC, Bonkovsky HL (2010) Parallel microRNA and mRNA expression profiling of (genotype 1b) human hepatoma cells expressing hepatitis C virus. Liver Int 30:1490–1504

Stirzaker C, Song JZ, Davidson B, Clark SJ (2004) Transcriptional gene silencing promotes DNA hypermethylation through a sequential change in chromatin modifications in cancer cells. Cancer Res 64:3871–3877

Swisshelm K, Disteche CM, Thorvaldsen J, Nelson A, Salk D (1990) Age-related increase in methylation of ribosomal genes and inactivation of chromosome-specific rRNA gene clusters in mouse. Mutat Res 237:131–146

Tao D, Lu J, Sun H, Zhao YM, Yuan ZG, Li XX, Huang BQ (2004) Trichostatin A extends the lifespan of Drosophila melanogaster by elevating hsp22 expression. Acta Biochim Biophys Sin (Shanghai) 36:618–622

Thevenet L, Mejean C, Moniot B, Bonneaud N, Galeotti N, Aldrian-Herrada G, Poulat F, Berta P, Benkirane M, Boizet-Bonhoure B (2004) Regulation of human SRY subcellular distribution by its acetylation/deacetylation. EMBO J 23:3336–3345

Trimarchi JM, Lees JA (2002) Sibling rivalry in the E2F family. Nat Rev Mol Cell Biol 3:11–20

Vaquero A, Scher M, Lee D, Erdjument-Bromage H, Tempst P, Reinberg D (2004) Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. Mol Cell 16:93–105

Vaquero A, Scher M, Erdjument-Bromage H, Tempst P, Serrano L, Reinberg D (2007) SIRT1 regulates the histone methyl-transferase SUV39H1 during heterochromatin formation. Nature 450:440–444

Villa R, Morey L, Raker VA, Buschbeck M, Gutierrez A, De Santis F, Corsaro M, Varas F, Bossi D, Minucci S, Pelicci PG, Di Croce L (2006) The methyl-CpG binding protein MBD1 is required for PML-RARalpha function. Proc Natl Acad Sci U S A 103:1400–1405

Vogt M, Haggblom C, Yeargin J, Christiansen-Weber T, Haas M (1998) Independent induction of senescence by p16INK4a and p21CIP1 in spontaneously immortalized human fibroblasts. Cell Growth Differ 9:139–146

Waki T, Tamura G, Sato M, Motoyama T (2003) Age-related methylation of tumor suppressor and tumor-related genes: an analysis of autopsy samples. Oncogene 22:4128–4133

Walter D, Matter A, Fahrenkrog B (2010) Bre1p-mediated histone H2B ubiquitylation regulates apoptosis in *Saccharomyces cerevisiae*. J Cell Sci 123:1931–1939

Wilson VL, Jones PA (1983) DNA methylation decreases in aging but not in immortal cells. Science 220:1055–1057

Wilson VL, Smith RA, Ma S, Cutler RG (1987) Genomic 5-methyldeoxycytidine decreases with age. J Biol Chem 262:9948–9951

Xu WS, Perez G, Ngo L, Gui CY, Marks PA (2005) Induction of polyploidy by histone deacetylase inhibitor: a pathway for antitumor effects. Cancer Res 65:7832–7839

Yamakuchi M, Ferlito M, Lowenstein CJ (2008) miR-34a repression of SIRT1 regulates apoptosis. Proc Natl Acad Sci U S A 105:13421–13426

Yang X, Phillips DL, Ferguson AT, Nelson WG, Herman JG, Davidson NE (2001) Synergistic activation of functional estrogen receptor (ER)-alpha by DNA methyltransferase and histone deacetylase inhibition in human ER-alpha-negative breast cancer cells. Cancer Res 61:7025–7029

Ye X, Zerlanko B, Zhang R, Somaiah N, Lipinski M, Salomoni P, Adams PD (2007) Definition of pRB- and p53-dependent and -independent steps in HIRA/ASF1a-mediated formation of senescence-associated heterochromatin foci. Mol Cell Biol 27:2452–2465

Yoshimi A, Kurokawa M (2011) Key roles of histone methyltransferase and demethylase in leukemogenesis. J Cell Biochem 112:415–424

Yuan F, Xie Q, Wu J, Bai Y, Mao B, Dong Y, Bi W, Ji G, Tao W, Wang Y, Yuan Z (2011) MST1 promotes apoptosis through regulating Sirt1-dependent p53 deacetylation. J Biol Chem 286:6940–6945

Zhang T, Kraus WL (2010) SIRT1-dependent regulation of chromatin and transcription: linking NAD(+) metabolism and signaling to the control of cellular functions. Biochim Biophys Acta 1804:1666–1675

Zhang R, Poustovoitov MV, Ye X, Santos HA, Chen W, Daganzo SM, Erzberger JP, Serebriiskii IG, Canutescu AA, Dunbrack RL, Pehrson JR, Berger JM, Kaufman PD, Adams PD (2005) Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev Cell 8:19–30

Zhang J, Liu Q, Zhang W, Li J, Li Z, Tang Z, Li Y, Han C, Hall SH, Zhang Y (2010) Comparative profiling of genes and miRNAs expressed in the newborn, young adult, and aged human epididymides. Acta Biochim Biophys Sin (Shanghai) 42:145–153

Zhao Y, Sun H, Lu J, Li X, Chen X, Tao D, Huang W, Huang B (2005) Lifespan extension and elevated hsp gene expression in Drosophila caused by histone deacetylase inhibitors. J Exp Biol 208:697–705