Chapter 16
Gene Expression, Epigenetics and Ageing

Babukrishna Maniyadath, Namrata Shukla, and Ullas Kolthur-Seetharam

Abstract As the popular adage goes, all diseases run into old age and almost all physiological changes are associated with alterations in gene expression, irrespective of whether they are causal or consequential. Therefore, the quest for mechanisms that delay ageing and decrease age-associated diseases has propelled researchers to unravel regulatory factors that lead to changes in chromatin structure and function, which ultimately results in deregulated gene expression. It is therefore essential to bring together literature, which until recently has investigated gene expression and chromatin independently. With advances in biomedical research and the emergence of epigenetic regulators as potential therapeutic targets, enhancing our understanding of mechanisms that 'derail' transcription and identification of causal genes/pathways during ageing will have a significant impact. In this context, this chapter aims to not only summarize the key features of age-associated changes in epigenetics and transcription, but also identifies gaps in the field and proposes aspects that need to be investigated in the future.

Keywords Ageing · Senescence · Gene expression · Epigenetics · Transcriptional Noise · Chromatin · Histone Variants · DNA methylation · 5hmC · SAHF

Introduction

Genetic and epigenetic information that is inherited across evolution and across life history in any given individual is essential to determine the expressability of genes, which then contribute to diverse physiological outputs. Therefore, it is not surprising that numerous studies have aimed at profiling transcripts to provide target pathways or genes that could be causal to a particular phenotype. Parallel efforts to unravel chromatin structure and functions have indeed catapulted our efforts at

B. Maniyadath · N. Shukla · U. Kolthur-Seetharam (✉)
Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, India
e-mail: ullas@tifr.res.in

understanding how signalling to chromatin, including environmental influences, shape gene expression patterns.

Ageing is a continuum of physiological processes, which over the lifespan of an organism, contributes to fitness that is essential for the survival of individuals. In fact ageing, which also ensures removal of unfit individuals, contributes to the overall fitness of the species (Croft et al. 2015; Flatt and Schmidt 2009; Parsons 2007; Zhang and Hood 2016). Ageing also increases the risk of diseases such as diabetes, obesity, cancer and neurodegeneration (Campisi 2013; Frasca et al. 2017; Niccoli and Partridge 2012; Wyss-Coray 2016).

Given this, the quest to identify molecular mechanisms and pathways that drive healthy ageing has motivated researchers to investigate causal genes. Efforts to profile alterations in gene expression and the underlying chromatin based mechanisms have therefore provided vast amount of information. In this article, we have attempted to not only review the current status of the field, but also raise open questions or gaps in the field that need to be addressed in the future. Specifically, we have tried to link functional outputs vis-à-vis gene expression with altered chromatin status during ageing, some of which are otherwise disparate.

Transcriptome and Ageing

The physiological state of cells or tissues is directly affected by gene expression and is therefore likely to impact ageing as well. It is also well established that changes in gene expression are contributed by chromatin structure (Sproul et al. 2005; Venkatesh and Workman 2015). Although, chromatin is known to be dynamic, alterations in gene expression are more variable. Similar to studies on chromatin (discussed below), most of the transcriptome profiling has been done on liver and brain in addition to senescent primary cells in culture. In this section, we have highlighted the alterations in transcriptome including the non-coding RNAs, which have been profiled in various studies.

Global Alterations in Gene Expression

Extensive analyses of transcriptional changes have been done on various model organisms and across ages. While, most of these studies have highlighted global alterations, whether these are causal or consequential to ageing is still unclear. Alterations in gene expression have been addressed at multiple levels: (a) turning on and off of specific genes or pathways that could contribute to ageing, (b) global deregulation vis-à-vis variance and an increase or decrease in noise and (c) expression of non-coding or repetitive elements in the genome. Although most of the

studies have addressed the first aspect, very little is known about the other two. Given that the non-coding component of the genome increased during evolution, it remains to be seen if expressability of such regions correlate with or contribute to organismal longevity.

While all of these studies have clearly demonstrated that young and old cells or tissues display distinct transcriptional profiles, very few studies have addressed the time window during ageing when these changes begin to manifest. Attempts at identifying age-wise changes in gene expression have been done in fruit flies, rodents and humans (Thomas et al. 2002). It is interesting to see that in humans, differential transcription is more pronounced and varied in middle-aged individuals, at least in the brain cortex. Individuals below 42 years and above 71 years of age were seen to be more homogenous and also showed complete switch in the expression vis-à-vis up- and down-regulated genes (Lu et al. 2004). In rat liver, gene-expression changes seem to be progressive, starting from 6–8 weeks of age and possibly suggest a higher mid-life variance (Kwekel et al. 2010). In contrast, although the pattern of heterogeneity in gene expression starts early in *Drosophila,* by the age of 13 days post eclosion, it changes little thereafter (Kim et al. 2005). Studies by both Lui and Finkielstain and their colleagues draw interesting correlations between the transcriptional programs during early post-natal development and ageing. It is shown that the transcriptional modules, which govern deceleration in growth in post-natal stages, are remarkably similar to those seen in aged tissues (Finkielstain et al. 2009; Lui et al. 2010). To our best knowledge there are no studies, which have systematically investigated short-lived and long-lived individuals in any given species or compared long-lived versus short-lived evolutionarily diverse organisms to even hypothesize if the overall lifespan impinges on rate of change of gene expression or vice versa.

There are very few studies that have highlighted sexual dimorphism in terms of both temporal and amplitudinal changes. Results from Kwekel et al. (2010) clearly demonstrate that male and female rats show maximal differences in their gene expression patterns between the ages of 15–52 weeks, which interestingly also marks the period of highest reproductive fitness. Intriguingly, a subset of DEGs (differentially expressed genes) showed age-dependent inversion by being highly expressed in young females but not males and were induced more in old males when compared to old females. Similar trends have been observed in ageing murine hearts, hinting at a conserved mechanism of sexual dimorphism across organs (Vijay et al. 2015). Studies in humans showed higher global gene expression in aged male brains than their female counterparts (Berchtold et al. 2008). These clearly suggest that age-dependent transcriptional controls are different in males and females. However, some studies do indicate that in extremely aged individuals the differences between the sexes disappear and the transcriptional pattern looks more feminized (Kwekel et al. 2010), a concept that is otherwise gaining ground.

Non-coding RNAs Associated with Ageing

Based on the literature accumulated over the past two to three decades it is now evident that non-coding RNAs, which include long non-coding (lncRNAs), retroviral transcripts and short non-coding RNAs such as microRNAs, circular RNAs, snoRNAs, tRNAs and rRNAs are now known to control the expression of genes transcribed from the coding part of the genome (Bonasio and Shiekhattar 2014; Kaikkonen et al. 2011; Patil et al. 2014; Rinn and Chang 2012). Importantly, ncRNAs themselves have turned out to be regulated both in terms of their expression and turnover across biological contexts and evolutionarily diverse organisms (Kutter et al. 2012; Spurlock et al. 2016). It is therefore not surprising to find that ncRNAs have also been associated with ageing.

Small Non-coding RNAs in Ageing

Studies in leukocytes showed that progressive differential expression of small non-coding RNAs (miRs and snoRNAs) during ageing seems to be well correlated with differentially expressed coding genes. Interestingly, these changes in ncRNAs are more pronounced around mid-life (Munoz-Culla et al. 2017).

Regulation of gene expression by microRNAs is well known (He and Hannon 2004; Valinezhad Orang et al. 2014) and there are several candidate-based studies, which have highlighted their roles in ageing (Harries 2014; Smith-Vikos and Slack 2012). Given the extremely diverse physiological contexts, and the genes and pathways that have been analyzed, it is difficult to comment on the overall directionality of miR changes that affect ageing from these reports. Nevertheless, it is clear that miR levels show drastic changes during ageing and their targets control some of these miRs, in a feed-back manner (Smith-Vikos and Slack 2012; Tsai et al. 2011). However, a few studies on non-coding RNAs, detailed above, have attempted to uncover global changes in miR expression and as mentioned changes in miRs and mRNAs do show correspondence.

One of the reasons for dysregulation of microRNA profiles in ageing individuals has been attributed to abnormal levels of circularRNAs (circRNA) (Panda et al. 2017b). Known for their miR-sponging function (Hansen et al. 2013; Memczak et al. 2013), increased levels of circRNA have been reported across species. For example, in mice and flies abundance of circRNAs increased in neurons, specifically in the cortex and hippocampus of mice (Gruner et al. 2016; Westholm et al. 2014). Although, these studies seem to suggest that this could be a tissue specific phenomenon, it remains to be seen if this is indeed true and whether it is present in humans as well. Reduction in the levels of a particular circRNA, circPVT1 in senescing human fibroblasts has been shown to affect the proliferative capacity of the cells. This has been attributed to upregulation of its target microRNA let-7, which antagonizes proliferative genes such as IGF2BP1, KRAS and HMGA2 (Panda et al. 2017a). It is important to note that these transcripts have indeed been shown to be down-regulated during ageing, by several independent reports

(Hansen et al. 2004; Nishino et al. 2008). circRNAs have also been recently implicated in fecundity or fertility. It was observed that oocytes retrieved from the granulosa cells of women above 38 years of age, who registered for *in vitro* fertilization, showed higher expression of circRNAs, which was inversely co-related with number of healthy zygotes obtained (Cheng et al. 2017).

Long Non-coding RNAs and Ageing

It is rather intriguing to note that there have been fewer studies that have analyzed lncRNAs during ageing. A whole transcriptome analysis in the liver by White et al. (2015) has revealed that several pseudogenes along with lncRNAs are differentially expressed between young and old tissues. It is interesting to note that their results indicate more ncRNAs were up-regulated during ageing than those, which had reduced expression. Moreover, highlighting a possible locus specific control of lncRNA transcription Meg3, Rian and Mirg, which are adjacent and are imprinted, were shown to be up-regulated. Similarly, this study also reported two novel lncRNAs, which flank the cdkna2 gene to be up-regulated. In this context, we would like to highlight that cdkna2 itself has been independently shown to be deregulated during ageing (Lui et al. 2010). Efforts to unravel differentially expressed genes in the cerebral cortex at 6, 12 and 28 months in mice seems to suggest that ncRNA expression, at least in this tissue, could be more pronounced during early ageing (Wood et al. 2013).

This raises the need to carry out a comprehensive analyses of changes in coding transcripts, which are contributed by both transcriptional (chromatin dependent) and post-transcriptional mechanisms (mediated by ncRNAs). Another aspect that needs to be addressed in the future is the involvement of ncRNAs in affecting chromatin structure and function, which will potentially influence transcription during ageing.

rDNA Expression, Energetics and Ageing

Involvement of rDNA and rRNA in ageing was first seen in yeast, which led to the identification of *Sir2*, whose orthologues and paralogues across species are known to be major determinants of organismal longevity (Banerjee et al. 2012; Gottlieb and Esposito 1989; Sinclair and Guarente 1997; Smith and Boeke 1997). Since there are excellent reviews on the role of Sirtuins and the mechanisms of ageing in yeast, we encourage the reader to refer to them (*see* Guarente 2007; Wierman and Smith 2014). Nevertheless, emerging studies including those from yeast clearly point out that ribosomal transcription has a major impact on ageing, which in part has been ascribed to the energetic cost of maintaining and transcribing rRNA genes (Larson et al. 2012; Sinclair and Guarente 1997; Warner 1999). Maintenance of nucleolar chromatin and structure that directly impacts rDNA expression has been speculated to be a key piece in the puzzle that link energetic cost of transcription to ageing.

Age Associated Transcriptional Deregulation of Pathway Genes

The main motivation to profile gene expression changes during ageing, as in any other physiological context, has been to identify pathways that are differentially regulated. Specifically, the goal is to discover either upstream transcriptional regulators or their target genes, which maybe causal to ageing and hence, manipulating them would potentially mitigate age-related pathophysiology. Here again, several global and candidate based analyses, across models, have provided a vast amount of information and we have attempted to summarize this to give a systems-level perspective (Fig. 16.1).

While efforts to catalogue age associated DEGs in lower metazoans have enabled us to fine map alterations as a continuum, they have lacked resolution vis-à-vis tissue specificity, with a few exceptions (Girardot et al. 2006; Golden and Melov 2004; Zhan et al. 2007). Despite this limitation, such whole organismal studies have unraveled genes or pathways, which are deregulated at a global level indicating their importance in contributing towards ageing (Girardot et al. 2006; Golden and Melov 2004; Zhan et al. 2007).

Intuitively, major classes of genes that were dysregulated, at least in some tissues, exemplified cell type specific alteration in physiology. For example, a com-
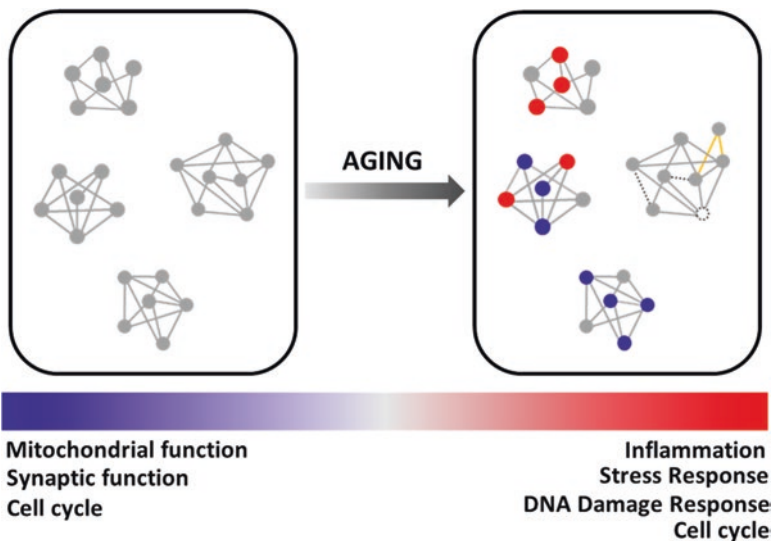

Mitochondrial function
Synaptic function
Cell cycle

Inflammation
Stress Response
DNA Damage Response
Cell cycle

Fig. 16.1 Transcriptional networks and ageing. Hierarchical/functional clustering of differentially expressed genes (DEGs) have clearly indicated that pathways, which contribute to overall fitness to be majorly deregulated. Decreased expression of 'protective' genes and hyper-induction of genes that cause exaggerated activation of stress response pathways is consistently observed in aged individuals. In addition, silencing or induction of key regulatory factors could lead to a restructuring of the gene network and hence the physiological output from the pathway

mon signature of altered liver physiology during ageing is seen by the up-regulation of stress response genes. Studies across species have shown a general trend towards increase in expression of genes involved in xenobiotic metabolism, oxidative stress and DNA damage. Genes such as for cytochrome P450, glutathione S transferase, superoxide dismutase *etc* have been regular candidates that are over-represented (Kwekel et al. 2010; Thomas et al. 2002). Genes coding for proteins important for synaptic plasticity, vesicle release and recycling including synapsin II, RAB3A *etc* have been seen to be down-regulated in ageing brain (Girardot et al. 2006; Lu et al. 2004). Another common feature associated with ageing is diminishing visual capacity (Owsley 2016). Fruit fly studies have shown several genes that are associated with the photo-response are progressively down-regulated in ageing *Drosophila*. The list includes guanylate cyclase, neurotransmitter secretion related protein, CAMKIIa as well as genes involved in DNA damage like mre11, rad50 and Ku80 (Hall et al. 2017; Kim et al. 2005). This hints towards not just a progressive decline in photo-responsiveness but also additional accumulation of DNA damage that ultimately may lead to poor vision in old age. Similarly, up-regulation of ECM genes like type 5 collagen and alpha-2 macroglobulin in the kidney (Rodwell et al. 2004) could contribute to fibrosis associated with old age.

Cell cycle genes are one of the most prominent that seem to be differentially expressed in aged tissues, as seen from both mice and human studies. This may not be surprising since *in vitro* systems have indeed pointed towards reduced proliferative potential as a major contributing factor for cellular senescence (Carlson and Conboy 2007; Kim et al. 2008; Maedler et al. 2006). Interestingly, this was observed in tissues such as liver, lung, pancreas, muscles and kidney and was conspicuously absent in brain (Enge et al. 2017; Lui et al. 2010; Welle et al. 2003; White et al. 2015). We surmise that this may be due to relatively fewer regenerative cells in the brain, which are also known to drastically decrease during ageing (Raz et al. 2004). It is interesting that genes required for progression, as well as those which inhibit cell cycle, were dysregulated within same tissues. For example, CDKN2A, which inhibits G1/S progression has been shown to be up-regulated across tissues in mammalian systems, wherein cell cycle progression genes like cdc5b, cdk6 and cyclin b1 are up-regulated. On the other hand the E2F family of transcription factors are also down-regulated (Baker et al. 2008; Lui et al. 2010; White et al. 2015). This discord could possibly be explained by one of the following: (a) a deregulation or lack directionality, (b) some of these genes may have cell cycle independent functions and (c) distinct cellular populations, with increased mitotic potential and enhanced senescent feature in these tissues could result in such heterogeneous outputs.

Across numerous studies, ontological analyses or functional clustering of genes that display maximal changes revealed enrichment in metabolic and mitochondrial genes. This is not surprising since inability to maintain metabolic fitness or altered dietary inputs have been well established as major drivers of ageing and age-related diseases (Banerjee et al. 2012; Barzilai et al. 2012; Lopez-Otin et al. 2013). Despite the tissue- and organism-specific signatures, genes involved in lipid, amino acid and carbohydrate metabolism have been seen to have abnormal expression in older

individuals. Some of these genes include glycolytic genes such as PFK, GAPDH1; TCA cycle components like PDH, malate dehydrogenase, fumarate hydratase *etc*; also those involved in mitochondrial electron transport chain and ATP synthesis, and those important for amino acid metabolism as well as for cholesterol biosynthesis such as HMGCR are also downregulated (Boisvert et al. 2018; Golden and Melov 2004; Kim et al. 2005; Welle et al. 2003; Zhan et al. 2007).

In a similar vein, nuclear encoded mitochondrial genes that modulate organelle biogenesis also emerge as a major subclass that is altered in an age-dependent manner (Hebert et al. 2010; Joseph et al. 2012; Ungvari et al. 2008). Even here, transcription of genes that are necessary for mitochondrial energetics and ROS homeostasis are affected predominantly (Thomas et al. 2002). Although mitochondria have now emerged as key signalling organelles, one exciting aspect that still needs careful analyses is the importance of retrograde signalling that originate in the mitochondria and impinge on nuclear transcription during ageing (Couvillion et al. 2016; Latorre-Pellicer et al. 2016; Richter-Dennerlein et al. 2016). One area of research, which has otherwise made significant progress but has not been thoroughly explored is that of global and tissue specific changes in mitochondrial transcription during ageing. Mitochondrial dysfunctions and the concept of nuclear-mitochondrial synchrony, which have been shown to be affected in ageing and in various diseases, needs to be investigated in light of mitochondrial cues controlling chromatin modifications and transcription (Jazwinski et al. 2017).

Similar to metabolic factors, genes that govern immune and inflammatory pathways are enriched in both up- and down-regulated categories from multiple studies, again across models. This is not surprising since many diseases and ageing have been traced to these pathways. Increased expression of genes such as for immunoglobulins and complement system components illustrate this feature (de Magalhaes et al. 2009; Rodwell et al. 2004). Elevated expression of antimicrobial peptides in fruit flies, which are a sign of activated immune system, is also commonly observed, especially in the gut, in old cohorts (Zhan et al. 2007). Inflammatory genes such as CTSS, FCGR2B, IGJ, C3, C1QA and C1QB have been shown to be up-regulated during ageing (de Magalhaes et al. 2009).

Reiterating the gender dependent alterations, gene expression pathways were shown to be deregulated differentially in males and females. While males show a down-regulation in overall metabolic and energetic pathway associated genes, immune pathways are more up-regulated in females (Berchtold et al. 2008).

As described above, classical approaches to group genes in well-defined pathways have clearly yielded high dividends in terms of enlisting them as being deregulated during ageing or in age-related diseases. However, it is now given that both upstream inputs/signals and downstream effectors that belong to all these pathways show a high degree of cross-talk. In this context, future studies should be focused at analyzing such changes at a systems level, which could include investigating chromatin contexts of loci that harbor these genes.

Elevated Transcriptional Noise

One of the characteristic features of ageing is the loss of transcriptional control, often manifested either in the form of aberrant gene expression or increase in heterogeneity within populations (at cellular and organismal levels). Studies across species have shown that with increasing age, gene expression tends to become more stochastic, as seen by the increased level of 'noise' in transcriptional profiles (Bahar et al. 2006; Martinez-Jimenez et al. 2017). This decrease in signal to noise ratio has been proposed as one of the leading causes of ageing and age-related diseases. There are reports, which argue in favor or against this hypothesis and most of these differences could be attributed to either tissue specific effects (Warren et al. 2007) or those stemming from experimental approaches that lack high sensitivity. However, emerging literature seems to indicate a significant correlation between transcriptional noise and ageing (Enge et al. 2017; Martinez-Jimenez et al. 2017; Southworth et al. 2009; Wang et al. 2011).

One of the key questions that remain to be comprehensively addressed is that of defining periods during ageing when noise becomes dominant and whether this precedes physiological attributes of ageing. In this regard, a multi-specie study published by Wang et al. (2011) highlighted the time windows during which the noisy gene expression begins to manifest. While deregulated transcription seemed to emerge around mid-life (~30 days) in *Drosophila*, in rodents this was observed by 10–15 months of age. Interestingly, similar to sex-specific onset in global transcriptional change, described above, emergence of transcriptional noise also seemed to be sexually dimorphic. Male mice were shown to be more predisposed to display this behavior, which occurred as early as 6 months of age, across tissues. On the other hand, noisy gene expression in females showed up only by 16 months of age.

It is interesting that pathways that show most dysregulation with age such as mitochondrial function, ribosomal biogenesis, inflammation *etc*. are also the ones that show maximum variation with age (Southworth et al. 2009). Southworth and colleagues also found that genes, which showed decreased correlation were clustered together on the same chromosome. This points towards a potential contribution by chromatin architecture or epigenetic mechanisms, which might result in increased noise in gene expression.

Moreover, with age, many genes in the same functional group have been shown to change expression in opposing direction, a phenomenon known as transcriptional drift, which has been shown to occur across tissues. Interestingly, lifespan expanding paradigms such as attenuation of insulin signalling and calorie restriction have been shown to suppress transcriptional drift (Murphy et al. 2003; Rangaraju et al. 2015).

It is also worthwhile to note that many of these studies indicate asynchrony in terms of different organ-systems or tissues, which show transcriptional noise, at least at the ages that were tested. This raises an extremely important question specifically in the context of deregulated transcription as a causal mechanism for ageing,

which is as follows: does the differential emergence of noise in different tissues contribute to tissue specific ageing and whether at an organismal context, there exists hierarchy for organ-system dependent transcription causing ageing?

Chromatin, Epigenetic Landscape and Ageing

It is not surprising to see that chromatin and ageing have created much interest amongst researchers, which is reflected by a vast number of papers and reviews in the field. Several review articles by others and ourselves have reflected the state of the field. Given this, we have restrained from reiterating some of the details, which have been already described in these reviews and we encourage the readers to consult them (*see* Feser and Tyler 2011; Fraga and Esteller 2007; Lazarus et al. 2013; O'Sullivan and Karlseder 2012; Pal and Tyler 2016). However, we have tried to provide a perspective based on some of the recent studies and link these chromatin changes with the functional or physiological attributes of chromatin.

Chromatin Compaction

The state of chromatin compaction is one of the best correlates of architectural change that is associated with ageing. For example, global changes in chromatin compaction have been studied across various tissues namely liver and brain in mice, and in primary cells in culture (Bochkis et al. 2014; Dell'Orco and Whittle 1982; Gaubatz et al. 1979; Ishimi et al. 1987; Thakur et al. 1999; Zongza and Mathias 1979). Classical nuclease digestion assays have revealed that there seems to be a general trend towards more compact chromatin in aged tissues (Gaubatz et al. 1979; Zongza and Mathias 1979). *In vitro* models of senescence specifically using human fibroblasts also showed that there is an age-dependent reduction in nuclease sensitivity (Dell'Orco and Whittle 1982; Ishimi et al. 1987). However, intriguingly the decrease in accessibility has been noted for both inter-nucleosomal and nucleosomal DNA depending upon the tissue or cells that were employed (Gaubatz et al. 1979; Ishimi et al. 1987; Thakur et al. 1999; Zongza and Mathias 1979). Although, some of these changes were found to be more or less pronounced in specific tissues or cell types, these early studies nevertheless indicated that ageing was indeed associated with hetero-chromatinization. These also hinted that the age-dependent changes could be brought about by alterations in nucleosomal occupancy and contributions made by non-histone proteins (Zongza and Mathias 1979).

With the advent of next generation sequencing platforms, recent studies have now unraveled loci that show differential nuclease sensitivities. It is interesting to note that maximal differences in nucleosomal occupancy, both gain and loss, were seen at distal regulatory elements (Bochkis et al. 2014). While it is probable that any differences in these studies could reflect biological heterogeneity between cells or

tissues, it is also likely to be a result of differential nuclease treatment itself. A recent report by Mieczkowski and colleagues clearly demonstrated that sequencing reads could be influenced by the extent of nuclease digestion (Mieczkowski et al. 2016).

Although, the field generally accepts the fact that chromatin may undergo compaction during ageing, loci or region specific changes have only recently begun to be unraveled. Moreover, interpreting chromatin to be more compact based on reduced nuclease accessibility is rather too simple. It is likely that some of these nucleosome bound regions still display high dynamicity and are amenable to transition to an open chromatin conformation under different physiological conditions, even in aged cells or tissues.

Chromatin Composition

It is obvious that any structural change in chromatin will be associated with histone and DNA modifications. Identification of DNA methylation and histone modifications, which define chromatin as being open or closed (hetero- or euchromatin), enabled the field to investigate if some of these were associated with global and/or loci specific alterations during ageing. It is important to note that given the recent advances in the field vis-à-vis novel modifications and along with differential deposition of histone variants, our current understanding is likely to expand immensely in the future. Further, given that some of these modifications are directly impacted by alterations in metabolism, it will be interesting to investigate if dietary inputs can encode chromatin states (Dai et al. 2014; Fan et al. 2015; Goudarzi et al. 2016; Li et al. 2016; Lu and Thompson 2012; Sabari et al. 2015; Sabari et al. 2017). This is relevant since modulations in diets have been well established to alter fitness and also contribute to organismal longevity (Banerjee et al. 2012; Fontana and Partridge 2015; Newman et al. 2017; Peleg et al. 2016).

DNA methylation

DNA methylations are well documented to influence gene expression via various mechanisms, depending on their abundance and whether they occur on inter-genic or intra-genic regions. Most of the current studies on young and old cohorts have largely investigated changes in CpG methylations (mCpG). Two prevalent views have emerged from the studies on various model systems: (a) that a global increase in promoter methylations could lead to gene expression changes in old age and (b) that a loss of DNA methylations at repeat elements seems to agree with the heterochromatin loss model of ageing (Kwabi-Addo et al. 2007; Pal and Tyler 2016; Rath and Kanungo 1989; Takasugi 2011; Wilson et al. 1987). However, more recent efforts indicate that both loci and age specific alterations in 5-methyl cytosine (5-mC) marks are more complex, wherein global bi-directional methylation changes

are variable within a population or an individual across tissues (Bjornsson et al. 2008; Christensen et al. 2009; Maegawa et al. 2010).

Studies in human peripheral blood cells have provided insights into age-dependent alterations in DNA methylation. Bjornsson et al. (2008) have reported global alterations and that the most prevalent differential methylations occurred near the transcription start site (TSS) of a number of immune-modulatory genes in aged human unfractionated peripheral blood cells. Efforts to identify age associated differentially methylated regions (aDMRs) revealed both hyper-aDMRs and hypo-aDMRs, most of which cluster around the TSS and is consistent with the previous study (Christensen et al. 2009; Rakyan et al. 2010). Further, addressing such changes in myeloid derived monocytes and lymphoid derived T-cells uncovered cell type changes (Rakyan et al. 2010). Studies have also revealed no specific bias towards enrichment in methylation at non-/CpG islands. Highlighting tissue/cell type specific variations, while the global DNA methylation did not alter significantly between young and old human epidermis, there were loci specific hyper and hypo methylated DMRs (Raddatz et al. 2013).

A relatively comprehensive analysis of CpG methylations in multiple human tissues revealed that genes associated with ageing show similar trends towards hypermethylation (Christensen et al. 2009). Intriguingly, while a positive correlation emerged between methylation and ageing for loci in the CpG islands (CGI), those that are not associated with CpG islands showed significant loss of methylation with age in solid tissues (Christensen et al. 2009). A comparative analysis of DNA methylation from young and old intestines, both in humans and in mice (Maegawa et al. 2010), showed that telomeres had maximum hypermethylation and it was least in centromeric regions. The physiological implications of these, both in terms of mechanisms and functional outcomes are still unclear. Intriguingly, while hypermethylation in human and mice intestines showed some concordance, there was no conservation for hypomethylated regions. However, consistent with the aforementioned study, a candidate based approach revealed that CpGs at specific gene-loci such as *ESR1, CDKN2A, P2RX7*, DMR1/2 of *IGF2 etc*, showed similar trends for either hyper- or hypo-methylation, across tissues in aged mice (Maegawa et al. 2010). While the results indicated that genes involved in development and differentiation were hyper-methylated, surprisingly, no such clustering was apparent for hypo-methylated loci (Maegawa et al. 2010).

Replicative senescence in mesenchymal stem cells (MSCs) was also shown to be associated with alterations in methylation at CGIs. Similar to ageing in tissues, even in the absence of a global change, methylations at specific CpGs showed senescence associated directional changes (Schellenberg et al. 2012). Comparative analysis between MSCs from adipose tissue and bone marrow showed tissue specific methylation changes and the regions that were differentially methylated during senescence showed enrichment for metabolic genes (Schellenberg et al. 2012).

It is not surprising to find that chromatin regulators are themselves under the control of epigenetic changes. In this context, age associated methylation changes were observed in various telomere maintenance and epigenetic regulatory genes

including TERT, ERCC1, DNMT3B *etc*. It was interesting to see the CpG methylation of the *de novo* DNA methyl transferase (DNMT3B) was itself significantly reduced during ageing, across tissues (Christensen et al. 2009). These suggest that epigenetic alterations at such loci could lead to a cascading effect resulting in global changes.

Recently identified 5-hydroxy methyl cytosine marks on DNA brought about by TET enzymes have enhanced our understanding about DNA modifications and their functional and regulatory roles (Pfeifer et al. 2013; Tan and Shi 2012). Unlike in other physiological contexts, the role of TET proteins and association of 5-hmC marks along with the process of cellular/organismal ageing has not been comprehensively investigated. Global changes in 5hmC marks have been assayed during ageing in specific regions of mice brain (Chen et al. 2012; Chouliaras et al. 2012a; Chouliaras et al. 2012b; Szulwach et al. 2011). A global increase in hippocampal 5hmC content in aged mice was seen, independent of any changes in TET expression (Chen et al. 2012). On the contrary, genome wide analysis and comparison of 5hmC marks in mice and human brain samples showed that these marks were relatively stable across development and ageing (Szulwach et al. 2011). Nevertheless, there were localized differentially hydroxy methylated regions (DhMRs) at the CGIs or TSS, indicating that loci specific 5hmC could be further acquired or lost with age (Szulwach et al. 2011).

Since oxidation of 5mC results in 5hmC, it will now be interesting to compare the regions that show age associated anti-correlative changes in these marks. Specifically at loci that show loss of methylation, a gain of 5hmC could indicate directional switch during ageing. Further, a meta-analysis of transcripts emerging from such regions might provide information about the importance of DNA methylation and de-methylation (to 5hmC) in regulating gene expression in aged tissues.

Histones and Modifications

Early studies, which assayed for nuclease sensitivity and nucleosomal packaging, as mentioned previously, displayed age-associated changes. These further led the field to check if overall nucleosomal content was altered during ageing. For example, a severe loss of nucleosomes owing to a reduction in core histone protein levels has been shown to be associated with replicative ageing in budding yeast (Dang et al. 2009; Feser et al. 2010; Hu et al. 2014). Although, studies in higher organisms have shown a prevalent decrease in the levels of histones H3 and H4 (but not of H1) during ageing (Liu et al. 2013; O'Sullivan et al. 2010), there are no reports to indicate altered nucleosomal deposition. Deregulated transcriptional, post-transcriptional and translational mechanisms have been attributed to this loss in histone proteins (Liu et al. 2013; O'Sullivan et al. 2010). Based on these observations, one could speculate that at least in higher eukaryotes, reduced histones along with altered

expression/activity of histone chaperones (O'Sullivan et al. 2010) may not necessarily lead to altered nucleosomal content, but may actually result in lower turnover and contribute to closed chromatin structures.

Histone Variants

In addition to changes in total histone levels, there is ageing dependent differential deposition of histone variants, which further strengthens the concept of altered nucleosomal composition during senescence. Independent studies have demonstrated a global decline in H3.1 and H3.2, the replication dependent H3 variants in higher organisms (Maze et al. 2015; Pina and Suau 1987; Rogakou and Sekeri-Pataryas 1999). Increase in H3.3 (a replication independent and constitutively expressed variant) in senescent cells/tissues, which no longer are mitotic seemed rather predictable (Duarte et al. 2014; Maze et al. 2015; Piazzesi et al. 2016; Pina and Suau 1987; Rogakou and Sekeri-Pataryas 1999). In senescent human fibroblasts and melanocytes, elevated H3.3 and its N-terminally cleaved forms (H3.3cs1/2) were associated with repressive marks and lead to down-regulation of cell cycle progression genes (Duarte et al. 2014). Piazzesi et al. (2016) showed that even at an organismal level, ageing leads to heightened levels of H3.3 in worms.

Histone H2A and its variants have been well documented to impact transcription, both positively and negatively. Specifically, H2A.Z at +1 and − 1 nucleosomes is considered as a hallmark of actively transcribing loci. H2A.Z levels were enhanced with ageing in human cells and mice brain (Rogakou and Sekeri-Pataryas 1999; Stefanelli et al. 2018), which was mostly seen at TSS of genes involved in transcription and chromatin regulation (like Crebbp, Dnmt3a *etc*) and ubiquitin mediated proteosomal degradation (Stefanelli et al. 2018). The canonical H2A variants, H2A.1 and H2A.2, showed opposing changes during ageing. Wherein the levels of H2A.1 showed progressive reduction during the course of senescence in human fibroblasts and rat cortical neurons, H2A.2 levels were elevated (Pina and Suau 1987; Rogakou and Sekeri-Pataryas 1999). However, functional specializations of the canonical variants have not been characterized to-date. Variant macroH2A1 showed a consistent age associated increase in its isoforms (mH2A1.1 and mH2A1.2) across higher mammals and during replicative senescence in cells (Chen et al. 2015; Kreiling et al. 2011; Zhang et al. 2005). Enhanced macroH2A1 was seen to be associated with facultative heterochromatin and lead to up-regulation of Senescence Associated Secretory Phenotype (SASP) genes in fibroblasts (Chen et al. 2015; Zhang et al. 2005).

Apart from core nucleosomal histones, variants of the linker histone H1 (H1.3, H1A, H1B and H1ᵒ) also showed alterations in aged chromatin. Although, cellular senescent models showed a loss of total H1 levels in senescent cells (Funayama et al. 2006), a global increase in H1 variants was seen in murine tissues during ageing (Medvedev and Medvedeva 1990). Altogether, age dependent elevation of histone variants that are primarily associated with heterochromatin formation or maintenance could aid in increased chromatin compaction (Table 16.1).

Table 16.1 Age-associated changes in histone variants

Variant	Model Organism	Tissue/Cell type	Change	Change Localised to	References
H3.1	Human	Lung fibroblasts	Decrease	Overall	Rogakou et al. (1999)
	Rat	Brain Cortical Neurons	Decrease	Overall	Pina and Suau (1987)
	Mouse and Human	NeuN+ Chromatin and whole brain	Decrease	Overall	Maze et al. (2015)
H3.2	Human	Lung fibroblasts	Slight Increase	Overall	Rogakou et al. (1999)
	Rat	Brain Cortical Neurons	Decrease	Overall	Pina and Suau (1987)
	Mouse and Human	NeuN+ Chromatin and whole brain	Decrease	Overall	Maze et al. (2015)
H3.3	Human	Lung fibroblasts	Increase	Overall	Rogakou et al. (1999)
	Human	Fibroblasts, Melanocytes	Increase in H3.3 and its cleaved forms	Euchromatic regions	Duarte et al. (2015)
	Mouse and Human	NeuN+ Chromatin and whole brain	Increase	Overall	Maze et al. (2015)
	Rat	Brain Cortical Neurons	Increase	Overall	Pina and Suau (1987)
	Worms	Whole Body, Nerve Ring, Foregut	Increase		Piazzesi et al. (2016)
H2A.1	Human	Lung fibroblasts	Decrease	Overall	Rogakou et al. (1999)
	Rat	Brain Cortical Neurons	Decrease	Overall	Pina and Suau (1987)
H2A.2	Human	Lung fibroblasts	Increase	Overall	Rogakou et al. (1999)
	Rat	Brain Cortical Neurons	Increase	Overall	Pina and Suau (1987)
H2A.Z	Human	Lung fibroblasts	Increase	Overall	Rogakou et al. (1999)
	Rat	Brain Cortical Neurons	No Change	Overall	Pina and Suau (1987)
	Mouse	Hippocampus	Increase	TSS, +1 and -1 nucleosome	Stefanelli et al. (2018)
H2AX	Human	Lung fibroblasts	Decrease	Overall	Rogakou et al. (1999)
	Rat	Brain Cortical Neurons	Increase	Overall	Pina and Suau (1987)

(continued)

Table 16.1 (continued)

Variant	Model Organism	Tissue/Cell type	Change	Change Localised to	References
macroH2A1	Human	IMR90-Fibroblasts	Increase in mH2A1.1 and mH2A1.2	Facultative Heterochromatin	Chen et al. (2015)
	Human	W138-Fibroblasts	Increase in mH2A1.2	Heterochromatin Foci	Zhang et al. (2005)
	Human	Fibroblasts	Increased but variable cell to cell intensity	Heterochromatin Foci	Kreiling et al. (2011)
	Mouse	Lung, Liver, Muscle	Increase in proportion of cells with higher intensity	Overall	Kreiling et al. (2011)
	Baboon	Muscle, Dermal Fibroblasts	Increase in proportion of cells with higher intensity in muscle, subtle increase in skin	Overall	Kreiling et al. (2011)
H1	Human	IMR90, MRC-5, BJ fibroblasts	Decrease	Overall	Funayama et al. (2006)
H1 A	Rat and Mice	Liver	Increase	Overall	Medvedev et al. (1990)
H1B	Rat and Mice	Liver	Increase	Overall	Medvedev et al. (1990)
H1°	Rat and Mice	Liver, Kidney	Increase	Overall	Medvedev et al. (1990)

Modifications

Ourselves and others have reviewed histone modifications and their association with cellular and organismal ageing extensively in the past (Lazarus et al. 2013; Pal and Tyler 2016). Hence, we have now tried to provide a more elaborate picture based on recent studies in this section, specifically in relation to alterations in gene expression. Histone modifications have also been implicated in the formation of senescence-associated heterochromatin foci (SAHF), which have been observed in senescing cells and are described in a later section.

Given the diversity of histone modifications, it is not surprising to see that some of these have been studied in a candidate based manner and have alluded to their roles in regulating expression of a subset of genes during ageing. Based on multiple studies to unravel global alterations in histone modifications, the findings can be grouped as below: (a) general euchromatic versus heterochromatic marks possibly involved in mediating chromatin architecture, (b) specific activatory or inhibitory marks mostly associated with immediate upstream regions, (c) distal regulatory regions such as silencers or enhancers and (d) at repeat and retroviral elements (Table 16.2).

Table 16.2 Age-dependent alterations in histone modifications

Modification	Model Organism	Tissue/Cell type	Change	Change Localised to	Assay Platform	References
Activatory Marks						
H3K4me3	Mouse	Hematopoietic stem cells	Spread of H3K4me3	TSS +1 and -1 nucleosome	Chip-seq	Sun et al. (2014)
	mouse	muscle stem cells	No change, reduced intensity	TSS +1 and -1 nucleosome	Chip-seq	Liu et al. (2013)
	Drosophila	Head	Overall reduction	TSS and downstream	ChIP-chip and WB	Wood et al. (2010)
	Human	Dermal Fibroblasts	Decreased but biphasic with increasing PDL	Overall	WB	Sullivan et al. (2010)
	Human	Fibroblast	Decrease	Overall	WB	Duarte et al. (2015)
	Worms	Whole Worms	No change	Overall	WB	
H3K9ac	Mice	Brain	Increase	Overall	WB	Rodrigues et al. (2014)
	Rat	Liver	Decrease	Overall	WB	Kawakami et al. (2009)
	Human	Dermal Fibroblasts	Slight increase	Overall	WB	Sullivan et al. (2010)
H3Ac	Rat	Pancreatic islets	Decrease	Enhancer at the Hnf4a Locus	ChIP	Sandovici et al. (2011)
H4K5Ac	Human	Dermal Fibroblasts	Increased with PDL	Overall	WB	Sullivan et al. (2010)
H3K18Ac	Human	IMR90 Fibroblast	Decrease	Overall	WB	Duarte et al. (2015)
H3K14ac	Human	IMR90 Fibroblast	Decrease	Overall	WB	Duarte et al. (2016)
	Rat	Liver	No change	Overall	WB	Kawakami et al. (2009)
H4K12Ac	mice	Hippocampus	No increase in response to Fear Conditioning	TSS and gene body downstream	WB and microarray and ChIP-seq	Peleg et al. (2010)
H4K16Ac	Human	Dermal Fibroblasts	Increased with PDL	Overall	WB	Sullivan et al. (2010)
	Yeast	Budding yeast cells	Increase	Overall	WB	Dang et al. (2009)
H3S10p	Rat	Liver	Increase	Overall	WB	Kawakami et al. (2009)
	Human	Dermal Fibroblasts	Decreases	Overall	WB	Sullivan et al. (2010)
H3K56Ac	Human	Dermal Fibroblasts	Decreased with PDL	Overall	WB	Sullivan et al. (2010)
	S.cer	Budding yeast cells	Decrease	Overall and at Telomeres	WB	Dang et al. (2009)
H3K36me3	Drosophila	Head region	Reduction majorly in the gene body	Mid gene body	ChIP-chip and WB	Wood et al. (2010)
N-Ac-H10	Rat and Mouse	Liver	Increase with age	Overall		Lindner et al. (1999)
H10 Asn-3/Asp-3		Kidney	Major Increase with age			

(continued)

Table 16.2 (continued)

Modification	Model Organism	Tissue/Cell type	Change	Change Localised to	Assay Platform	References
			Inhibitory marks			
H3K27me3	Mouse	muscle stem cells	Majorly Increase but decrease at specific loci	Spread in to gene body and upstream	Chip-seq	Sun et al. (2014)
	mouse	QSC and ASC of muscle	Spread but no overall change in levels	Less Focussed and spread around TSS	Chip-seq	Liu et al. (2013)
	Human	Embryonic Fibroblasts	Reduction across PDLs	TSS and promoter of INK4b-p16-ARF cluster	ChIP-PCR	Bracken et al. (2006)
	Human	Dermal Fibroblasts	Increased in specific cell cycle stage-leading to overall increase	Overall	WB	Sullivan et al. (2010)
	Mouse	b-cells	Increases	Near proximal promoter region	ChIP-Seq	Avrahami et al. (2015)
	Human	IMR90 Fibroblast	Increase	Overall	WB	Duarte et al. (2015)
	Rat	Pancreatic islets	Increases	Enhancer/Promoter at Hnf4a locus	ChIP	Sandovici et al. (2011)
H3K9me3	Mice	Brain	Slight Reduction	Overall	WB	Rodrigues et al. (2014)
	Drosophila	Head region	No overall change, loss w.r.t to HP1 was more	Overall	ChIP-chip and WB	Wood et al. (2010)
		Fat body	On WB there is an increase	Spread by IF	IF	
	Rat	Liver	No change	Overall	WB	Kawakami et al. (2009)
	Human	Multiple skin fibroblasts	Overall decrease with age but increase with PDL in cells derived from aged donors	Overall	IF	Scaffidi (2006)
	Rat	Pancreatic islets	Increases	Enhancer/Promoter at Hnf4a locus	ChIP	Sandovici et al. (2011)
H3K9me1, me2	Human	Dermal Fibroblasts	Decreases	Overall	WB	Sullivan et al. (2010)
			Increases with PDL			
uH2A and uH2B	Mice	Brain	Increase	Overall	WB	Morimoto et al. (1993)
H4K20me3	Human	Dermal Fibroblasts	Decreases	Overall	WB	Sullivan et al. (2010)
H4K20me2	Rats	Liver, Kidney	Increase	Overall	Mass-spec	Sarg et al. (2002)
			No Change			
H4K20me1			Increase			

(continued)

Table 16.2 (continued)

Modification	Model Organism	Tissue/Cell type	Change	Change Localised to	Assay Platform	References
Distal Regulatory Marks						
H3K4me1	Rat	Pancreatic islets	Decrease	Enhancer at Hnf4a locus	ChIP	Sandovici et al. (2011)
	Human	Dermal Fibroblasts	Decreased but biphasic in cell cycle with increasing PDL	Overall	WB	Sullivan et al. (2010)
At Repeat Elements						
uH2B	Yeast	Budding yeast cells	Increase with age	At the telomeres	WB	Ho Rhie et al. (2013)
H4K16Ac	Yeast	Budding yeast cells	Increase with age	At telomeres and rDNA loci	ChIP	Dang et al. (2009)
H4K16Ac	Human	Dermal Fibroblasts	Increase with PDIs	At the telomeres	ChIP and Dot Blot	Sullivan et al. (2010)
H4K20me2	Human	Dermal Fibroblasts	No change in levels but shift in cell cyce phase	At the telomeres	ChIP and Dot Blot	Sullivan et al. (2011)
H3K79me2	Human	Dermal Fibroblasts	Increase with PDIs	At the telomeres	ChIP and Dot Blot	Sullivan et al. (2010)

The prominent euchromatin marks like K4me3, K9Ac, K14Ac, K36me3 on histone H3 and acetylations on K5, K12 and K16 of histone H4 have been widely assayed in the context of ageing (Duarte et al. 2014; Kawakami et al. 2009; Liu et al. 2013; O'Sullivan et al. 2010; Sun et al. 2014; Wood et al. 2010). Several studies across tissues and cell types have reported an overall reduction in the transcriptional activation mark H3K4me3, at the proximal promoter regions (Duarte et al. 2014; Liu et al. 2013; O'Sullivan et al. 2010; Sun et al. 2014; Wood et al. 2010). The H3K4me3 peak at the TSS is now considered as a bona-fide mark of active transcription. ChIP-Seq analysis in young and old hematopoietic stem cells indicated that, while some regions had decreased H3K4me3, these were much fewer when compared to the percentage of genes, which showed an age associated gain in this mark (Sun et al. 2014). Interestingly, both reduced levels and spread of H3K4me3 (away from its typical +1 and − 1 nucleosomes) have been reported specifically in old stem cells (Liu et al. 2013; Sun et al. 2014). In addition to the findings from Sun and colleagues on promoter switching and expression of isoforms in old HSCs (Sun et al. 2014), this spread of K4me3 raises the possibility that TSS in aged cells could be altered or ill defined (Fig. 16.2). Efforts to investigate the H3K4-me1/me2/me3 in cellular models of senescence added a layer of complexity with regards to cell cycle phase dependent alterations at a global level (O'Sullivan et al. 2010). It remains to be seen if such changes are found in other cell/tissue types and whether
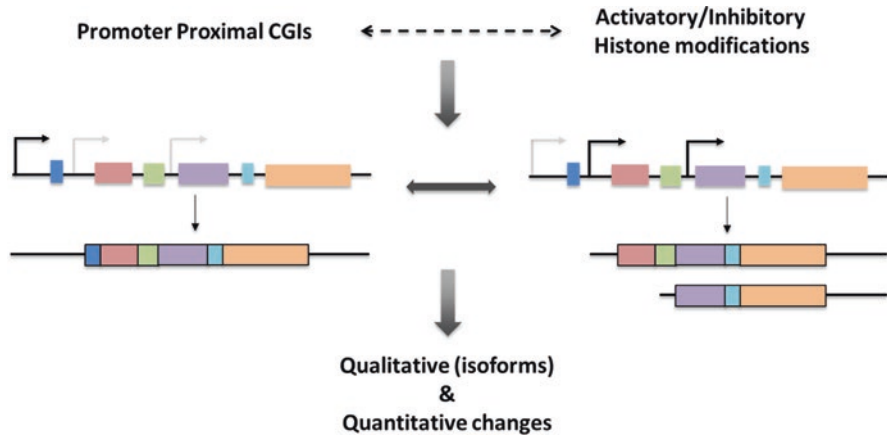
Fig. 16.2 Alternate start site specification. Loss of 5mC at CpG islands (CGIs) and spilling of H3K4me3, beyond the typical di-nucleosomal context, could lead to specification of novel or aberrant transcription start sites (TSS). Specification of such TSS have been proposed to generate alternate transcripts, which could have differential localizations, stabilities and in some instances code for different isoforms of proteins

these lead to increase in noise vis-à-vis both abundance and type of transcripts produced during ageing (Fig. 16.3).

Limited numbers of studies that have investigated the changes in histone H3 lysine-9 acetylation, an open chromatin signature, illustrate it to be more variable across cell/tissue types during ageing (Kawakami et al. 2009; O'Sullivan et al. 2010; Rodrigues et al. 2014). In aged rat liver there was a global decrease in K9Ac, whereas it showed an overall increase in nuclei from old mice brains (Kawakami et al. 2009; Rodrigues et al. 2014). Although there seems to be a very slight accumulation of K9Ac in senescent human fibroblasts, the changes were also cell cycle dependent (O'Sullivan et al. 2010). In the absence of genome wide studies to define loci-specific changes in H3K9Ac, at present, there is poor concordance between organismal ageing and replicative senescence.

Across models, other activatory modifications on H3 showed a general trend towards decrease during ageing. For example, reduced global H3 acetylation (Sandovici et al. 2011), K18Ac, K14Ac (Duarte et al. 2014) K56Ac (Dang et al. 2009; O'Sullivan et al. 2010) and K36me3 (Wood et al. 2010) were observed. On the contrary, activatory marks on histone H4 such as K5Ac and K16Ac were shown to increase with age (Dang et al. 2009; O'Sullivan et al. 2010). In addition to H4 marks, deamidation and N-terminal acetylation of H1 also showed a substantial increase with age in mice and rat tissues (Lindner et al. 1999). Even though this
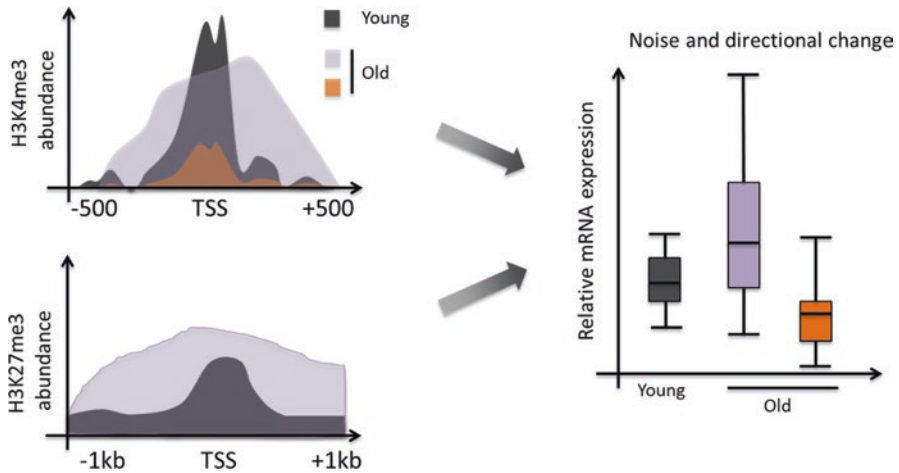
Fig. 16.3 Epigenetics as causal mechanism for age associated transcriptional deregulation: Perturbations in histone modifications around the upstream cis-regulatory elements lead to transcriptional noise and altered expression. An increase in the repressive mark H3K27me3 and a decrease in the active mark H3K4me3 occur during ageing. Intuitively, these could result in both up- and down-regulation of genes, which have been seen independently. In addition to these, H3K4me3 that is typically present in −1/+1 nucleosomes also seems to spread around the TSS. Similarly, H3K27me3 is also shown to spread in aged tissues/cells. It is therefore plausible that altered regulatory histone modifications cause the age-associated elevation in transcriptional noise

seems to indicate a non-directional perturbation, whether these changes happen at same or different loci needs to be addressed. Irrespectively, this once again points towards deregulated signatures that may contribute to aberrant or noisy transcription.

With the general understanding that heterochromatin content increases during ageing, there has been a special effort to unravel the importance of repressive histone modifications in this regard. H3K27me3, a polycomb dependent repressive chromatin mark, showed consistent increase during ageing, both in stem cells and differentiated cells (Avrahami et al. 2015; Duarte et al. 2014; Liu et al. 2013; O'Sullivan et al. 2010; Sandovici et al. 2011; Sun et al. 2014). More detailed analysis at specific loci across the genome revealed that unlike in younger cells, methylation at this residue tend to be less focused around the promoter proximal sites and spread in to upstream and downstream regions (with reference to TSS) in older cells (Liu et al. 2013, Sun et al. 2014). Heterochromatic H3K9me3 and H4K20me3 marks showed an overall decrease in fibroblasts with higher population doubling

levels (PDLs). Interestingly, this was associated with a concomitant increase in mono- and/or di-methylations (O'Sullivan et al. 2010; Rodrigues et al. 2014; Sarg et al. 2002; Scaffidi and Misteli 2006). On the contrary, aged tissues such as *Drosophila* heads and rat liver showed no apparent change in total K9me3 levels (Kawakami et al. 2009; Wood et al. 2010). But fat body and pancreatic islets from aged *Drosophila* and rats, respectively, showed enhanced tri-methylation (Kawakami et al. 2009; Wood et al. 2010). Assaying for H3K9me3 by immune-fluorescence in *Drosophila* fat body showed an increased intensity and dispersed localization of this mark in old flies (Wood et al. 2010).

Ubiquitination on H2A, a co-signature of polycomb associated K27me3 mark, and on H2B also increased with age and this seemed to be consistent across cell/ tissue type (Morimoto et al. 1993; Rhie et al. 2013). Although, these observations raise the possibility of combinatorial/interdependent histone marks to be similarly regulated during ageing, this requires further comprehensive analysis. Taken together, the preponderance of inhibitory chromatin marks are in concordance with increased chromatin compaction and heterochromatinization in aged cells/tissues. This notion is also supported by the observation that both H3K27me3 and H3K9me3 showed a general trend of dispersed localization on chromatin during the process of ageing (O'Sullivan et al. 2010; Wood et al. 2010). Albeit that some of these studies highlight a lack of correlation regarding either an increase or decrease in repressive modifications, it is important to note that assaying for these by biochemical or cell biological methods does not provide the required resolution. Hence, a thorough high-throughput based analysis will provide information about regions that show a gain or loss of heterochromatic marks.

There is enough evidence in the literature demonstrating interdependence of DNA methylations and histone modifications, that eventually result in different chromatin states (Balasubramanian et al. 2012; King et al. 2016; Morselli et al. 2015; Rose and Klose 2014). With this premise, studies that focused on aged chromatin have tried to draw correlations between bi-directional changes in the levels of DNA methylation and states of chromatin (w.r.t. histone marks). Across cell types, age dependent hypo-DMRs were strongly correlated with histone modifications such as H3K4me1/3 and K27Ac that mark actively transcribing regions or active enhancers (Avrahami et al. 2015; Raddatz et al. 2013; Schellenberg et al. 2012; Sun et al. 2014). Not surprisingly, heterochromatin marks like H3K9me3 and K27me3 were highly associated with hyper-aDMRs (Raddatz et al. 2013; Rakyan et al. 2010; Schellenberg et al. 2012; Sun et al. 2014). Interestingly, poised/bivalent regions that harbor both H3K27me3 and H4K4me3 marks at the promoters showed elevated CpG methylation during ageing (Raddatz et al. 2013; Rakyan et al. 2010). These results indicate that the intricate crosstalk between modifications on DNA and histones (Table 16.3) bring about chromatin remodeling, with an overall trend towards heterochromatinization, during ageing (Fig. 16.4). Furthermore, it is still not clear as to which of these modifications drive ageing associated chromatin compaction.

Physiologically, key cellular and molecular dysfunctions ranging from stem cell fatigue to DNA damage and mitochondrial/metabolic outputs have been shown to be major drivers of ageing (Balaban et al. 2005; Beerman et al. 2014; Bratic and

Table 16.3 Association of DMRs with histone modifications

DNA methylation status	Model Organism	Tissue/Cell type	Associated Histone marks	Loci	References
Hypo-DMRs	Mouse	β-cells	H3K4me1, H3K27Ac	Active Loci	Avrahami et al. (2015)
	Human	MSC-AT and ESCs	H3K4me3	Active Loci	Schellenberg et al. (2011)
	Human	Epidermis	H3K27Ac, H3K4me1	Enhancers	Raddatz et al. (2013)
	Human	HSCs	H3K4me3, H3K27me3	Promoters and Poised	Sun et al. (2014)
Hyper DMRs	Human	CD4+ T Cells	H3K9me3, H3K27me3, H3K4me3	Heterochromatic and poised	Rakyan et al. (2010)
	Human	MSC-AT and ESCs	H3K9me3, H3K27me3,	Heterochromatic	Schellenberg et al. (2011)
	Human	Epidermis	H3K27me3, H3K4me3, H3K27Ac	Poised Promoters	Raddatz et al. (2013)
	Human	HSCs	H3K27me3	Heterochromatic	Sun et al. (2014)
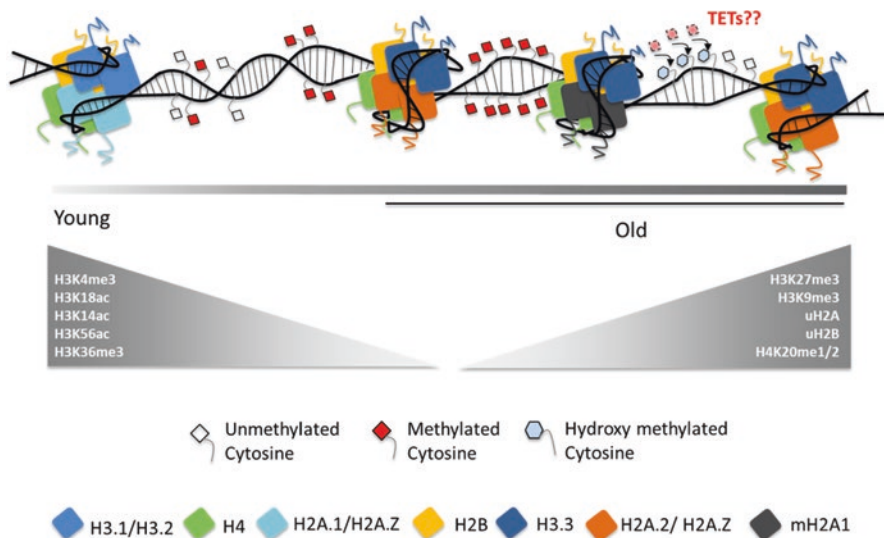
Fig. 16.4 Chromatin composition and modifications drive global compaction during ageing. Globally, chromatin gets compacted during ageing. This is associated with altered nucleosomal composition and a gain in heterochromatic marks, as detailed in the figure and the article. Some of these alterations are also evident in the form of senescence associated heterochromatin foci (SAHF) that are mostly seen in *in vitro* models of ageing. It is still unclear if the loss of 5mC at some regions correlates with a gain in 5hmC at the very same loci. Meta-analyses of recruitment of chromatin modifiers, along with these marks and in an age-dependent manner, will be critical to assess the progression in altered chromatin architecture

Trifunovic 2010; Chambers et al. 2007; Chen et al. 2007; Di Micco et al. 2008; Finkel 2015; Latella et al. 2017; Pollina and Brunet 2011). In this regard, very few existing and emerging studies have tried to link the changes in chromatin to these, possibly mediated by altered gene expression patterns and deregulated DNA damage response (Burgess et al. 2012; Gorbunova and Seluanov 2016; O'Sullivan et al. 2010). Independently, age dependent reduction in genomic integrity and alterations in histone modifications that affect DNA damage response are well documented (Oberdoerffer et al. 2008; Pan et al. 2014; Schotta et al. 2008; Van Meter et al. 2014). However, very few studies have linked these together at phenomenological and mechanistic levels during ageing. Recent reports illustrate that both DNA and histone modifications are visibly dissimilar in young and old stem cells. Modifications such as H3K4me3, K9me3 and K27me3, which are central determinants of gene expression and genome integrity, show dramatic alterations during ageing that together could influence proliferation and differentiation potentials (Liu et al. 2013; Schellenberg et al. 2012; Sun et al. 2014). Here again, combining transcriptome data with ChIP-seq analyses for histone modifications will be crucial to unravel the importance of chromatin structure in driving age-associated transcriptional changes.

Senescence Associated Heterochromatin Foci (SAHF)

Formation of dense and compact DNA foci, now called Senescence Associated Heterochromatin Foci (SAHFs) is considered to be one of the better-characterized correlates of irreversible chromatin changes during senescence. Therefore, it is not surprising that several review articles, including ours (Lazarus et al. 2013), have elaborated on the identification and composition vis-à-vis histone variants, modifications and non-histone proteins (Adams 2007; Funayama and Ishikawa 2007; Narita 2007; O'Sullivan and Karlseder 2012; Pal and Tyler 2016; Rastogi et al. 2006; Sadaie et al. 2013) (Table 16.4). In this light, we have only reviewed more recent studies and specifically highlight aspects that provide novel insights.

SAHFs have been mostly observed and described for *in vitro* models of senescence and there are no studies about their presence in aged tissues as far as our literature survey goes (Funayama et al. 2006; Narita et al. 2006; Narita et al. 2003; Zhang et al. 2007; Zhang et al. 2005). However, in a report by Kreiling et al. (2011) it seemed as if mH2A, a component of SAHFs, was enhanced and strongly associated with heterochromatic foci in aged liver and muscle. Although, the authors of the study do not comment if these could be typical SAHFs, this needs further investigation in the future.

It is important to note that very few reports to-date, have tried to address the functional relevance, if any, of formation of these foci. Further, there is no clear understanding of the regions that contribute to the formation of these foci, although heterochromatinization itself is a generic event during ageing. Given that heterochromatin signatures like H3K9me2/3 and H3K27me3 are enriched in SAHFs, a study by Chandra and colleagues methodically demonstrated the spatial

Table 16.4 Senescence-associated heterochromatin foci from *in vitro* models

Model Organism	Tissue/Cell type	Associated Histone Variants	Associated Histone Modifications	Associated Proteins	Additional Observations	Genes Affected	Ref	Paradigm
Human	IMR90, WI38 fibroblasts		H3K9me3	HP1α,β,γ		Up: p21 Down: E2F responsive genes like cyclin E	Narita et al. (2003)	Ras induced and late passage
Human	WI38 fibroblasts	macroH2A1.2	H3K9me3	HP1α,β,γ, HIRA, ASF1a	ASF1a H3 binding function is necessary		Zhang et al. (2005)	Ras induced and late passage
Human	WI38 fibroblasts		Marks are distinct from mitotic/apoptotic condensed chromatin		Whole chromosome condense in to SAHF		Funayama et al. (2006)	Ras induced senescence
Human	IMR90, BJ fibroblasts		H3K9me3	HMGA1, HMGA2		Down regulation of Cyclin A, MCM3	Narita et al. (2006)	Ras induced senescence
Human	MCF7			Prohibitin, HP1γ	Prohibitin was indispensable & associated with HP1	Represses E2F mediated Transcription	Rastogi et al. (2006)	Adriamycin induced Senescence
Human	WI38 fibroblasts	macroH2A1.2	H3K9me2/3 HP1γS9p	HP1α,β,γ, HIRA, ASF1a	Each chromosome condenses in to SAHF focus		Zhang et al. (2007)	Ras induced senescence
Human	IMR90 fibroblasts		H3K9me2/3, H3K27me3, HP1γ		K9me3 limited to the core of SAHF, K9me2 formed a layer around it, K27me3 formed the outer shell	Down regulation of CCNA2, Up regulation of p16	Chandra et al. (2012)	Ras induced senescence
Human	WI38 fibroblasts				Reduced intra-TAD interactions increased inter-TAD interactions		Chandra et al. (2015)	WI-38hTERT/GFP-RAF1-ER moel
Human	IMR90 fibroblasts				Reduced LaminB1 in K9me3 regions and Increase at K27me3 regions		Sadaie et al. (2013)	Ras induced senescence

organization and senescence-associated repositioning of these modifications (Chandra et al. 2012). Interestingly, H3K27me3 that formed the 'outermost shell' of the repressive core in these SAHFs (Chandra et al. 2015; Chandra et al. 2012) could act as potential barriers to regions of transcriptional activity.

Attempts to unravel if specific regions of chromosomes or entire chromosomes are packaged into SAHFs have been scarce. Early studies have shown that whole chromosomes condense to form a single SAHF (Funayama et al. 2006; Zhang et al. 2007). But recent observations with inactivated X chromosome in senescent cells showed that involvement of whole chromosomes might not be necessary for SAHF remodeling (Chandra et al. 2012). However, whether this is true for all chromosomes and cells in a population and the heterogeneity associated, have not been investigated thus far.

Unlike euchromatin, quite expectedly heterochromatin forms a dense structure, which has led researchers to investigate Topologically Associated Domains (TADs) in SAHFs. A very recent study demonstrated the architectural changes in the genome during cellular senescence (Chandra et al. 2015). With no apparent change in global chromosomal interactions, these results revealed striking changes in the strength of intra-TAD interactions. While they saw a loss of interactions within TADs, senescent cells seemed to gain cross boundary interactions across TADs. Domains, which lost boundary strength with ageing, were enriched in H3K9me3 and Lamin Associated domains (LADs). Whereas the TAD boundaries that attained strength with age, showed enrichment in H3K36me3 marks. Very interestingly, the study demonstrated that a LAD that drifted away from the nuclear periphery during senescence was in close proximity to the *CDKN2A* locus, expression from which has been well documented to be altered during ageing (Chandra et al. 2015). These findings have defined yet another layer of complexity to the mechanisms that can possibly lead to altered gene expression and thus the pathophysiology of ageing.

Concluding Remarks

Interactions between several transcription factors and plasticity of chromatin have only recently begun to be appreciated. Such interactions are likely to be more complex during ageing since physiological manifestations are a summation of both short term and long term impact on mechanisms that determine gene expression. This brings in the burden of ensuring accuracy and responsivity throughout the lifespan of an individual.

While most of the current literature provides a general picture, it will be essential to investigate such changes in gene expression and chromatin architecture in response to altered environmental inputs. This will be key, because ageing is affected by a plethora of factors. Moreover, such analyses will also likely provide information vis-à-vis memory and plasticity of gene expression mechanisms. As pointed out earlier, a systems level approach, including help from mathematical modeling, will be critical in analyzing both existing and newly generated high-throughput data.

References

Adams PD (2007) Remodeling of chromatin structure in senescent cells and its potential impact on tumor suppression and ageing. Gene 397:84–93. https://doi.org/10.1016/j.gene.2007.04.020

Avrahami D et al (2015) Ageing-Dependent Demethylation of Regulatory Elements Correlates with Chromatin State and Improved beta Cell Function. Cell Metab 22:619–632. https://doi.org/10.1016/j.cmet.2015.07.025

Bahar R et al (2006) Increased cell-to-cell variation in gene expression in ageing mouse heart. Nature 441:1011–1014. https://doi.org/10.1038/nature04844

Baker DJ, Jin F, van Deursen JM (2008) The yin and yang of the Cdkn2a locus in senescence and ageing. Cell Cycle 7:2795–2802. https://doi.org/10.4161/cc.7.18.6687

Balaban RS, Nemoto S, Finkel T (2005) Mitochondria, oxidants, and ageing. Cell 120:483–495. https://doi.org/10.1016/j.cell.2005.02.001

Balasubramanian D et al (2012) H3K4me3 inversely correlates with DNA methylation at a large class of non-CpG-island-containing start sites. Genome Med 4:47. https://doi.org/10.1186/gm346

Banerjee KK, Ayyub C, Ali SZ, Mandot V, Prasad NG, Kolthur-Seetharam U (2012) dSir2 in the adult fat body, but not in muscles, regulates life span in a diet-dependent manner. Cell Rep 2:1485–1491. https://doi.org/10.1016/j.celrep.2012.11.013

Barzilai N, Huffman DM, Muzumdar RH, Bartke A (2012) The critical role of metabolic pathways in ageing. Diabetes 61:1315–1322. https://doi.org/10.2337/db11-1300

Beerman I, Seita J, Inlay MA, Weissman IL, Rossi DJ (2014) Quiescent hematopoietic stem cells accumulate DNA damage during ageing that is repaired upon entry into cell cycle. Cell Stem Cell 15:37–50. https://doi.org/10.1016/j.stem.2014.04.016

Berchtold NC et al (2008) Gene expression changes in the course of normal brain ageing are sexually dimorphic. Proc Natl Acad Sci U S A 105:15605–15610. https://doi.org/10.1073/pnas.0806883105

Bjornsson HT et al (2008) Intra-individual change over time in DNA methylation with familial clustering. JAMA 299:2877–2883. https://doi.org/10.1001/jama.299.24.2877

Bochkis IM, Przybylski D, Chen J, Regev A (2014) Changes in nucleosome occupancy associated with metabolic alterations in aged mammalian liver. Cell Rep 9:996–1006. https://doi.org/10.1016/j.celrep.2014.09.048

Boisvert MM, Erikson GA, Shokhirev MN, Allen NJ (2018) The ageing astrocyte transcriptome from multiple regions of the mouse brain. Cell Rep 22:269–285. https://doi.org/10.1016/j.celrep.2017.12.039

Bonasio R, Shiekhattar R (2014) Regulation of transcription by long noncoding RNAs. Annu Rev Genet 48:433–455. https://doi.org/10.1146/annurev-genet-120213-092323

Bratic I, Trifunovic A (2010) Mitochondrial energy metabolism and ageing. Biochim Biophys Acta 1797:961–967. https://doi.org/10.1016/j.bbabio.2010.01.004

Burgess RC, Misteli T, Oberdoerffer P (2012) DNA damage, chromatin, and transcription: the trinity of ageing. Curr Opin Cell Biol 24:724–730. https://doi.org/10.1016/j.ceb.2012.07.005

Campisi J (2013) Ageing, cellular senescence, and cancer. Annu Rev Physiol 75:685–705. https://doi.org/10.1146/annurev-physiol-030212-183653

Carlson ME, Conboy IM (2007) Loss of stem cell regenerative capacity within aged niches. Ageing Cell 6:371–382. https://doi.org/10.1111/j.1474-9726.2007.00286.x

Chambers SM, Shaw CA, Gatza C, Fisk CJ, Donehower LA, Goodell MA (2007) Ageing hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. PLoS Biol 5:e201. https://doi.org/10.1371/journal.pbio.0050201

Chandra T et al (2012) Independence of repressive histone marks and chromatin compaction during senescent heterochromatic layer formation. Mol Cell 47:203–214. https://doi.org/10.1016/j.molcel.2012.06.010

Chandra T et al (2015) Global reorganization of the nuclear landscape in senescent cells. Cell Rep 10:471–483. https://doi.org/10.1016/j.celrep.2014.12.055

Chen JH, Hales CN, Ozanne SE (2007) DNA damage, cellular senescence and organismal ageing: causal or correlative? Nucleic Acids Res 35:7417–7428. https://doi.org/10.1093/nar/gkm681

Chen H, Dzitoyeva S, Manev H (2012) Effect of ageing on 5-hydroxymethylcytosine in the mouse hippocampus. Restor Neurol Neurosci 30:237–245. https://doi.org/10.3233/RNN-2012-110223

Chen H, Ruiz PD, McKimpson WM, Novikov L, Kitsis RN, Gamble MJ (2015) MacroH2A1 and ATM Play Opposing Roles in Paracrine Senescence and the Senescence-Associated Secretory Phenotype. Mol Cell 59:719–731. https://doi.org/10.1016/j.molcel.2015.07.011

Cheng J et al (2017) Circular RNA expression profiling of human granulosa cells during maternal ageing reveals novel transcripts associated with assisted reproductive technology outcomes. PLoS One 12:e0177888. https://doi.org/10.1371/journal.pone.0177888

Chouliaras L et al (2012a) Age-related increase in levels of 5-hydroxymethylcytosine in mouse hippocampus is prevented by caloric restriction. Curr Alzheimer Res 9:536–544

Chouliaras L et al (2012b) Prevention of age-related changes in hippocampal levels of 5-methylcytidine by caloric restriction. Neurobiol Ageing 33:1672–1681. https://doi.org/10.1016/j.neurobiolageing.2011.06.003

Christensen BC et al (2009) Ageing and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. PLoS Genet 5:e1000602. https://doi.org/10.1371/journal.pgen.1000602

Couvillion MT, Soto IC, Shipkovenska G, Churchman LS (2016) Synchronized mitochondrial and cytosolic translation programs. Nature 533:499–503. https://doi.org/10.1038/nature18015

Croft DP, Brent LJ, Franks DW, Cant MA (2015) The evolution of prolonged life after reproduction. Trends Ecol Evol 30:407–416. https://doi.org/10.1016/j.tree.2015.04.011

Dai L et al (2014) Lysine 2-hydroxyisobutyrylation is a widely distributed active histone mark. Nat Chem Biol 10:365–370. https://doi.org/10.1038/nchembio.1497

Dang W et al (2009) Histone H4 lysine 16 acetylation regulates cellular lifespan. Nature 459:802–807. https://doi.org/10.1038/nature08085

de Magalhaes JP, Curado J, Church GM (2009) Meta-analysis of age-related gene expression profiles identifies common signatures of ageing. Bioinformatics 25:875–881. https://doi.org/10.1093/bioinformatics/btp073

Dell'Orco RT, Whittle WL (1982) Micrococcal nuclease and DNase I digestion of DNA from ageing human diploid cells. Biochem Biophys Res Commun 107:117–122

Di Micco R, Cicalese A, Fumagalli M, Dobreva M, Verrecchia A, Pelicci PG, di Fagagna F (2008) DNA damage response activation in mouse embryonic fibroblasts undergoing replicative senescence and following spontaneous immortalization. Cell Cycle 7:3601–3606. https://doi.org/10.4161/cc.7.22.7152

Duarte LF et al (2014) Histone H3.3 and its proteolytically processed form drive a cellular senescence programme. Nat Commun 5:5210. https://doi.org/10.1038/ncomms6210

Enge M, Arda HE, Mignardi M, Beausang J, Bottino R, Kim SK, Quake SR (2017) Single-Cell Analysis of Human Pancreas Reveals Transcriptional Signatures of Ageing and Somatic Mutation Patterns. Cell 171:321–330 e314. https://doi.org/10.1016/j.cell.2017.09.004

Fan J, Krautkramer KA, Feldman JL, Denu JM (2015) Metabolic regulation of histone post-translational modifications. ACS Chem Biol 10:95–108. https://doi.org/10.1021/cb500846u

Feser J, Tyler J (2011) Chromatin structure as a mediator of ageing. FEBS Lett 585:2041–2048. https://doi.org/10.1016/j.febslet.2010.11.016

Feser J, Truong D, Das C, Carson JJ, Kieft J, Harkness T, Tyler JK (2010) Elevated histone expression promotes life span extension. Mol Cell 39:724–735. https://doi.org/10.1016/j.molcel.2010.08.015

Finkel T (2015) The metabolic regulation of ageing. Nat Med 21:1416–1423. https://doi.org/10.1038/nm.3998

Finkielstain GP et al (2009) An extensive genetic program occurring during postnatal growth in multiple tissues. Endocrinology 150:1791–1800. https://doi.org/10.1210/en.2008-0868

Flatt T, Schmidt PS (2009) Integrating evolutionary and molecular genetics of ageing. Biochim Biophys Acta 1790:951–962. https://doi.org/10.1016/j.bbagen.2009.07.010

Fontana L, Partridge L (2015) Promoting health and longevity through diet: from model organisms to humans. Cell 161:106–118. https://doi.org/10.1016/j.cell.2015.02.020

Fraga MF, Esteller M (2007) Epigenetics and ageing: the targets and the marks. Trends Genet 23:413–418. https://doi.org/10.1016/j.tig.2007.05.008

Frasca D, Blomberg BB, Paganelli R (2017) Ageing, Obesity, and Inflammatory Age-Related Diseases. Front Immunol 8:1745. https://doi.org/10.3389/fimmu.2017.01745

Funayama R, Ishikawa F (2007) Cellular senescence and chromatin structure. Chromosoma 116:431–440. https://doi.org/10.1007/s00412-007-0115-7

Funayama R, Saito M, Tanobe H, Ishikawa F (2006) Loss of linker histone H1 in cellular senescence. J Cell Biol 175:869–880. https://doi.org/10.1083/jcb.200604005

Gaubatz J, Ellis M, Chalkley R (1979) Nuclease digestion studies of mouse chromatin as a function of age. J Gerontol 34:672–679

Girardot F, Lasbleiz C, Monnier V, Tricoire H (2006) Specific age-related signatures in Drosophila body parts transcriptome. BMC Genomics 7:69. https://doi.org/10.1186/1471-2164-7-69

Golden TR, Melov S (2004) Microarray analysis of gene expression with age in individual nematodes. Ageing Cell 3:111–124. https://doi.org/10.1111/j.1474-9728.2004.00095.x

Gorbunova V, Seluanov A (2016) DNA double strand break repair, ageing and the chromatin connection. Mutat Res 788:2–6. https://doi.org/10.1016/j.mrfmmm.2016.02.004

Gottlieb S, Esposito RE (1989) A new role for a yeast transcriptional silencer gene, SIR2, in regulation of recombination in ribosomal DNA. Cell 56:771–776

Goudarzi A et al (2016) Dynamic competing histone H4 K5K8 acetylation and butyrylation are hallmarks of highly active gene promoters. Mol Cell 62:169–180. https://doi.org/10.1016/j.molcel.2016.03.014

Gruner H, Cortes-Lopez M, Cooper DA, Bauer M, Miura P (2016) CircRNA accumulation in the ageing mouse brain. Sci Rep 6:38907. https://doi.org/10.1038/srep38907

Guarente L (2007) Sirtuins in ageing and disease. Cold Spring Harb Symp Quant Biol 72:483–488. https://doi.org/10.1101/sqb.2007.72.024

Hall H et al (2017) Transcriptome profiling of ageing Drosophila photoreceptors reveals gene expression trends that correlate with visual senescence. BMC Genomics 18:894. https://doi.org/10.1186/s12864-017-4304-3

Hansen TV et al (2004) Dwarfism and impaired gut development in insulin-like growth factor II mRNA-binding protein 1-deficient mice. Mol Cell Biol 24:4448–4464

Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J (2013) Natural RNA circles function as efficient microRNA sponges. Nature 495:384–388. https://doi.org/10.1038/nature11993

Harries LW (2014) MicroRNAs as Mediators of the Ageing Process. Genes (Basel) 5:656–670. https://doi.org/10.3390/genes5030656

He L, Hannon GJ (2004) MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 5:522–531. https://doi.org/10.1038/nrg1379

Hebert SL, Lanza IR, Nair KS (2010) Mitochondrial DNA alterations and reduced mitochondrial function in ageing. Mech Ageing Dev 131:451–462. https://doi.org/10.1016/j.mad.2010.03.007

Hu Z et al (2014) Nucleosome loss leads to global transcriptional up-regulation and genomic instability during yeast ageing. Genes Dev 28:396–408. https://doi.org/10.1101/gad.233221.113

Ishimi Y, Kojima M, Takeuchi F, Miyamoto T, Yamada M, Hanaoka F (1987) Changes in chromatin structure during ageing of human skin fibroblasts. Exp Cell Res 169:458–467

Jazwinski SM, Jiang JC, Kim S (2017) Adaptation to metabolic dysfunction during ageing: making the best of a bad situation. Exp Gerontol 107:87–90. https://doi.org/10.1016/j.exger.2017.07.013

Joseph AM et al (2012) The impact of ageing on mitochondrial function and biogenesis pathways in skeletal muscle of sedentary high- and low-functioning elderly individuals. Ageing Cell 11:801–809. https://doi.org/10.1111/j.1474-9726.2012.00844.x

Kaikkonen MU, Lam MT, Glass CK (2011) Non-coding RNAs as regulators of gene expression and epigenetics. Cardiovasc Res 90:430–440. https://doi.org/10.1093/cvr/cvr097

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. https://doi.org/10.1007/s10522-008-9176-0

Kim SN et al (2005) Age-dependent changes of gene expression in the Drosophila head. Neurobiol Ageing 26:1083–1091. https://doi.org/10.1016/j.neurobiolageing.2004.06.017

Kim MJ, Kim MH, Kim SA, Chang JS (2008) Age-related deterioration of hematopoietic stem cells. Int J Stem Cells 1:55–63

King AD et al (2016) Reversible Regulation of Promoter and Enhancer Histone Landscape by DNA Methylation in Mouse Embryonic Stem Cells. Cell Rep 17:289–302. https://doi.org/10.1016/j.celrep.2016.08.083

Kreiling JA et al (2011) Age-associated increase in heterochromatic marks in murine and primate tissues. Ageing Cell 10:292–304. https://doi.org/10.1111/j.1474-9726.2010.00666.x

Kutter C et al (2012) Rapid turnover of long noncoding RNAs and the evolution of gene expression. PLoS Genet 8:e1002841. https://doi.org/10.1371/journal.pgen.1002841

Kwabi-Addo B, Chung W, Shen L, Ittmann M, Wheeler T, Jelinek J, Issa JP (2007) Age-related DNA methylation changes in normal human prostate tissues. Clin Cancer Res 13:3796–3802. https://doi.org/10.1158/1078-0432.CCR-07-0085

Kwekel JC, Desai VG, Moland CL, Branham WS, Fuscoe JC (2010) Age and sex dependent changes in liver gene expression during the life cycle of the rat. BMC Genomics 11:675. https://doi.org/10.1186/1471-2164-11-675

Larson K et al (2012) Heterochromatin formation promotes longevity and represses ribosomal RNA synthesis. PLoS Genet 8:e1002473. https://doi.org/10.1371/journal.pgen.1002473

Latella L et al (2017) DNA damage signalling mediates the functional antagonism between replicative senescence and terminal muscle differentiation. Genes Dev 31:648–659. https://doi.org/10.1101/gad.293266.116

Latorre-Pellicer A et al (2016) Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing. Nature 535:561–565. https://doi.org/10.1038/nature18618

Lazarus A, Banerjee KK, Kolthur-Seetharam U (2013) Small changes, big effects: chromatin goes ageing. Subcell Biochem 61:151–176. https://doi.org/10.1007/978-94-007-4525-4_8

Li Y et al (2016) Molecular coupling of histone crotonylation and active transcription by AF9 YEATS Domain. Mol Cell 62:181–193. https://doi.org/10.1016/j.molcel.2016.03.028

Lindner H, Sarg B, Grunicke H, Helliger W (1999) Age-dependent deamidation of H1(0) histones in chromatin of mammalian tissues. J Cancer Res Clin Oncol 125:182–186

Liu L et al (2013) Chromatin modifications as determinants of muscle stem cell quiescence and chronological ageing. Cell Rep 4:189–204. https://doi.org/10.1016/j.celrep.2013.05.043

Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of ageing. Cell 153:1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Lu C, Thompson CB (2012) Metabolic regulation of epigenetics. Cell Metab 16:9–17. https://doi.org/10.1016/j.cmet.2012.06.001

Lu T, Pan Y, Kao SY, Li C, Kohane I, Chan J, Yankner BA (2004) Gene regulation and DNA damage in the ageing human brain. Nature 429:883–891. https://doi.org/10.1038/nature02661

Lui JC, Chen W, Barnes KM, Baron J (2010) Changes in gene expression associated with ageing commonly originate during juvenile growth. Mech Ageing Dev 131:641–649. https://doi.org/10.1016/j.mad.2010.08.010

Maedler K, Schumann DM, Schulthess F, Oberholzer J, Bosco D, Berney T, Donath MY (2006) Ageing correlates with decreased beta-cell proliferative capacity and enhanced sensitivity to apoptosis: a potential role for Fas and pancreatic duodenal homeobox-1. Diabetes 55:2455–2462. https://doi.org/10.2337/db05-1586

Maegawa S et al (2010) Widespread and tissue specific age-related DNA methylation changes in mice. Genome Res 20:332–340. https://doi.org/10.1101/gr.096826.109

Martinez-Jimenez CP et al (2017) Ageing increases cell-to-cell transcriptional variability upon immune stimulation. Science 355:1433–1436. https://doi.org/10.1126/science.aah4115

Maze I et al (2015) Critical Role of Histone Turnover in Neuronal Transcription and Plasticity. Neuron 87:77–94. https://doi.org/10.1016/j.neuron.2015.06.014

Medvedev ZA, Medvedeva MN (1990) Age-related changes of the H1 and H1(0) histone variants in murine tissues. Exp Gerontol 25:189–200

Memczak S et al (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495:333–338. https://doi.org/10.1038/nature11928

Mieczkowski J et al (2016) MNase titration reveals differences between nucleosome occupancy and chromatin accessibility. Nat Commun 7:11485. https://doi.org/10.1038/ncomms11485

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

Morselli M et al (2015) In vivo targeting of de novo DNA methylation by histone modifications in yeast and mouse. Elife 4:e06205. https://doi.org/10.7554/eLife.06205

Munoz-Culla M et al (2017) Progressive changes in non-coding RNA profile in leucocytes with age. Ageing (Albany NY) 9:1202–1218. https://doi.org/10.18632/ageing.101220

Murphy CT et al (2003) Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature 424:277–283. https://doi.org/10.1038/nature01789

Narita M (2007) Cellular senescence and chromatin organisation. Br J Cancer 96:686–691. https://doi.org/10.1038/sj.bjc.6603636

Narita M et al (2003) Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 113:703–716

Narita M et al (2006) A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. Cell 126:503–514. https://doi.org/10.1016/j.cell.2006.05.052

Newman JC et al (2017) Ketogenic diet reduces midlife mortality and improves memory in ageing mice. Cell Metab 26:547–557 e548. https://doi.org/10.1016/j.cmet.2017.08.004

Niccoli T, Partridge L (2012) Ageing as a risk factor for disease. Curr Biol 22:R741–R752. https://doi.org/10.1016/j.cub.2012.07.024

Nishino J, Kim I, Chada K, Morrison SJ (2008) Hmga2 promotes neural stem cell self-renewal in young but not old mice by reducing p16Ink4a and p19Arf Expression. Cell 135:227–239. https://doi.org/10.1016/j.cell.2008.09.017

Oberdoerffer P et al (2008) SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during ageing. Cell 135:907–918. https://doi.org/10.1016/j.cell.2008.10.025

O'Sullivan RJ, Karlseder J (2012) The great unravelling: chromatin as a modulator of the ageing process. Trends Biochem Sci 37:466–476. https://doi.org/10.1016/j.tibs.2012.08.001

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. https://doi.org/10.1038/nsmb.1897

Owsley C (2016) Vision and ageing. Annu Rev Vis Sci 2:255–271. https://doi.org/10.1146/annurev-vision-111815-114550

Pal S, Tyler JK (2016) Epigenetics and ageing. Sci Adv 2:e1600584. https://doi.org/10.1126/sciadv.1600584

Pan L, Penney J, Tsai LH (2014) Chromatin regulation of DNA damage repair and genome integrity in the central nervous system. J Mol Biol 426:3376–3388. https://doi.org/10.1016/j.jmb.2014.08.001

Panda AC et al (2017a) Identification of senescence-associated circular RNAs (SAC-RNAs) reveals senescence suppressor CircPVT1. Nucleic Acids Res 45:4021–4035. https://doi.org/10.1093/nar/gkw1201

Panda AC, Grammatikakis I, Munk R, Gorospe M, Abdelmohsen K (2017b) Emerging roles and context of circular RNAs. Wiley Interdiscip Rev RNA 8:e1386. https://doi.org/10.1002/wrna.1386

Parsons PA (2007) The ecological stress theory of ageing and hormesis: an energetic evolutionary model. Biogerontology 8:233–242. https://doi.org/10.1007/s10522-007-9080-z

Patil VS, Zhou R, Rana TM (2014) Gene regulation by non-coding RNAs. Crit Rev Biochem Mol Biol 49:16–32. https://doi.org/10.3109/10409238.2013.844092

Peleg S, Feller C, Ladurner AG, Imhof A (2016) The Metabolic Impact on Histone Acetylation and Transcription in Ageing. Trends Biochem Sci 41:700–711. https://doi.org/10.1016/j.tibs.2016.05.008

Pfeifer GP, Kadam S, Jin SG (2013) 5-hydroxymethylcytosine and its potential roles in development and cancer. Epigenetics Chromatin 6:10. https://doi.org/10.1186/1756-8935-6-10

Piazzesi A, Papic D, Bertan F, Salomoni P, Nicotera P, Bano D (2016) Replication-Independent Histone Variant H3.3 Controls Animal Lifespan through the Regulation of Pro-longevity Transcriptional Programs. Cell Rep 17:987–996. https://doi.org/10.1016/j.celrep.2016.09.074

Pina B, Suau P (1987) Changes in histones H2A and H3 variant composition in differentiating and mature rat brain cortical neurons. Dev Biol 123:51–58

Pollina EA, Brunet A (2011) Epigenetic regulation of ageing stem cells. Oncogene 30:3105–3126. https://doi.org/10.1038/onc.2011.45

Raddatz G et al (2013) Ageing is associated with highly defined epigenetic changes in the human epidermis. Epigenetics Chromatin 6:36. https://doi.org/10.1186/1756-8935-6-36

Rakyan VK et al (2010) Human ageing-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. Genome Res 20:434–439. https://doi.org/10.1101/gr.103101.109

Rangaraju S et al (2015) Suppression of transcriptional drift extends C elegans lifespan by postponing the onset of mortality. Elife 4:e08833. https://doi.org/10.7554/eLife.08833

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. https://doi.org/10.1128/MCB.02142-05

Rath PC, Kanungo MS (1989) Methylation of repetitive DNA sequences in the brain during ageing of the rat. FEBS Lett 244:193–198

Raz N, Rodrigue KM, Head D, Kennedy KM, Acker JD (2004) Differential ageing of the medial temporal lobe: a study of a five-year change. Neurology 62:433–438

Rhie BH, Song YH, Ryu HY, Ahn SH (2013) Cellular ageing is associated with increased ubiquitylation of histone H2B in yeast telomeric heterochromatin. Biochem Biophys Res Commun 439:570–575. https://doi.org/10.1016/j.bbrc.2013.09.017

Richter-Dennerlein R et al (2016) Mitochondrial protein synthesis adapts to influx of nuclear-encoded protein. Cell 167:471–483 e410. https://doi.org/10.1016/j.cell.2016.09.003

Rinn JL, Chang HY (2012) Genome regulation by long noncoding RNAs. Annu Rev Biochem 81:145–166. https://doi.org/10.1146/annurev-biochem-051410-092902

Rodrigues HF, Souza TA, Ghiraldini FG, Mello ML, Moraes AS (2014) Increased age is associated with epigenetic and structural changes in chromatin from neuronal nuclei. J Cell Biochem 115:659–665. https://doi.org/10.1002/jcb.24705

Rodwell GE et al (2004) A transcriptional profile of ageing in the human kidney. PLoS Biol 2:e427. https://doi.org/10.1371/journal.pbio.0020427

Rogakou EP, Sekeri-Pataryas KE (1999) Histone variants of H2A and H3 families are regulated during in vitro ageing in the same manner as during differentiation. Exp Gerontol 34:741–754

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

Sabari BR et al (2015) Intracellular crotonyl-CoA stimulates transcription through p300-catalyzed histone crotonylation. Mol Cell 58:203–215. https://doi.org/10.1016/j.molcel.2015.02.029

Sabari BR, Zhang D, Allis CD, Zhao Y (2017) Metabolic regulation of gene expression through histone acylations. Nat Rev Mol Cell Biol 18:90–101. https://doi.org/10.1038/nrm.2016.140

Sadaie M et al (2013) Redistribution of the Lamin B1 genomic binding profile affects rearrangement of heterochromatic domains and SAHF formation during senescence. Genes Dev 27:1800–1808. https://doi.org/10.1101/gad.217281.113

Sandovici I et al (2011) Maternal diet and ageing alter the epigenetic control of a promoter-enhancer interaction at the Hnf4a gene in rat pancreatic islets. Proc Natl Acad Sci U S A 108:5449–5454. https://doi.org/10.1073/pnas.1019007108

Sarg B, Koutzamani E, Helliger W, Rundquist I, Lindner HH (2002) Postsynthetic trimethyl-ation of histone H4 at lysine 20 in mammalian tissues is associated with ageing. J Biol Chem 277:39195–39201. https://doi.org/10.1074/jbc.M205166200

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

Schellenberg A, Stiehl T, Horn P, Joussen S, Pallua N, Ho AD, Wagner W (2012) Population dynamics of mesenchymal stromal cells during culture expansion. Cytotherapy 14:401–411. https://doi.org/10.3109/14653249.2011.640669

Schotta G et al (2008) A chromatin-wide transition to H4K20 monomethylation impairs genome integrity and programmed DNA rearrangements in the mouse. Genes Dev 22:2048–2061. https://doi.org/10.1101/gad.476008

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

Smith JS, Boeke JD (1997) An unusual form of transcriptional silencing in yeast ribosomal. DNA Genes Dev 11:241–254

Smith-Vikos T, Slack FJ (2012) MicroRNAs and their roles in ageing. J Cell Sci 125:7–17. https://doi.org/10.1242/jcs.099200

Southworth LK, Owen AB, Kim SK (2009) Ageing mice show a decreasing correlation of gene expression within genetic modules. PLoS Genet 5:e1000776. https://doi.org/10.1371/journal.pgen.1000776

Sproul D, Gilbert N, Bickmore WA (2005) The role of chromatin structure in regulating the expression of clustered genes. Nat Rev Genet 6:775–781. https://doi.org/10.1038/nrg1688

Spurlock CF, 3rd, Crooke PS, 3rd, Aune TM (2016) Biogenesis and transcriptional regulation of long noncoding RNAs in the human immune system J Immunol 197:4509–4517 doi:https://doi.org/10.4049/jimmunol.1600970

Stefanelli G et al (2018) Learning and age-related changes in genome-wide H2A.Z binding in the mouse hippocampus. Cell Rep 22:1124–1131. https://doi.org/10.1016/j.celrep.2018.01.020

Sun D et al (2014) Epigenomic profiling of young and aged HSCs reveals concerted changes during ageing that reinforce self-renewal. Cell Stem Cell 14:673–688. https://doi.org/10.1016/j.stem.2014.03.002

Szulwach KE et al (2011) 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and ageing. Nat Neurosci 14:1607–1616. https://doi.org/10.1038/nn.2959

Takasugi M (2011) Progressive age-dependent DNA methylation changes start before adulthood in mouse tissues. Mech Ageing Dev 132:65–71. https://doi.org/10.1016/j.mad.2010.12.003

Tan L, Shi YG (2012) Tet family proteins and 5-hydroxymethylcytosine in development and disease. Development 139:1895–1902. https://doi.org/10.1242/dev.070771

Thakur MK, Asaithambi A, Mukherjee S (1999) Sex-specific alterations in chromatin conformation of the brain of ageing mouse. Mol Biol Rep 26:239–247

Thomas RP, Guigneaux M, Wood T, Evers BM (2002) Age-associated changes in gene expression patterns in the liver. J Gastrointest Surg 6:445–453 discussion 454

Tsai K-L, Chen L-H, Chen Y-C, Kao C-L, Chen L-K, Chiou S-H (2011) The role of microRNAs in modulating sirtuin 1 expression. Journal of Clinical Gerontology and Geriatrics 2:71–75. https://doi.org/10.1016/j.jcgg.2011.06.004

Ungvari Z, Labinskyy N, Gupte S, Chander PN, Edwards JG, Csiszar A (2008) Dysregulation of mitochondrial biogenesis in vascular endothelial and smooth muscle cells of aged rats. Am J Physiol Heart Circ Physiol 294:H2121–H2128. https://doi.org/10.1152/ajpheart.00012.2008

Valinezhad Orang A, Safaralizadeh R, Kazemzadeh-Bavili M (2014) Mechanisms of miRNA-Mediated Gene Regulation from Common Downregulation to mRNA-Specific Upregulation. Int J Genomics 2014:970607. https://doi.org/10.1155/2014/970607

Van Meter M, Kashyap M, Rezazadeh S, Geneva AJ, Morello TD, Seluanov A, Gorbunova V (2014) SIRT6 represses LINE1 retrotransposons by ribosylating KAP1 but this repression fails with stress and age. Nat Commun 5:5011. https://doi.org/10.1038/ncomms6011

Venkatesh S, Workman JL (2015) Histone exchange, chromatin structure and the regulation of transcription. Nat Rev Mol Cell Biol 16:178–189. https://doi.org/10.1038/nrm3941

Vijay V, Han T, Moland CL, Kwekel JC, Fuscoe JC, Desai VG (2015) Sexual dimorphism in the expression of mitochondria-related genes in rat heart at different ages. PLoS One 10:e0117047. https://doi.org/10.1371/journal.pone.0117047

Wang Q, Huang J, Zhang X, Wu B, Liu X, Shen Z (2011) The spatial association of gene expression evolves from synchrony to asynchrony and stochasticity with age. PLoS One 6:e24076. https://doi.org/10.1371/journal.pone.0024076

Warner JR (1999) The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 24:437–440

Warren LA, Rossi DJ, Schiebinger GR, Weissman IL, Kim SK, Quake SR (2007) Transcriptional instability is not a universal attribute of ageing. Ageing Cell 6:775–782. https://doi.org/10.1111/j.1474-9726.2007.00337.x

Welle S, Brooks AI, Delehanty JM, Needler N, Thornton CA (2003) Gene expression profile of ageing in human muscle. Physiol Genomics 14:149–159. https://doi.org/10.1152/physiolgenomics.00049.2003

Westholm JO et al (2014) Genome-wide analysis of drosophila circular RNAs reveals their structural and sequence properties and age-dependent neural accumulation. Cell Rep 9:1966–1980. https://doi.org/10.1016/j.celrep.2014.10.062

White RR, Milholland B, MacRae SL, Lin M, Zheng D, Vijg J (2015) Comprehensive transcriptional landscape of ageing mouse liver. BMC Genomics 16:899. https://doi.org/10.1186/s12864-015-2061-8

Wierman MB, Smith JS (2014) Yeast sirtuins and the regulation of ageing. FEMS Yeast Res 14:73–88. https://doi.org/10.1111/1567-1364.12115

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

Wood JG et al (2010) Chromatin remodeling in the ageing genome of Drosophila. Ageing Cell 9:971–978. https://doi.org/10.1111/j.1474-9726.2010.00624.x

Wood SH, Craig T, Li Y, Merry B, de Magalhaes JP (2013) Whole transcriptome sequencing of the ageing rat brain reveals dynamic RNA changes in the dark matter of the genome. Age (Dordr) 35:763–776. https://doi.org/10.1007/s11357-012-9410-1

Wyss-Coray T (2016) Ageing, neurodegeneration and brain rejuvenation. Nature 539:180–186. https://doi.org/10.1038/nature20411

Zhan M, Yamaza H, Sun Y, Sinclair J, Li H, Zou S (2007) Temporal and spatial transcriptional profiles of ageing in Drosophila melanogaster. Genome Res 17:1236–1243. https://doi.org/10.1101/gr.6216607

Zhang Y, Hood WR (2016) Current versus future reproduction and longevity: a re-evaluation of predictions and mechanisms. J Exp Biol 219:3177–3189. https://doi.org/10.1242/jeb.132183

Zhang R et al (2005) Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Dev Cell 8:19–30. https://doi.org/10.1016/j.devcel.2004.10.019

Zhang R, Chen W, Adams PD (2007) Molecular dissection of formation of senescence-associated heterochromatin foci. Mol Cell Biol 27:2343–2358. https://doi.org/10.1128/MCB.02019-06

Zongza V, Mathias AP (1979) The variation with age of the structure of chromatin in three cell types from rat liver. Biochem J 179:291–298