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Epigenetic regulation of ageing: linking environmental inputs to genomic stability

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Preface

Ageing is affected by both genetic and non-genetic factors. Here, we review the chromatin-based epigenetic changes that occur during ageing, the role of chromatin modifiers in modulating lifespan and the importance of epigenetic signatures as biomarkers of ageing. We also discuss how epigenome remodeling by environmental stimuli impacts several aspects of transcription and genomic stability, with important consequences on longevity, and outline epigenetic differences between the 'mortal soma' and the 'immortal germline'. Finally, we discuss the inheritance of ageing characteristics and potential chromatin-based strategies to delay or reverse hallmarks of ageing or age-related diseases.

Introduction

The lifespan of an organism encompasses a period of growth that culminates in sexual maturity, a period of maximal fitness and fertility, and a period of ageing that is characterized by functional decline and an increased probability of death. Ageing is associated with loss of functionality at the cellular, tissue and organismal level and a wide range of diseases, including cardiovascular and neurodegenerative diseases, metabolic disorders and many cancers. Highly relevant to human ageing, 'healthspan' is the duration of disease-free physiological health (for example high cognition and mobility). Understanding the changes that occur during ageing, and identifying regulators of lifespan and healthspan, should pave the way for interventions to promote a longer youthful period, increase vigor and, potentially, reverse some hallmarks of ageing.

The discovery of long-lived mutants in invertebrate model systems supports the idea that the ageing process can be genetically modulated[1]. In addition to genetic inputs, evidence implicates non-genetic factors in ageing. Indeed, studies in humans have estimated the non-heritable portion of lifespan regulation to be ~70%[2]. Environmental stimuli, such as dietary

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manipulations or stress, can potently influence the lifespan and healthspan of animals across various species[3]. The importance of non-genetic factors is further underscored in eusocial insects that have a caste system with queens and workers (for example, honey bees), where individuals with similar genomes exhibit large differences in lifespan (~10 fold difference between queen and workers)[4]. Such differences can be triggered by early exposure to environmental stimuli and are relatively stable throughout life, although some remodeling is still possible later on[4]. Even in relatively controlled environments, lifespan among isogenic individuals is highly variable with large differences between the first and last death[5]. Although these differences could be purely stochastic, this result suggests that even minute environmental variations may cumulatively influence lifespan. While a portion of this non-genetic variation may stem from somatic mutations, the majority likely results from other non-genetic, regulated factors that stably impact healthspan and longevity.

In this Review, we examine evidence for an epigenetic component in the regulation of ageing. We will use the term epigenetic broadly to refer to changes in genomic regulation, although we note that in its strictest definition, this term encompasses only heritable phenotypic changes without changes to the underlying gene sequence[6]. Among the different modes of genome regulation, we will particularly focus on chromatin (Box 1) for several reasons. First, changes to chromatin states influence transcription and could underlie at least in part the transcriptional changes that are observed with ageing[7, 8]. Second, as chromatin can be influenced by the environment[9], it could act as an interface through which environmental signals interact with genetic components throughout lifespan. Finally, stable changes to the chromatin landscape could preserve memory of past environmental exposures, leading to long-lasting phenotypic effects[10, 11] that may be particularly relevant for ageing.

We will bring ageing and epigenetic research together to provide an integrated picture of the non-genetic regulation of lifespan and healthspan. We describe chromatin changes that occur during ageing and present emerging evidence for a role of chromatin modifiers in modulating lifespan. We also highlight the use of epigenetic signatures as ‘ageing clocks’ (Box 2). We then explore the influence of environmental inputs that affect ageing on epigenetic remodeling, and the potential consequences of this remodeling on genome stability and transcription. We evaluate the contributions of epigenetics in long-lasting, even heritable longevity phenotypes. Finally, we discuss remaining outstanding questions and challenges, including how to develop epigenetic strategies to counter or reverse age-related diseases. Understanding the importance of chromatin-based epigenetic mechanisms in ageing is timely because of recent advances in ultra-high throughput technology (Supplementary information S1 (Box)), which have revolutionized our knowledge of epigenetic factors and their relationships to gene regulation. Reference epigenomes provided by ENCODE and Roadmap epigenomics[12, 13] should also aid our understanding of the remodeling and persistence of epigenomic patterns during ageing. These questions are all the more pressing given the increasing life expectancy in all parts of the world, and the socioeconomic and medical challenges to meet growing health demands.

Chromatin modifications and ageing

A role for epigenetics in ageing is supported by evidence that chromatin is altered during the ageing process and that interfering with chromatin regulatory complexes impacts lifespan. We will present findings from model organisms (yeast, worms, flies, and mice) and from cellular models of replicative senescence. We will also discuss results from organismal and cellular models of diseases with accelerated signs of ageing (progeroid syndromes), such as Hutchinson-Gilford progeria syndrome (HGPS) or Werner syndrome (WS). In presenting this evidence, we note that the precise role of chromatin in lifespan is difficult to establish. Indeed, chromatin-modifying enzymes can often also affect non-chromatin substrates, or target multiple chromatin sites that could influence ageing. Furthermore, swapping modified DNA or histone residues by ones that cannot be modified, and to do so in a locus-specific manner, is technically difficult to achieve. Nevertheless, the evidence accumulated so far highlights the importance of many chromatin features during ageing.

DNA methylation in ageing

Methylation of the 5-carbon of cytosines in CpG dinucleotide sites is a conserved epigenetic modification that is classically linked to transcriptional silencing in vertebrates (Box 1). DNA methylation undergoes remodeling during ageing in various tissues in mice and humans (Table 1 **and** supplementary information S2 (table)). In human cells, the global levels of 5-methylcytosine (5-mC) are reduced in senescent compared to actively cycling cells[14, 15] (Table 1). Studies probing the methylation status of specific CpG sites across the genome suggest that the methylation of such sites outside of promoter CpG 'islands' tends to decrease in a variety of tissues in humans[16]. In contrast, in both mice and humans, CpG 'islands' near promoters are typically hypermethylated with age, most notably on genes involved in differentiation or development[16–18]. Although changes in DNA methylation in heterogeneous tissues may partly reflect alterations in cell composition[19], it is interesting to note that age-dependent changes in DNA methylation are detected in more homogeneous cell populations, such as hematopoietic stem cells (HSCs)[20, 21]. The consequences of age-dependent changes in DNA methylation for cellular function are not yet completely clear, but interestingly, in HSCs, regions with altered DNA methylation overlap binding sites of specific sets of transcription factors[21]. This observation raises the possibility that these methylation changes could influence the targeting of transcription factors, and thereby contribute to misregulation of gene expression during ageing[21]. While many studies have focused on 5-mC, the discovery of other forms of DNA methylation, including 5-hydroxymethylcytosine (5-hmC), cytosine methylation at non-CpG dinucleotides[22], and N6-methylation of adenines (6-mA)[23–25], deserve exploration in the context of ageing. Interestingly, recent studies in humans suggest a link between 5-mC DNA methylation, chronological age, and even 'biological' age[26–28] (Box 2). Thus, DNA methylation could represent an 'ageing clock'.

Traditional model organisms for ageing are not well-suited for studying the role of 5-mC methylation in organismal lifespan, as this modification has not been observed in *Saccharomyces cerevisiae*[29] or *Caenorhabditis elegans*[30]. However, DNA methylation is present in *Drosophila melanogaster*[29], another invertebrate model for ageing. In *D.*

melanogaster, overexpression of the putative DNA methyltransferase *dDnmt2* increases longevity, whereas flies lacking a functional copy of the gene are short-lived[31] (Table 2 **and** Supplementary information S3 (table)). Whether lifespan changes in *dDnmt2* mutants result from changes in DNA methylation is still subject to debate. While an initial study identified a potential role for *dDnmt2* in the methylation of CpG at transposons[32], a subsequent study using bisulfite sequencing on wild-type and *dDnmt2* mutant flies did not[33]. Additional evidence supports a role for dDNMT2 in tRNA methylation[33]. Thus, how CpG methylation impacts longevity remains unclear, but the relationship between DNA methylation and biological age in humans (Box 2) suggests that it could be used as a biomarker of ageing.

Core histone expression in ageing and longevity

The folding of cellular genomes into higher-order chromatin structure affects nearly all cellular processes that are linked to ageing, including transcription, DNA repair and DNA replication[34]. Age-dependent changes in chromatin structure, which are mediated in part by changes in histone expression, are linked to ageing phenotypes in mammalian cells and to lifespan regulation in yeast (Table 1 **and** Supplementary information S2 (table)). The expression of core histones is reduced during replicative ageing in yeast, as well as in other species and cell types[35–39] (Table 1). In yeast, decreased histone expression is coupled to decreased nucleosome occupancy and aberrant upregulation of associated genes[36]. Interfering with complexes that control the exchange and deposition of histones onto chromatin also influences *S. cerevisiae* lifespan: the histone chaperone ASF1 (Anti-Silencing Function), which promotes histone deposition and stability, is required for normal replicative lifespan, whereas the HIR complex (Histone Information Regulator), which represses histone expression, limits yeast replicative lifespan[40]. Interestingly, increasing the cellular supply of histone H3 and H4, but not of histone H2A and H2B, increases the replicative lifespan of yeast by up to 50%[40] (Table 2 **and** Supplementary information S3 (table)). The oligomerization of histones into chromatin is initiated by the deposition of H3–H4 dimers, which then allows the recruitment of H2A–H2B dimers[41]. Thus, an increased supply of H3–H4 dimers may promote nucleosome deposition in old cells and protect the genome against unwarranted genome activation. To our knowledge, whether histone expression levels limit metazoan longevity has not been investigated. However, because histone expression declines in some mammalian cells during ageing[37, 38] (Table 1), this process may also impact ageing in mammals.

Histone methylation in ageing and longevity

Histone methylation is associated with active or repressed genome regions, depending on the residue affected and the level of methylation (Box 1), and it can be dynamically regulated by histone methyltransferases and histone demethylases (reviewed in[42]). The global level or genomic distribution of many histone methylations change in organismal and cellular models of ageing (Table 1). Furthermore, the manipulation of histone methyltransferases and histone demethylases can modulate longevity of model organisms (Table 2).

Data from both physiological and accelerated models of ageing generally support the so-called ‘heterochromatin loss model of ageing’[43] (Table 1). This model postulates that decreased heterochromatin and/or the inappropriate redistribution of heterochromatin

silencing proteins may cause cellular dysfunction with age. The mechanisms underlying heterochromatin remodeling are not fully understood, but may involve interactions between the chromatin machinery and nuclear periphery proteins such as nuclear lamins. Such interactions can establish nuclear microdomains that delineate regions of active and repressed gene expression[44]. For example, reducing Lamin B1 levels in proliferating fibroblasts can induce locus-specific remodeling of H3K4me3 and H3K27me3, which mimic alterations observed in senescent cells[39]. Interestingly, widespread alterations in heterochromatin organization are observed in mesenchymal stem cells derived from an embryonic stem cell (ESC) model of Werner syndrome, including a generalized reduction of H3K9me3 and decreased interactions with inner nuclear membrane proteins[45]. It will be important to determine the mechanisms that trigger large-scale changes in repressive chromatin with ageing.

Consistent with a model that loss of repressive chromatin is detrimental during ageing, the manipulation of chromatin regulators leading to increased 'repressive' histone methylation, such as H3K27me3, is often linked to increased longevity[46–48] (Table 2). For example, reduced expression of the H3K27me3 demethylase *utx-1* promotes *C. elegans* longevity[46, 47]. Interestingly, during worm ageing, a global decrease in somatic H3K27me3[47, 48] and an increase in *utx-1* expression[46] is observed, suggesting that *utx-1* could limit worm longevity. However, results from several organisms indicate that the impact of H3K27me3 on ageing is likely more complex. Opposite to what could be expected from results with *utx-1*, reduction of *mes-2*, the *C. elegans* ortholog of the H3K27 trimethyltransferase component of Polycomb, extends the lifespan of sterile worms[48]. Furthermore, mutation in the Polycomb group protein E(Z) also increases lifespan in flies[49]. Finally, global levels of H3K27me3 increase in the muscle stem cells of old mice[38] and in brains of old African killifish[50], which contrasts with the age-related H3K27me3 decrease in *C. elegans*[47, 48]. That H3K27me3 levels can both be associated with lifespan extension and shortening suggests that different H3K27me3 regulators may influence lifespan through specific loci and/or in specific cells (for example, stem cells versus differentiated cells). It is also possible that some H3K27me3 regulators could exert their effect on lifespan via other, H3K27me3-independent effects.

Age-associated loss of heterochromatin is often coupled to remodeling of histone methylations associated with 'active' chromatin. Indeed, redistribution of the active histone modification H3K4me3 (a mark of accessible promoters) is observed during ageing and in cellular senescence[39, 48, 51] (Table 1). For example, new widespread regions of H3K4me3 emerge in senescent human fibroblasts, even when normalized to the declining total histone H3 levels[39]. Spreading of H3K4me3 domains is also observed during ageing in mouse HSCs[21], although it will be important to compare the magnitude of these changes to those in histone H3 levels. As broad H3K4me3 domains mark genes that are important for cell identity in many cell types[52], the spreading of H3K4me3 during ageing could influence cell function. Targeted RNAi screens probing the effects of histone methyltransferases and demethylases on longevity in flies and worms have revealed that regulators of H3K4me3 can modulate lifespan[46–48, 53] (Table 2).

Specifically, a longevity screen in fertile well-fed *C. elegans* hermaphrodites revealed that knock-down or mutation of genes encoding members of the COMPASS H3K4me3 methyltransferase complex[54] (ASH-2, SET-2 - the worm homolog of SETD1A and SETD1B in humans - and WDR-5) increases lifespan, whereas knock-down of the gene encoding H3K4me3 demethylase RBR-2 shortens lifespan[53]. In addition, overexpressing RBR-2 in the germline extends lifespan[53], supporting the idea that H3K4me3 demethylation in the germline promotes somatic maintenance. Consistently, male *D. melanogaster* deficient for the ortholog of RBR-2, LID, also exhibit shortened lifespan[55], although female flies are unaffected. However, H3K4me3 regulators may have different effects depending on the H3K4me3 complex or conditions. For example, deficiency in *Trx*, which is part of another type of H3K4me3 methyltransferase complex in *D. melanogaster*[54], does not significantly impact the lifespan of male flies[49]. In addition, several studies indicate that *rbr-2* RNAi can actually also extend lifespan in worms under some experimental conditions[48, 56, 57]. These discrepancies may result from differences in H3K4me3 complexes and/or conditions, such as nutrient intake or reproductive status.

Maintaining the levels of another active histone methylation, H3K36me3, which is linked to transcriptional elongation, is required for healthy ageing in yeast and worms. Indeed, mutation of the yeast *RPH1* gene, which encodes a H3K36 demethylase, extends yeast replicative lifespan, and yeast carrying H3 mutant forms that cannot be H3K36-methylated are short-lived[58]. In *C. elegans*, somatic levels of H3K36me3 moderately decrease with age[48], and appear to be particularly reduced at genes that are deregulated with age[51] (Table 1). Consistently, knock-down or mutation of the gene that encodes the putative *C. elegans* enzyme depositing the H3K36me3 mark, *met-1*, shortens worm lifespan[51]. These results suggest that the correct maintenance of H3K36me3 may be a critical process during ageing.

Thus, altering specific histone methyltransferases or demethylases modulates lifespan in yeast, worms, and flies. However, apart from studies in yeast, it is unclear whether changes in histone methylation directly impact ageing. Future studies should determine whether the role of histone methylation complexes in lifespan regulation is conserved throughout evolution.

Histone acetylation in ageing and lifespan regulation

Histone acetylation directly impacts the physical association of histones and DNA. Histone acetylation is a key, conserved player in longevity, and evidence suggests that its pattern changes during normal ageing (Table 1 and Table 2). In yeast, the global levels of H3K56ac decrease during replicative ageing, while that of H4K16ac increase, leading to de-silencing of telomeric repeats[59]. In contrast, the role of H4K16ac in mammalian ageing may be distinct from its functions in telomere maintenance. Global H4K16ac levels decrease during normal ageing and in a mouse model of HGPS and may be linked, at least in the progeroid model, to a decreased association of histone acetyltransferases with the nuclear periphery[60]. Following contextual fear conditioning, older mice fail to upregulate H4K12ac, a mark that promotes transcriptional elongation[61], in their hippocampus, and this correlates with altered gene expression and learning impairment[62]. Changes in histone acetylation thus may be a consequence and a cause of the failure of older cells to transduce external stimuli to

downstream transcriptional responses, a process that is particularly detrimental for rapid cell-to-cell signaling in the brain.

Both histone acetyltransferases (HAT) and deacetylases (HDAC) modulate lifespan and metabolic health (Table 2). For example, H4K16ac is deacetylated by the sirtuin SIR2[63], and increased *SIR2* dosage extends yeast lifespan[64] by limiting aberrant recombination at the ribosomal DNA (rDNA) locus. Although SIR2 and its orthologs also have non-histone substrates[65], SIR2-mediated deacetylation of H4K16ac, which is counteracted by SAS2-mediated acetylation, is important in regulating yeast lifespan[59]. Indeed, the lifespan of yeast mutants carrying an H4 gene mimicking constitutive H4K16ac is not altered by *SIR2* deletion, suggesting that SIR2 regulates lifespan through its activity on acetylated H4K16[59].

While the role of class III HDACs (also known as 'sirtuins') in lifespan modulation in metazoans has been challenged[66], new evidence supports a role of SIR2 orthologs, such as SIRT1 in mice, in promoting health and lifespan under standard conditions or at least in the context of dietary restriction (DR)-mediated longevity and health[67] (Table 2). The role of mammalian SIRT6 in regulating longevity is clearer. SIRT6 can deacetylate H3K9ac[68] and H3K56ac[69]. SIRT6 is the only sirtuin for which a deficiency induces a progeroid phenotype in mice[70]. Conversely, *Sirt6* transgenic male mice are long-lived[71]. The mechanism by which SIRT6 promotes longevity is unclear, but may stem from its role in supporting genomic stability[70, 72], partly by recruiting chromatin remodelers at sites of DNA-damage[69]. More generally, sirtuins may have a pro-longevity role by promoting increased genomic stability[69, 70, 72–74] (see also section below).

In general, deregulation of both HATs and HDACs, through genetic manipulation or targeting by chemicals or drugs, has been associated with significant changes in longevity across taxa (Table 2). However, in most cases, whether modulation of these enzymes regulates lifespan by acting solely on chromatin or also by regulating non-histone substrates warrants further studies.

Enzymes regulating other histone modifications

Regulators of other types of histone modifications could also impact ageing, although this has been much less studied (Table 2). For instance, O-N-acetyl-glucosamine (O-GlcNac) can be deposited on histones H2A, H2B and H4 and is enriched at promoters of genes that are important for ageing and stress response in *C. elegans*, notably targets of insulin-signaling[75]. Specifically, mutation of the gene encoding the enzyme that deposits O-GlcNac (*ogt-1*) shortens worm lifespan[75, 76], and mutation of the gene encoding the enzyme that removes O-GlcNac (*oga-1*) can extend lifespan in worms depending on the genetic background[75, 76]. However, O-GlcNac can also modify metabolic enzymes in addition to histones[76]. Thus, the exact mechanism by which O-GlcNac cycling impacts lifespan remains to be explored.

Role of nucleosome remodelers

Nucleosome remodelers also modify chromatin and their perturbation can regulate lifespan in yeast and worms (Table 2). Chromatin remodelers can modify nucleosome positioning,

chromatin higher-order structure and overall nuclear organization (reviewed in[77]). Changes to heterochromatin in ageing models have been linked to activity of nucleosome remodelers, such as the NuRD complex[78]. NuRD components RBBP4 and RBBP7 are downregulated in fibroblasts isolated from HGPS patients and healthy aged donors, and depletion of NuRD by RNAi in HeLa cells recapitulates the loss of heterochromatin in HGPS cells[78]. Inactivation of the chromatin remodeling ISWI complex has recently been shown to promote lifespan in *S. cerevisiae* and *C. elegans*[79]. Indeed, deletion of *ISW2*, a gene encoding a subunit of the ISWI complex, in yeast leads to shifts in nucleosome positioning at thousands of stress-response genes and to increased replicative lifespan[79]. Disruption of the homologous complex in *C. elegans* through another subunit leads to increased longevity[79]. On the contrary, SWI/SNF complexes are crucial for promoting longevity in the context of the Insulin–FOXO pathway in *C. elegans*[80]. Indeed, both the transcriptional changes and the lifespan extension promoted by DAF-16 (the worm FOXO transcription factor), require functional SWI/SNF[80]. These studies suggest that the regulation of nucleosome positioning and chromatin compaction by remodelers can both impact pro-ageing or pro-longevity genes. However, how these complexes are targeted to specific genes, how their different activities compete, and whether they have a role in the physiological regulation of ageing remains to be explored.

Environment and age-related epigenetics

Emerging evidence highlights the importance of non-genetic cues in the regulation of ageing and longevity, including diet[3], exercise[81], sexual stimuli[82], and circadian rhythms[83] (Figure 1). Many proteins that directly or indirectly induce epigenetic remodeling are also modulated by environmental cues. However, causal evidence linearly linking external cues to specific chromatin changes, and subsequently to alterations in ageing and longevity, is still missing. We discuss a subset of environmental signals that may act, at least in part, through changes in chromatin to promote healthspan and lifespan.

Nutrient intake, ageing and chromatin

Dietary restriction (DR) – a reduction in food intake without malnutrition – extends lifespan and delays signs of ageing in many organisms[3]. DR induces changes in gene expression across tissues (reviewed in[10]), including the expression of genes regulating metabolism, stress responses, DNA repair, and chromatin structure. These DR-induced transcriptional changes are consistent with a global preservation of genome integrity and chromatin structure[84]. Thus, the age-delaying effect of DR may be due to increased genomic stability[10, 84]. DR can also impact the chromatin landscape (reviewed in[10]). For instance, DR induces positional shifts of nucleosomes at thousands of genes in yeast, which may potentiate a stress response at the transcriptional level[79]. Interestingly, shifts at partially overlapping locations are observed in long-lived *ISW2* deletion mutants in yeast, and the effects of DR and *ISW2* mutation on longevity are epistatic[79]. Thus, nucleosome rearrangements may partly underlie DR-induced longevity in yeast[79].

DR may also affect the global chromatin landscape in metazoans. A study using a position variegation effect reporter suggests that DR may delay the age-related loss of facultative heterochromatin in flies[85]. Diet-switch experiments between an unrestricted diet and DR

suggest that diet-regulated changes in heterochromatin may be rapid (within 3 days) and reversible[85], which is suggestive of short-term adaptations. High nutrient intake in humans, using increased body mass index as a proxy, also seems to induce an 'aged-like' DNA methylation profile in liver[86]. Thus, nutrient intake has important ties to the regulation of both longevity and chromatin structure. However, it is unclear whether the observed changes are directly responsible for ageing and longevity changes. An important step in understanding how diet impacts longevity will be to establish the molecular link between changes in chromatin that are induced by diet and organismal ageing.

Energy and nutrient sensing and chromatin modulation

Consistent with the widespread anti-ageing effect of DR, many proteins that modulate longevity are linked to nutrient sensing and metabolic regulation. These proteins may integrate metabolic signals into chromatin responses. The detection of DR can occur through the systemic sensing of nutrient availability (for example, via signaling pathways) and/or the direct sensing of cellular energy levels that are reflected by changes in NADH:NAD+ or ATP:ADP:AMP ratios (reviewed in[10]). Although these pathways also have well-known non-chromatin substrates, their impact on ageing and longevity may be partly mediated through chromatin.

The insulin and insulin-like growth factor (IGF) signaling pathways are well characterized nutrient-sensing pathways that regulate lifespan across evolution[1]. Critical effectors of insulin signaling are FOXO transcription factors. In high nutrient conditions, FOXO transcription factors (FOXOs) are phosphorylated and excluded from the nucleus; in low nutrient conditions they re-localize to the nucleus[87]. Although FOXOs are not classical chromatin modifiers, FOXO1 can directly decrease chromatin compaction of the mouse *Igfbp1* promoter[88]. Furthermore, DAF-16, the worm FOXO transcription factor, directly recruits the SWI/SNF remodeling complex to target genes, and this complex is required for FOXO-mediated longevity[80].

Sirtuins rely on NAD+ as a cofactor and are inhibited by nicotinamide; thus, they are direct sensors of the cellular metabolic state[63]. Sirtuins are important mediators of DR-induced longevity across species[89, 90] (Table 2). Although they also regulate non-histone substrates, sirtuins have a strong functional link to chromatin regulation, which is required for their effect on lifespan, at least in yeast[59]. In mice, SIRT6 can recruit the SWI/SNI subunit SNF2H to DNA break sites and prevent genomic instability through chromatin remodeling and local deacetylation of H3K56ac[69]. Whether, as a rule, sirtuins promote DR-induced longevity by directly acting on chromatin remains unclear.

The energy-sensor AMP-activated protein kinase (AMPK) is necessary for longevity in response to several DR regimens in worms[91] and flies[92]. In addition, metformin, an AMPK activator, extends mouse lifespan[93], although metformin may have additional targets. AMPK can modify several chromatin regulators. In yeast, AMPK promotes histone acetylation by controlling the genomic occupancy and activity of the HAT GCN5[94] (Table 2). In mammals, AMPK can also inhibit histone deacetylation by phosphorylating HDAC4, HDAC5 or HDAC7[95, 96]. Interestingly, AMPK has several other substrates that are directly or indirectly implicated in chromatin regulation and genomic stability, including histone

H2B itself[97], SIRT1[98], and O-GlcNAc transferase (OGT)[99]. Whether AMPK promotes longevity by modifying chromatin targets or its non-chromatin substrates remains unknown.

Metabolites and metabolic enzymes may also directly regulate age-related epigenetics. Glycolytic enzymes, such as GAPDH (glyceraldehyde-3-phosphate dehydrogenase) or HK (Hexokinase), when mutated, can extend replicative lifespan in yeast[100, 101]. These enzymes can also modulate transcription in the nucleus in a metabolism-dependent manner (reviewed in[10]). For instance, GAPDH directly promotes the transcription of H2B genes during S-phase[102]. The nuclear functions of these enzymes may be important to integrate metabolic input and chromatin regulation. Metabolites generated by mitochondrial respiration have also been implicated in longevity and chromatin regulation. For instance, the Krebs cycle intermediate citrate can be converted to acetyl-CoA in mammalian cells, and this process can in turn modulate global levels of histone acetylation[103]. Several other Krebs cycle intermediates may also impact the global levels of DNA and histone methylation because they can act as obligatory co-substrates (such as α-ketoglutarate) or potent inhibitors (such as succinate) of the Ten Eleven Translocation (TET) enzymes (which are involved in DNA demethylation) and the KDM2/7 histone demethylases[104]. Thus, perturbation of the Krebs cycle during ageing may induce stochastic or aberrant chromatin remodeling. Interestingly, supplementation with Krebs cycle metabolites (such as oxaloacetate, malate, fumarate and α-ketoglutarate) can extend worm lifespan through the genetic pathways that mediate DR-induced longevity[105–107]. Thus, strong molecular ties link DR, metabolism, longevity and the regulation of chromatin states. Future studies should establish whether these links are fortuitous, or whether DR requires chromatin remodeling to have a lasting impact on longevity.

The circadian clock and ageing

The circadian clock controls many physiological and behavioral systems and is highly linked to energy metabolism[108] and chromatin[83]. Disrupting circadian rhythms negatively impacts health and longevity across species (reviewed in[108]). Conversely, restoring a functional circadian clock in ageing animals improves health and/or lifespan[109, 110]. Furthermore, isogenic mice with innate circadian periods closest to 24h live longer than their littermates with shorter or longer innate circadian periods[5], further highlighting that a robust circadian clock is key to longevity.

At a molecular level, the circadian clock regulates a circadian epigenome and gene program, which requires the rhythmic recruitment of protein complexes to chromatin[111]. CLOCK, a primary molecular component of the clock, has H3K9 and H3K14 HAT activity[112, 113]. The circadian epigenome can be influenced by metabolic cues, and several energy sensors that mediate DR-induced longevity also modulate the circadian machinery and the chromatin state of circadian promoters. For instance, SIRT1 deacetylates the circadian clock components PER2 and BMAL1[113, 114]. Activation of SIRT1 leads to decreased H3 acetylation, and thus repression of circadian genes in a time-specific manner[115]. Other energy sensors that modulate the clock and have links to chromatin include AMPK[116]. Thus, although the mechanism linking circadian rhythms to health and longevity is elusive, the circadian clock may promote longevity by modulating the epigenome.

Impact of physical exercise on ageing and chromatin

Physical activity promotes healthy ageing in mammals. For example, voluntary exercise promotes neurogenesis and healthy cognitive ageing in mice[117, 118]. Exercise may also prevent cognitive decline in humans[119], and regular physical activity is associated with longevity, a 30% reduction in all-cause mortality, and a dose-response improvement in overall health in humans[120].

Evidence suggests that exercise may influence chromatin dynamics, though the underlying mechanisms are unknown. In rat skeletal muscle, ageing is associated with decreased SIRT1 activity, and exercise can counteract this effect[121]. In humans, exercise is associated with enhanced AMPK activity, nuclear exclusion of HDAC4 and HDAC5, and increased H3K36 acetylation in skeletal muscle tissue[122]. The molecular mechanisms underlying the beneficial effects of exercise during ageing and whether they involve chromatin changes are still unresolved questions, but future work is likely to explore this connection.

Longevity modulation by pheromones

The longevity of worms and flies can be modulated by the opposite sex[82, 123, 124], a process that can involve pheromone production and/or sensing[82, 124]. In the presence of males in *C. elegans*, hermaphrodites are short-lived with signs of premature ageing, and the expression of the H3K27 demethylase *utx-1* is highly upregulated[82]. Interestingly, knock-down of *utx-1* in hermaphrodites extends longevity even in the presence of males[82], suggesting that the effect of males on hermaphrodite longevity may involve chromatin remodeling. As a portion of the premature demise induced by the presence of males is dependent on the sensing of male pheromones by hermaphrodites[82], it is possible that pheromones may influence chromatin states to regulate premature ageing.

Pheromones are not only involved in sexual sensing, but also in sensing of animal density ('crowding') in *C. elegans*[125]. Interestingly, exposure to pheromones involved in crowding (ascaroside #2 or ascaroside #3) is sufficient to extend *C. elegans* lifespan[126]. This pheromone-induced longevity requires the histone deacetylase gene *sir2.1*, but is independent of insulin-signaling[126]. Thus, chromatin modifiers may mediate the effect of pheromones, although whether pheromones directly impact chromatin marks or the overall chromatin structure is unknown. While ascarosides are nematode-specific molecules, their chemical structures resemble that of metabolites and they are part of a class of molecules termed 'secondary metabolites'. As secondary metabolites are present across evolution, the potential link between pheromonal signaling, chromatin, and ageing deserves further exploration in other species.

Systemic regulation of ageing: an epigenetic connection?

Sex steroid hormones (for example, estrogens and androgens) are systemic factors that decline with age in humans. Indeed, the cessation of ovarian lifespan (menopause in humans) ends its endocrine activity and shuts down the bulk synthesis of female sex steroid hormones. Estrogens act mainly through nuclear receptors (for example, the estrogen receptor (ER)), which can impact transcription by directly modulating chromatin states[127] and higher order chromatin structure[128]. Interestingly, global histone acetylation levels

decrease with age in rat brains, and estradiol promotes histone acetylation in *ex-vivo* brain slices from young, but not old, rats[129]. Estrogens are thought to limit the prevalence or age-of-onset of osteoporosis, sarcopenia, cardiovascular diseases, immune decline and neurodegeneration[130]. In reproductively senescent women, age-related decrease in estrogens may induce ageing phenotypes, partly due to decreased activity of estrogen receptors on chromatin. More studies are needed to understand the mechanistic impact of sex steroid hormones on human ageing and the chromatin changes induced by the age-induced drop in estrogen synthesis.

Environmental factors, thus, seem to modulate the activity of chromatin modifiers and organismal longevity. However, whether longevity is achieved through chromatin changes or via other mechanisms is still very much unexplored.

Epigenetics and genomic stability in ageing

Epigenomic changes during ageing can generate instability at two levels; age-dependent changes in chromatin may increase the susceptibility of the genome to mutation and may reduce transcriptional precision. Epigenomic states can themselves be unstable; indeed, 'epimutations', which correspond to aberrant or atypical changes in epigenetic states[131], appear to stochastically increase throughout life[132]. Thus, accumulated epimutations during ageing may induce further genomic instability (Figure 2).

Epigenetic instability and DNA mutations

The accumulation of mutations and epimutations with age can result from errors in DNA repair and failure to correctly replicate the genome and epigenome[133, 134]. While the extent and importance of mutations during ageing is debated, persistent DNA-damage signaling during ageing may drive local changes in chromatin structure and epigenetic modifications[132, 134]. DNA-damage signaling may also promote specific chromatin alterations, such as recruitment of chromatin modifiers, including Polycomb, SIRT1, SIRT6, and DNA-methyltransferases (reviewed in[134]). The resulting aberrant chromatin environment may in turn further increase susceptibility of DNA to damage[134]. Consistent with the idea that DNA damage can globally impact chromatin structure, deficiency in the WRN DNA repair helicase in mesenchymal stem cells leads to global loss of chromatin compaction[45], decreased levels of the heterochromatin marks H3K9me3 and H3K27me3, and increased phosphorylation of the H2A.X variant at centromeric loci[45].

Different types of genomic instability, such as single nucleotide mutations, aneuploidy, and transposition events increase with age and might contribute to ageing phenotypes (reviewed in[133]). Age-dependent genomic instability is due in part to the progressive failure of DNA repair pathways[135]. The importance of accumulated DNA damage in ageing is illustrated by several progeroid phenotypes, which are induced by mutations in DNA repair enzymes[136], although it is unclear whether increased DNA repair itself can extend lifespan. Interestingly, metabolites linked to the regulation of longevity, such as NAD or α-ketoglutarate, modulate the activity of proteins involved in both DNA repair and chromatin remodeling[137]. For example, the recruitment of NAD-dependent deacetylases SIRT1 and SIRT6 may directly promote genomic stability[69, 70, 73, 74]. By triggering chromatin remodeling, the

accumulation of mutations with age may also induce changes in gene expression, driving some aspects of age-related functional decline.

Epigenetic instability and transposition

Another source of genomic instability during ageing comes from the activation of endogenous mobile genetic elements (transposable elements (TE)). In eukaryotes, 30–80% of the genome is composed of TEs[138], which are transcribed and can regulate the expression of nearby genes. Active TEs induce extensive genomic instability[139] and they are normally kept in check, especially in the germline, by heterochromatin marks such as H3K9me3[140]. Interestingly, transposition can increase during ageing in several species[72, 139, 141–144], and TE activation correlates with neurodegenerative disorders in humans[145]. Conversely, DR may counteract the age-linked derepression of TEs in the liver and skeletal muscle of aged mice[144]. TE reactivation during ageing may result at least in part from heterochromatin loss. It may also be due to loss of SIRT6 specifically at TEs, as SIRT6 normally promotes heterochromatin formation and transcriptional repression in fibroblasts, heart, liver and brain from young mice[72]. Consistent with the idea that TE activation may negatively impact organismal lifespan, flies that are deficient for the Argonaute gene *Ago2* exhibit exacerbated transposition and a shortened lifespan[143]. This result suggests that activation of TEs may cause certain aspects of ageing, although the shortening of lifespan could also result from defects in small RNA pathways due to *Ago2* deficiency. Finally, in senescent human fibroblasts, chromatin accessibility increases at retrotransposon insertion sites, leading to increased TE transcription and transposition[146]. Together, these observations suggest that increased transposition during ageing is partially associated with aberrant chromatin remodeling and may promote ageing-phenotypes.

Epigenetics and transcriptional instability

Age-related epimutations could also impact ageing by causing stereotypical changes in gene expression[7, 21, 38], as well as by adversely affecting transcriptional precision. The integrity of transcriptional networks seems to decline with age in *C. elegans*[147] and in mice[8], as does coordinated gene expression within cells of a tissue[148]. Interestingly, an increase in cell-to-cell transcriptional noise was observed at 11 out of 15 tested genes in cardiomyocytes from old mice[149]. However, transcriptional noise was not increased, at least in the 6 assayed genes, in HSCs from old mice[150]. Because these pioneering studies were limited to only a few genes and cell types, whether transcriptional noise increases globally or at subsets of genes during ageing remains unclear. Genome-wide studies are needed to assess the significance and impact of alterations to transcriptional noise during ageing.

While transcriptional instability may be a byproduct of genomic instability[149, 151], it could also result from the accumulation of epimutations over the lifetime of an organism. Aspects of transcriptional precision may be affected by age-dependent changes in chromatin modifiers. For example, age-dependent fluctuations in gene expression are accompanied by decreases in H3K36me3 levels in worms. Maintenance of H3K36me3 levels restricts these fluctuations in gene expression and promotes longevity[51]. In yeast, sustained H3K36me3 levels are required to suppress cryptic transcription, and deletion of the H3K36me3 demethylase promote longer lifespan[58]. Transcriptional precision may also be affected by

other chromatin regulators, including HDACs[152], and modifiers of H3K4me3 breadth[52], and it will be interesting to determine if these chromatin modifiers are themselves altered with ageing. Future studies will need to systematically elucidate the link between changes in transcriptional precision and in the ageing chromatin landscape to determine whether chromatin regulates transcriptional precision during ageing.

Transgenerational regulation of ageing

Because the germline propagates intact genetic material throughout generations[153, 154], there has been interest in understanding the differential mechanisms of chromatin regulation in somatic versus germline cells during ageing (Box 3). Fertilization, which is mimicked to some degree by *in vitro* reprogramming, requires the resetting of age-dependent perturbations such as protein aggregates, chromatin disorganization, and mitochondrial dysfunction, and this reset may not always be complete. Intriguingly, some age-related phenotypes, including changes in lifespan and fertility, may be inherited through generations in model organisms. Thus, while the bulk of the epigenome is reset between generations, select epigenomic loci escape reprogramming or are re-established through unidentified triggers in subsequent generations. For example, in *S. pombe, C. elegans*, and mice, various chromatin marks (H3K9me3, H3K27me3, and DNA methylation) may be transmitted through mitosis and meiosis to the next generation[155–157]. We discuss examples of transgenerational inheritance of ageing phenotypes that are triggered by perturbations in chromatin regulators. As the molecular nature of the inherited signals for ageing phenotypes remains unknown, it is important to note that the mechanisms underlying this transgenerational inheritance could be independent of chromatin and could even involve cryptic genetic or microbiome-based transmission.

Progressive germline mortality

The germline is normally ‘immortal’ (Box 3). However, there are examples of progressive sterility over generations from worms to mammals, a phenomenon called ‘germline mortality’. Understanding progressive germline mortality could give insights into the transmission of chromatin changes to the next generation, which could in turn affect somatic maintenance. Recently, epigenetic mechanisms, including small RNAs and chromatin modifiers, were shown to contribute to germline mortality in *C. elegans* (reviewed in[158]). Mutations of *spr-5*, one of the worm homologs of the H3K4me2 demethylase LSD1, increase H3K4me2 levels in whole worms and primordial germ cells and induce sterility by generation 20[159, 160]. A predicted null mutation of the *rbr-2* H3K4me3 demethylase gene, as well as simultaneous loss of *spr-5* and *rbr-2*, result in progressive sterility at higher temperatures[57], as does loss of function of the *set-2* H3K4me2/3 methyltransferase[161]. Germline maintenance throughout generations may partly depend on the coordinated control of H3K4 methylation and repressed chromatin states. Accordingly, deletion of a predicted H3K9-demethylase in *C. elegans* can suppress the fertility defects of *spr-5* mutants, whereas loss of H3K9-methyltransferases leads to loss of fertility at earlier generations[160, 162]. These results suggest that the presence of H3K4me2 in H3K9-methylated regions promotes inappropriate gene activation that is detrimental for germ cells[160, 163]. Whether the marks themselves or small RNA intermediates cause the transgenerational phenotype is unknown,

but it is interesting that feedback loops exist between chromatin and small RNAs[164, 165]. It will be important to determine the role of chromatin states in germline function across generations in other species, including mammals.

Epigenetic memory of longevity

Several phenotypes associated with somatic maintenance, including lifespan and stress resistance, can be inherited in a transgenerational manner. In *C. elegans*, wild-type descendants from ancestors deficient for members of the H3K4 trimethyltransferase complex have a lifespan extended by up to four generations[166]. This extension can be reverted by transient inactivation of the *rbr-2* demethylase[166]. The mechanisms of lifespan extension are unclear, since global levels of H3K4me3 are unchanged in worms descended from ancestors with deficiencies in H3K4me3 modifiers[166]. However, a small number of genes are aberrantly expressed in genetically wild-type descendants from mutant ancestors in the fourth, but not the fifth, generation[166]. Whether specific changes to H3K4me3-marked loci escape the reprogramming of germ cells during meiosis to influence the expression of the associated genes is unknown. It is noteworthy that transgenerationally-regulated genes are enriched in genes involved in metabolic pathways[166], suggesting that loss of H3K4me methyltransferases in the parental generation may alter diet and/or trigger a metabolic cascade that affects lifespan independently of H3K4me3.

Consistent with the possibility that metabolic states could be inherited and thereby affect lifespan, a recent study indicated that worms whose grandparents were starved display increased organismal lifespan up to the third generation, although the extent of lifespan extension varies between replicates (22–70% extension)[167]. The transgenerational inheritance of metabolic states has also been observed in mammals (reviewed in[154]) and one hypothesis to explain this, at least in mice, is that metabolic changes in the parental generation influence chromatin states in germ cells, and that this permits the transmission of a previous environmental state to offspring[157, 168].

A phenotype associated with longevity is stress resistance. In *C. elegans*, exposure of the parental generation to high glucose promotes resistance to oxidative stress and can reduce neurodegeneration in the F1 progeny[169]. The transmission of stress-resistance requires intact *sir-2.1* and genes encoding COMPASS H3K4 methyltransferase complex components WDR-5 and SET-2, although changes in global H3K4me3 levels are not inherited along with stress resistance[169]. In flies, exposure to heat shock or osmotic stress induces heterochromatin changes that persist over several generations in the absence of stress[11]. In another model, chemical stress in flies induces the derepression of Polycomb-targeted genes, a subset of which remains derepressed in the absence of the stressor over multiple generations[170]. Thus, the memory of environmental exposures that affect lifespan might alter chromatin states in a manner that could be maintained through generations.

Many of these phenotypes revert after a certain number of generations, raising the questions of why and how the stimulus ceases to be passed on. Future research should identify the mechanisms of inheritance and establish whether chromatin directly mediates the inheritance of longevity phenotypes.

Concluding remarks

Epigenetic marks are remodeled during ageing and experimental perturbation of chromatin modifiers can directly impact lifespan. Thus, a compelling hypothesis is that the observed epigenomic changes throughout lifespan, and in the context of life-extending regimens, may actively modulate ageing. Throughout lifespan, environmental insults cause stochastic epimutations, which could contribute to functional decline at different scales, from cells to organs. These events can have systemic effects on ageing at the organismal level. While the sequence of events leading to genomic and transcriptional instability during ageing is unclear, genome surveillance and epigenetic remodeling demonstrably influence each other. Thus, we propose that that environment-induced epigenomic instability throughout life is a major driver of the ageing process, which is supported by the accuracy of the DNA-methylation clock in humans (Box 2).

In general, the plasticity of ageing chromatin states, notably upon environmental changes or cellular reprogramming, suggests that chromatin-regulating enzymes may be important therapeutic targets in the pursuit of healthy ageing. A variety of drugs that target chromatin modifiers have been identified[171, 172], and a number of these increase the lifespan of model organisms (see Table 2). Improving the specificity of such drugs and testing their efficacy in different cellular contexts could help treat age-related diseases, as proposed for Alzheimer's disease[173]. A major goal for drug design will be the specificity of delivery given their differential effects in various cells and tissues.

Despite recent progress, a full understanding of how epigenetic information integrates environmental input in the context of a genetic background during ageing is still in its infancy. This is partly due to the technical challenge of definitively studying how alterations in chromatin state may cause in ageing phenotypes. Among these challenges, the high copy number of histone genes in metazoan genomes prohibits simple 'gene-swap' experiments to study the function of specific residues in the regulation of longevity and ageing. Moreover, when perturbing chromatin modifying complexes we need to disentangle how to direct the activity of the enzyme to a particular histone residue and how to target the inhibition or activation of a mark at a subset of loci of interest rather than genome-wide. Finally, it will be important to further develop techniques to probe the chromatin landscape at the single cell and single molecule levels[174, 175]. This will help distinguish between three biological scenarios: the consistent chromatin remodeling in all ageing cells within a population, the emergence or disappearance of specific subpopulations during ageing, or the allele-specific epigenetic regulation changes during ageing in humans or outbred populations.

Reference epigenomes have transformed our understanding of transcription and highlighted key differences in chromatin states between tissues in young individuals. To better understand the integrative changes that occur during ageing, epigenome maps throughout lifespan would be particularly helpful. Such an ageing roadmap should help define better strategies for delaying or reversing aspects of ageing and age-related diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Lifespan refers to the time elapsed between birth and death in time units. In yeast, lifespan can be measured in two ways: chronological lifespan, which corresponds to the length of time a non-dividing yeast cell can survive; replicative lifespan, which corresponds to the number of mitotic cell divisions a mother cell has undergone.

Healthspan corresponds to the duration of disease-free physiological health within the lifespan of an individual. In humans, for instance, this corresponds to the period with high cognitive abilities, immune competence, and peak physical condition.

Isogenic	characterized by essentially identical genetic material. Highly inbred populations are usually considered to be isogenic.
Epigenetic	the broad definition of the term corresponds to modes of genomic regulation not directly encoded in DNA. We use it specifically to refer to chromatin-level regulation. In its strictest definition, epigenetic encompasses strictly heritable changes without changes to the underlying gene sequence.
Chromatin	a complex between DNA and histone proteins that packages genetic information. Chromatin can be broadly stratified in two states: euchromatin, a loose and transcription-permissive compartment, and heterochromatin, a dense and compact compartment that harbors repressed DNA.
Epigenome	corresponds to the epigenetic landscape of a cell, including the genome-wide distributions of chromatin marks, such as histone modifications or DNA methylation.
Replicative senescence	a limitation in the number of time a cell can divide. Replicative senescence has sometimes been used as an *in vitro* proxy of ageing for dividing mammalian cells such as fibroblasts or stem cells.
CpG islands	clusters of CpG dinucleotides, usually found in the promoter regions of some genes. They are typically defined by a minimum length (~200bp–500bp) and an unusual enrichment of CpG (>60%). They are usually unmethylated in the germline or pluripotent state.
Chronological age	the time elapsed since an individual's birth. Chronological age is often compared to biological age.
Biological age	is used as a proxy for individual health. It is expressed as the age of the average population that is most similar to the individual under observation. Younger biological ages are linked to high performance and health, while older biological ages correlate with disease onset.
Histone chaperones	proteins that aid the folding or dimerization of histones, as well as their loading onto the chromatin fiber. Different histones have different chaperones.
Sirtuin	the family of class III histone deacetylases (HDACs), the founding member of which is the yeast Sir2p. The specificity of these HDACs is their dependency on NAD+ as a co-factor to catalyze deacetylation or other enzymatic reactions, including ADP-ribosylation.
Dietary restriction	a reduction in food intake without malnutrition. There are many types of DR-regimens, such as intermittent-fasting, global caloric restriction or specific reduction of a nutrient type.

Position Variegation Effect	variability in the activation state of a gene depending on its proximity to a heterochromatin domain.
Insulin and insulin-like growth factor (IGF) signaling pathways	important conserved signaling pathways that are sensitive to nutrient levels and have been linked to longevity and stress resistance across metazoans. Primary components relevant to ageing include the insulin and IGF1 receptors, the activation of which triggers a phosphorylation cascade involving PI3-Kinase, AKT and ultimately the FOXO transcription factors.
Pheromones	secreted chemical factors that can trigger a response in other members of a given species. Pheromone signaling has been linked to sexual behavior, feeding and stress.
Epimutations	aberrant or atypical changes in epigenetic states, often stochastic.
Transposable Elements	endogenous DNA elements that change their position or amplify their copy number within a host genome. There are two main types of transposable elements: Type I elements, which function through an RNA intermediate (LINEs, LTRs; copy–paste mechanism) and Type II elements, which function through a DNA intermediate (cut–paste mechanism). They usually occupy a large portion of eukaryotic genomes.
Genomic instability	random loss of integrity of genetic material, whether through large-scale rearrangements (such as chromosomal translocations, large inversions and deletions, and so on), site-specific changes (such as single nucleotide changes) or transposon insertions. Genomic instability may be a driver of ageing and age-related diseases, such as cancer.

Biographies

Bérénice A. Benayoun is a post-doctoral fellow in Anne Brunet's lab in the Department of Genetics at Stanford University. She performed her graduate work in Reiner Veitia's lab at the Jacques Monod Institute (France), studying ovarian ageing. She joined the Brunet laboratory to study the genetics and epigenetics of ageing.

Elizabeth A. Pollina is a graduate student in the Cancer Biology Program at Stanford University. She joined Anne Brunet's lab in the Department of Genetics to study the role of chromatin in ageing, with particular interest in the central nervous system.

Anne Brunet is a professor of Genetics at Stanford University and the co-director of the Glenn Laboratories for Ageing Biology at Stanford. Her lab is interested in the mechanisms of ageing and longevity.

Box 1

Chromatin structure and regulation

Chromatin is the nucleo-proteic complex that allows the genome to be packaged inside the nucleus. It can be broadly categorized into two states: euchromatin, a loose and transcription-permissive compartment, and heterochromatin, a dense and compact compartment that harbors repressed DNA. The basic repeating units of chromatin, 'nucleosomes', consist of ~150bp of DNA wrapped around histone protein octamers (octamers contain two each of the H2A, H2B, H3 and H4 histone proteins)[176]. Post-translational modifications of histone protein N-terminal tails are thought to regulate the accessibility and expression potential of underlying genes[177], a hypothesis referred to as the 'histone code' hypothesis[177]. Interestingly, in addition to canonical histone proteins, the transcription of which is usually restricted to S-phase, some histone variants can be expressed at any point during the cell cycle. These specific histone variants have been involved in regulatory aspects of gene expression (for example, H2A.Z or H3.3) or chromatin structure (for example, CENP-A)[178]. The best-studied core histone modifications include Lys and Arg methylation, Lys acetylation, Ser/Thr phosphorylation and Lys poly- or mono-ubiquitinylation (reviewed in[179]). These modifications can be added and removed by 'chromatin modifiers', including histone acetyltransferases (HAT), histone deacetylases (HDAC), histone methyltransferases (HMT) and histone demethylases (KDM).

Another layer of regulation resides in the methylation status of Cytosines in CG dinucleotides (referred to as 'CpG'), and the most frequent type of methylation occurs on carbon 5 of cytosine (5-mC)[180]. In mammals, DNA methylation is generally associated with gene repression, though whether methylation plays an instructive role in repression is still unclear[180]. Approximately 60–90% of all CpGs are methylated in mammalian genomes, with the notable exception of hypomethylated 'CpG islands' (large regions with concentrated CpGs in gene-rich regions)[180]. DNA-methyltransferases (DNMTs) methylate DNA, and the removal process is initiated by the Ten-Eleven Translocation (TET) proteins[180].

Finally, chromatin can be regulated through the positioning of nucleosomes with respect to regulatory sequences and the compaction of chromatin in a 3D structure. ATP-dependent chromatin remodelers (for example, SWI/SNF or ISWI) can impact the chromatin and transcriptional landscape by modifying nucleosome positioning, chromatin higher-order structure and overall nuclear organization[77].

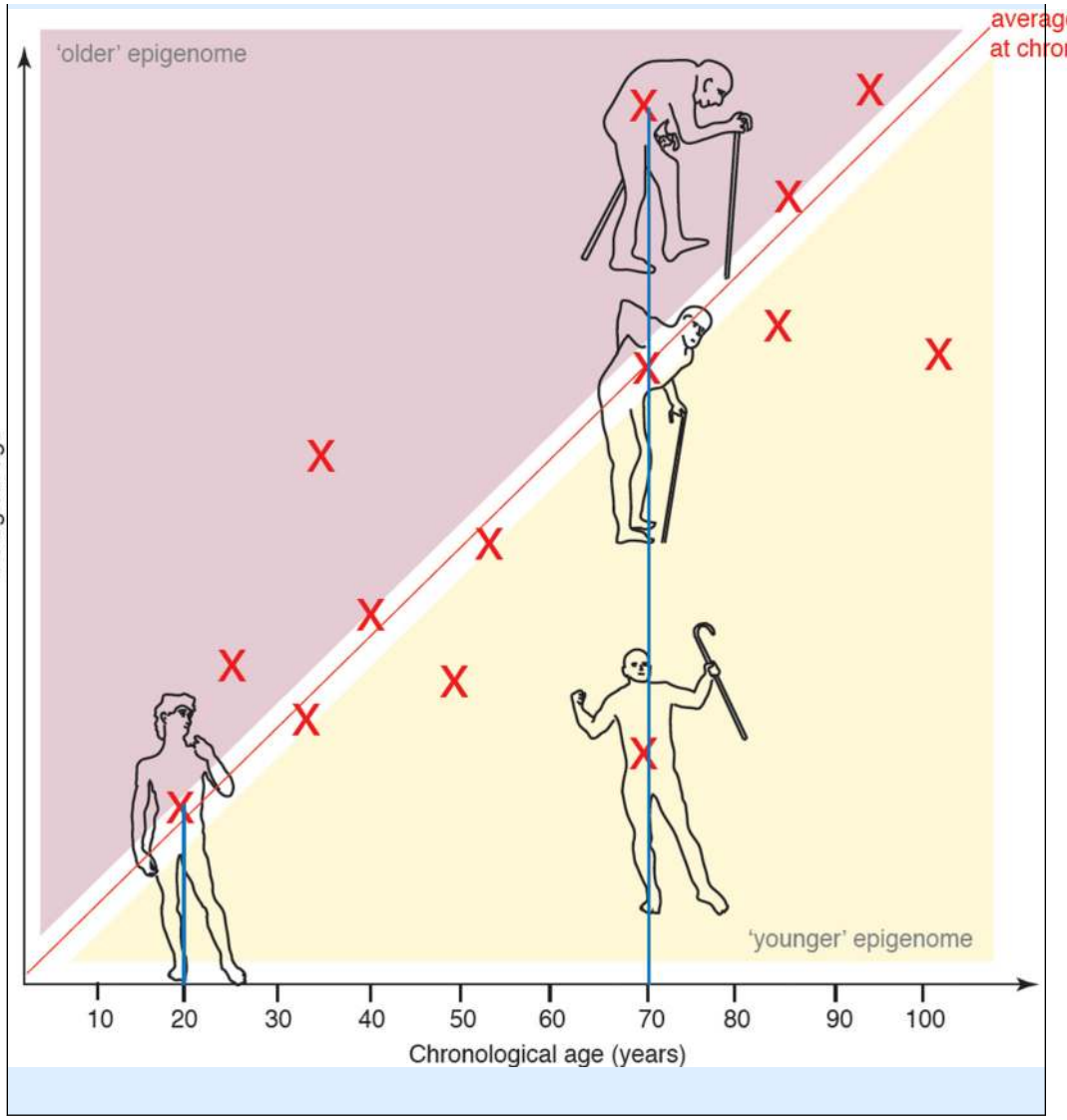
Box 2

Epigenetic modifications as biomarkers of age

Epigenetic modifications can persist in cells even between cell divisions, and an emerging hypothesis is that epigenetic landscapes are 'biological sensors' that reflect cellular identity, cell health and youthfulness. But do chromatin landscapes track with chronological age (that is, the age of the individual in time units) or biological age (that is, the age in years of the population average most similar to the individual) (see model in the figure)?

Studies have explored the use of changes in DNA methylation (DNA methylation age) as a sensor for both chronological age and biological age across species and cell types. Based on the methylation status of a defined number of CpGs, highly accurate predictors of chronological age have been constructed in a given tissue, such as saliva[181] or peripheral blood cells[26–28]. Surprisingly, these types of analyses have resulted in accurate estimators of age that seems to apply to human tissues and cell types regardless of their developmental potential[28, 86]. For example, embryonic stem cells and induced pluripotent stem cells are estimated to be 'ageless' by such models, and the DNA methylation age of sperm cells (which are part of the 'immortal' germline) is estimated to be far younger than that of the somatic cells of the same donor[26, 28]. While DNA methylation allows accurate modeling of chronological age with high correlation coefficients[27, 28], there is significant deviation from the linear fit, suggesting that methylation patterns may also reflect youthfulness or health (biological age) (see model in figure). Indeed, the use of DNA methylation age may predict the health of various tissues. For example, the liver tissue of obese subjects displays increased DNA methylation age compared to muscle or blood tissue from the same individual[86]. Furthermore, an increased DNA methylation age of blood cells can be observed in subjects with some progeroid diseases, including aplastic anemia and dyskeratosis congenita[26], or Down syndrome[182]. Moreover, DNA methylation-based age of blood cells can predict mortality by all causes in later life, even after adjusting for traditional risk factors and blood cell counts[183].

Previously proposed biomarkers of ageing include expression levels of the cell cycle inhibitor p16-INK4A[184] and telomere length attrition rate[185]. However, CpG DNA-methylation outperforms these molecular markers as predictors of chronological age for unknown reasons[28, 86, 183]. Whether greater deviations between chronological ('real') age and biological (predicted) age reflect actual disparities in ageing rates or represent potential oversimplifications (overfitting) of specific models remains unclear. Other DNA modifications, such as 5-hmC and other new oxidation derivatives[186] or 6-mA[23–25], could also be integrated into models of 'epigenetic' ageing clocks. Finally, although histone modification patterns are still unexplored as epigenetic biomarkers of age, they may increase the accuracy and scope of current biological clock models. As 'clock measurements' are mostly taken on entire tissues, it will be crucial to determine the role of age-related changes in cell composition within tissues in the predictive nature of the epigenetic clocks[19].



Box 3

Epigenetics of the 'mortal soma' and 'immortal germline'

An emerging question in the field of ageing epigenetics is whether different epigenetic mechanisms act in somatic cells and germ cells during ageing. Regulatory mechanisms that maintain germ cells may be more robust[187] and a number of studies (reviewed in[188]) have explored the hypothesis that aged somatic cells could 'rejuvenate' through reprograming, the process by which differentiated cells are converted back to a germ or embryonic stem cell (ESC)-like state via the re-expression of pluripotency regulators[189]. Indeed, both senescent and aged fibroblasts undergo some aspects of chromatin rejuvenation upon reprogramming and the subsequent re-differentiation into fibroblasts, including restoration of DNA methylation patterns at the loci of the pluripotency genes *OCT4* and *NANOG* and disappearance of senescence-associated heterochromatin foci[188]. Aged HSCs, which normally show persistent age-dependent gene expression changes, produce induced pluripotent stem cells (iPSCs) with similar transcriptional profiles to both normal ESCs and iPSCs clones derived from young HSCs, suggesting that chromatin modifications that influence transcriptional states may have been reset by reprogramming[190]. The differentiation potential of iPSCs derived from aged HSCs, both *in vitro* and through embryos, is similar to lines derived from young cells[190], consistent with a stable re-setting of epigenetic states rather than a masking of defects in the pluripotent state. However, it cannot be excluded that seemingly rejuvenated properties of iPSCs generated from aged donor cells results from the selection of cells with more 'youthful' chromatin states during reprogramming rather than an active reset process. Nevertheless, together, these studies highlight that the pluripotent state possesses unique properties for the erasure and re-establishment of epigenetic marks.

Despite their unique modes of chromatin regulation, germ cells may also undergo epigenetic alterations with ageing. Longitudinal studies of DNA methylation changes in human sperm reveal global hypermethylation at repetitive elements and site-specific CpG hypomethylation with age, particularly at the regions retaining canonical nucleosomes (as opposed to smaller protamines that normally replace bulky nucleosomes in mature sperm chromatin to promote compaction)[191]. Oocytes isolated from aged mice also display disordered chromatin and changes in the levels of certain histone modifications, including decreased histone methylation levels[192, 193]. Moreover, transcriptional profiling of oocytes from young and old mice reveal that several genes encoding chromatin modifiers are down-regulated with age, such as Polycomb genes *Ezh2* and *Bmi1* and DNA methyltransferase genes *Dnmt1* and *Dnmt3l*[194]. These studies suggest that the deregulation of chromatin states in ageing germ cells may be more common than previously thought. Delineating which epigenetic alterations are reset, and which are fixed and transmittable, will be invaluable for understanding how lifespan trajectories evolved, and whether certain mechanisms can be harnessed to induce 'erasure' of ageing signs.

Key points

- Widespread epigenomic remodeling, at the level of DNA or histone protein modification, has been observed during ageing across species and cell types. Some epigenetic states can serve as “molecular clocks”.
- The experimental perturbation of chromatin modifiers can influence the lifespan of model organisms.
- Environmental inputs, such as diet, physical activity, hormones or pheromones, have been linked to remodeling of the epigenome as well as to changes in lifespan. Chromatin may thus act as a molecular integrator of environmental exposures.
- Random epigenetic changes, or epimutations, throughout life may trigger increases in transcriptional and genomic instability.
- The unique regulation of chromatin in germline cells might protect these cells more than somatic cells during ageing.
- Some ageing or age-related phenotypes, such as lifespan, fertility, and stress-resistance, may be inherited through successive generations in model organisms, through non-genetic mechanisms.
- The use of epigenetic drugs or epigenome-editing technologies may be a promising avenue for age-related therapeutics.

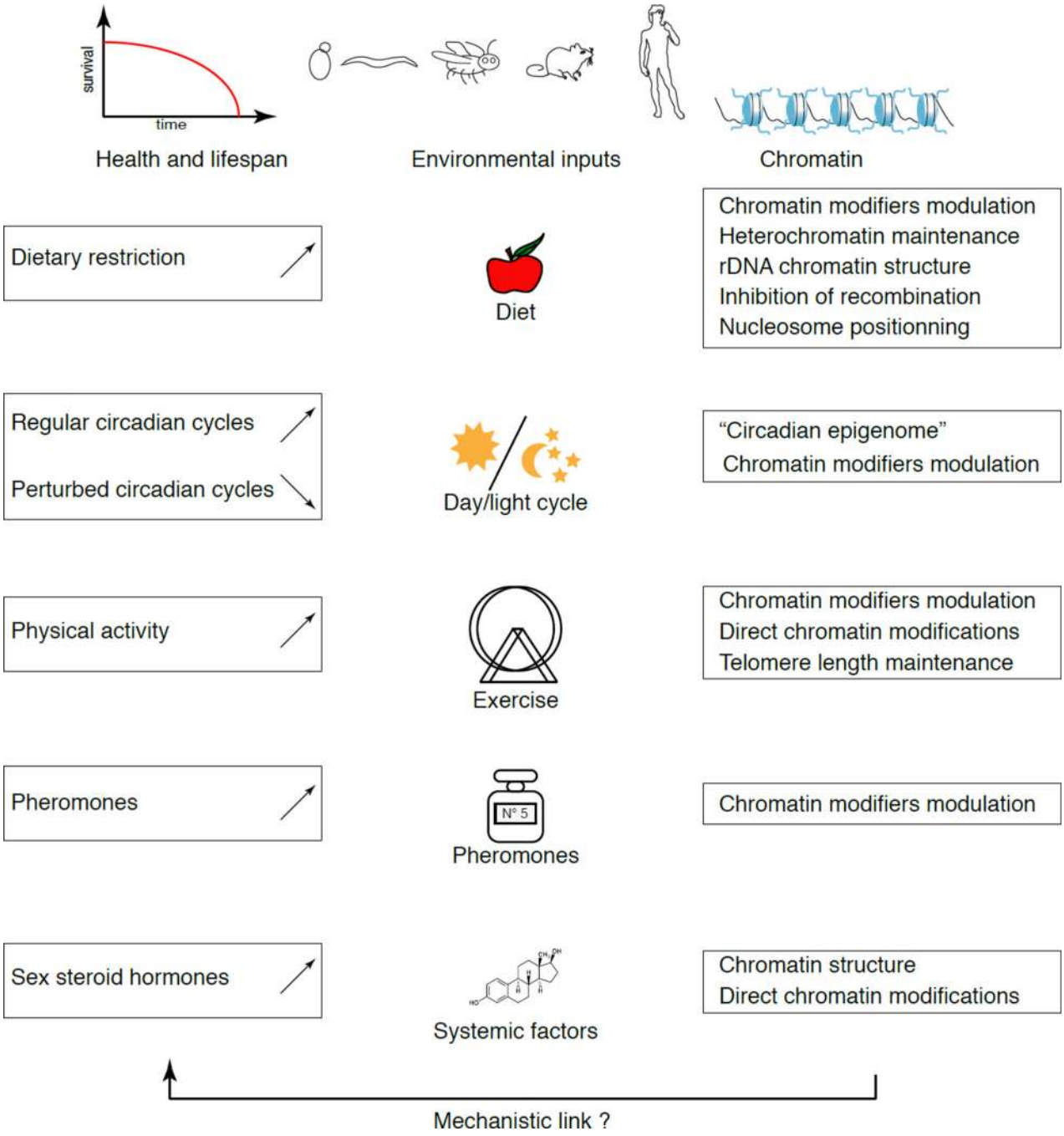


Figure 1. Environmental inputs that impact longevity can also affect the chromatin landscape

Environmental signals that modulate lifespan might do so by modulating chromatin. Dietary restriction increases lifespan in a range of organisms, and DR has also been linked to changes in the chromatin landscape. Those include changes in the expression of chromatin modifiers, for example increased expression of several sirtuins, or an increased maintenance of heterochromatin. Robust circadian light cycles also promote health and lifespan and are linked to circadian epigenomic changes (for example, periodic increases in H3K14 acetylation of circadian promoters by the CLOCK protein) and the modulation of the

activity of chromatin modifiers such as SIRT1. Physical activity is also beneficial to health and lifespan, and has been associated with changes in chromatin modifications (for example, increased H3K36ac in human skeletal muscle) and with the regulation of chromatin modifiers (for example, induced nuclear exclusion of HDAC4 and HDAC5 in human skeletal muscle). Recent work has shown that, in *C. elegans*, pheromones may increase lifespan, through a mechanism requiring chromatin-modifying enzymes (for example, SIR-2.1 is required for lifespan extension following exposure to ascarosides #2 or #3). Finally, in women, the strong decrease in production of sex steroid hormones with age, such as estrogens, contributes to age-related diseases, and estrogens can directly remodel chromatin at target genes through their receptor. Whether there is a linear pathway from the environmental output to the changes in chromatin and, ultimately, to lifespan extension, remains untested.

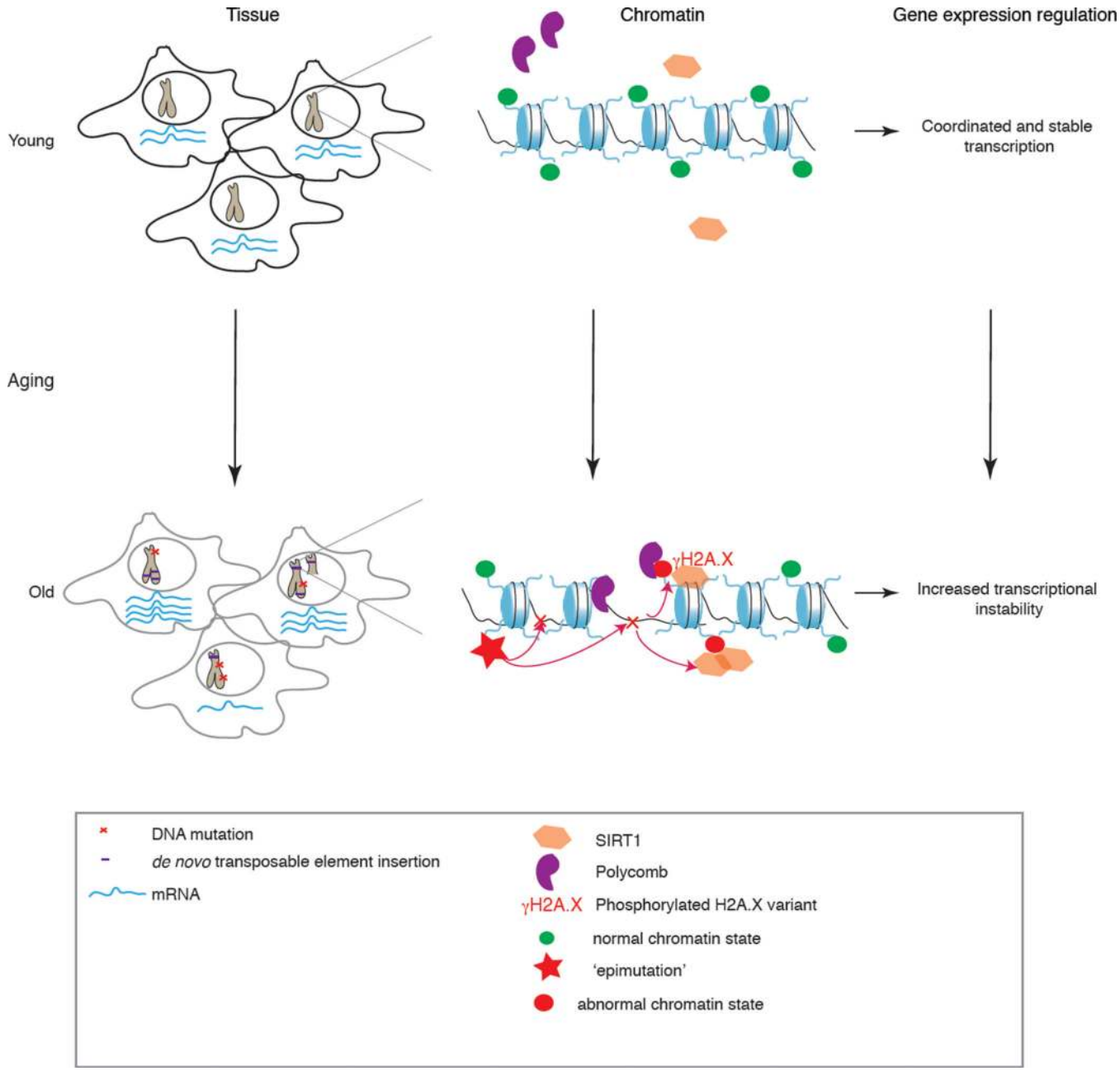


Figure 2. Model for the possible cross-talk between chromatin changes and transcriptional and genomic instability during ageing

A possible model is the following: in cells from young organisms (top left), transcriptional programs are robustly defined and precise between cells (depicted by consistent mRNA levels). The genomic integrity is maintained (depicted by intact chromosomes), because mutations are rare or correctly repaired. As a result, 'normal' chromatin states are found throughout the genome. With increased age (bottom left), transcriptional instability is increased between cells of a tissue (depicted by variable mRNA levels between cells). Genomic instability is also a hallmark of ageing and is increased at both a macro level (for example, aneuploidies depicted by partial chromosome duplication), increased transposable

element insertions and, more locally by DNA mutations in the form of single nucleotide mutations, small insertions or deletions (indels). DNA damage can trigger the recruitment of chromatin modifiers, and the acquisition of abnormal chromatin states. Thus, genomic instability could modify the epigenetic landscape of old cells. Reciprocally, aberrant changes in epigenetic marks, known as ‘epimutations’, can further promote the accumulation of DNA mutations in a feedback loop mechanism. The epigenetic changes acquired during ageing could also decrease the transcriptional precision of neighboring genes.

Table 1

Epigenetic changes observed during ageing[¥]

Epigenetic mark	Functional role	Ageing paradigm	Change in epigenetic mark with ageing	Change observed in tissue/cells	Detection method	Species
5-mC	Transcriptional repression (?)	Organismal (WS)	No global change	MSCs	RRBS	*H. sapiens*
		Cellular (senescence)	Global decrease, local increases	Fibroblasts	Immunostaining, WGBS, chromatography	*H. sapiens, M. musculus, M. auratus*
		Organismal	Minor global increase, local increasesand some local decreases	HSCs	RRBS, WGBS	*M. musculus*
		Organismal	Local increases and some local decreases	Small intestine, Colon, Lung, Liver, Spleen, Brain, Blood, Kidney, Muscle	Pyrosequencing, MCAM, Beadchip arrays	*H. sapiens, M. musculus*
		Organismal	Increase (LINEs), local decreases and rare local increases	Sperm	Pyrosequencing (LINEs), Beadchip arrays	*H. sapiens*
		Organismal	No global change	Cortex	WGBS	*M. musculus, H. sapiens*
5-hmC	Transcriptional activation (?)	Organismal	Minor increase (SINEs, LTRs)	Cerebellum	Immunostaining, Chemical tagging/ sequencing	*M. musculus*
		Organismal	Minor global decrease	HSCs	HPLC-MS	*M. musculus*
mCH (where H represents A,C,T)	(?)	Organismal	Minor global decrease	Cortex	WGBS	*H. sapiens, M. musculus*
*Histone H2A	Core chromatin component	Cellular (senescence)	Decrease	Fibroblasts	Western blot	*H. sapiens*
		Organismal (replicative lifespan)	Decrease	Yeast cells	Western blot	*S. cerevisiae*
*Histone H2B	Core chromatin component	Cellular (senescence)	Decrease	Fibroblasts	Western blot	*H. sapiens*
		Organismal	Decrease	Muscle stem cells	Transcriptional profiling	*M. musculus*
*Histone H3	Core chromatin component	Cellular (senescence)	Decrease	Epidermis, Fibroblasts	Immunostaining, Western blot, SILAC-MS	*H. sapiens*
		Organismal (replicative lifespan)	Decrease, changes in occupancy	Yeast cells	Western blot, SILAC-MS	*S. cerevisiae*

Epigenetic mark	Functional role	Ageing paradigm	Change in epigenetic mark with ageing	Change observed in tissue/cells	Detection method	Species
		Organismal	Decrease	Soma, Whole male flies, Muscle stem cells	Western blot (normalization to tubulin), Transcriptional profiling	*C. elegans, D. melanogaster, M. musculus*
		Organismal	No Change	Head	Western blot (normalization to total protein or DNA)	*D. melanogaster*
H4	Core chromatin component	Cellular (senescence)	Decrease	Fibroblasts	Western blot, SILAC-MS	*H. sapiens*
macroH2A	Component of heterochromatin	Cellular (senescence)	Increase	Fibroblasts	Immunostaining, Western blot	*H. sapiens*
		Organismal	Increase	Lung, Liver	Immunostaining, Western blot	*M. musculus*
*HP1	Component of heterochromatin	Organismal	Remodeling	Head, Gut, Fat cells	Immunostaining, ChIP-chip	*D. melanogaster*
HP1α	Component of heterochromatin	Organismal (HGPS, WS)	Decrease	Fibroblasts, MSCs	Immunostaining, Western blot	*H. sapiens*
		Organismal	Decrease	MSCs	Western blot	*H. sapiens*
HP1β	Component of heterochromatin	Cellular (senescence)	Increase	Fibroblasts	Immunostaining, Western blot	*H. sapiens*
HP1γ	Component of heterochromatin	Organismal (HGPS)	Decrease	Fibroblasts	Immunostaining	*H. sapiens*
		Organismal	Decrease	Fibroblasts	Immunostaining	*H. sapiens*
H3K9me1	Enriched in euchromatin along gene bodies, transcriptional repression	Cellular (senescence)	Increase	Fibroblasts	Western blot, SILAC-MS	*H. sapiens*
H3K9me2	Enriched in euchromatin along gene bodies, transcriptional repression	Cellular (senescence)	Decrease	Fibroblasts	Western blot, SILAC-MS	*H. sapiens*
		Organismal	Decrease	Whole male flies	Western blot	*D. melanogaster*
H3K9me3	Enriched in heterochromatin regions, transcriptional silencing	Organismal (HGPS, WS)	Decrease	Fibroblasts, MSCs	Immunostaining, Western blot, ChIP-seq	*H. sapiens*
		Cellular (senescence)	Decrease	Fibroblasts	SILAC-MS, Western blot	*H. sapiens*
		Organismal	Increase, remodeling	Head	Western blot, ChIP-chip	*D. melanogaster*
		Organismal	Decrease	Fibroblasts, Soma	Immunostaining, Western blot	*H. sapiens, C. elegans*

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Epigenetic mark	Functional role	Ageing paradigm	Change in epigenetic mark with ageing	Change observed in tissue/cells	Detection method	Species
H3K27me3	Enriched in euchromatin along gene bodies, transcriptional repression	Organismal (HGPS, WS)	Decrease, remodeling	Fibroblasts, MSCs	Immunostaining, ChIP-Seq	*H. sapiens*
		Cellular (senescence)	Remodeling	Fibroblasts	ChIP-Seq	*H. sapiens*
		Organismal	Decrease	Soma	Western blot	*C. elegans*
		Organismal	Increase, remodeling	Brain, Muscle stem cells, HSCs	Immunostaining, ChIP-Seq	*N. furzeri, M. musculus*
H4K20me2	DNA repair and genomic stability	Cellular (senescence)	Increase	Fibroblasts	Western blot, SILAC-MS	*H. sapiens*
H4K20me3	Enriched in pericentric heterochromatin	Organismal (HGPS)	Increase	Fibroblasts	Western blot, Immunostaining	*H. sapiens*
		Cellular (senescence)	Decrease	Fibroblasts	Western blot, SILAC-MS	*H. sapiens*
		Organismal	Increase	Liver, Kidney	Liquid chromatography	*R. norvegicus*
H3K4me2	Enriched in euchromatin along gene body, transcriptional activation	Organismal	Global increase, remodeling	Cortex	ChIP-Seq	*M. mulatta*
H3K4me3	Enriched in euchromatin at promoters, transcriptional activation	Organismal (WS)	No global change	MSCs	ChIP-Seq	*H. sapiens*
		Cellular (senescence)	Remodeling	Fibroblasts	ChIP-Seq	*H. sapiens*
		Organismal	No change	Soma	Western blot	*C. elegans*
		Organismal	Decrease, remodeling	Head	ChIP-chip	*D. melanogaster*
		Organismal	Minor global increase, remodeling	Neurons, HSCs, Muscle stem cells	ChIP-Seq	*H. sapiens, M. musculus*
H3K36me3	Enriched in euchromatin along gene body, transcriptional elongation	Organismal (replicative lifespan)	No global change, remodeling	Yeast cells	ChIP-Seq	*S. cerevisiae*
		Organismal	Minor global decrease, remodeling	Soma, Head	Western blot, ChIP-chip, ChIP-Seq	*C. elegans, D. melanogaster*
H3K56ac	DNA replication, DNA damage response, nucleosome assembly	Cellular (senescence)	Decrease	Fibroblasts	Immunostaining, Western blot	*H. sapiens*
		Organismal (replicative lifespan)	Decrease	Yeast cells	Western blot	*S. cerevisiae*

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Epigenetic mark	Functional role	Ageing paradigm	Change in epigenetic mark with ageing	Change observed in tissue/cells	Detection method	Species
H4K16ac	Regulation of telomere silencing, regulation of chromatin compaction (?)	Organismal (HGPS)	Decrease	Fibroblasts, Liver	Immunostaining, Western blot	*M. musculus*
		Cellular (senescence)	Decrease	Fibroblasts	Western blot	*H. sapiens*
		Organismal (replicative lifespan)	Increase	Yeast cells	Western blot	*S. cerevisiae*
		Organismal	Decrease	Liver, Kidney	Western blot	*H. sapiens, M. musculus*
H4K12ac	Enriched in euchromatin along gene body, transcriptional elongation	Organismal	Decrease upon contextual fear conditioning	Hippocampus	Western blot	*M. musculus*

¥ see supplementary information S2 (table) for a fully referenced version of this table.

Note that the changes in chromatin reported for WS come from mesenchymal cells derived from an ESC model of WS. Chromatin changes reported for HGPS come from patient-derived cells or a genetic mouse model of the disease.

* Reported studies of core histone changes correspond to all variants of a particular histone family (not assessed separately).

(?) Functional role of epigenetic mark unclear

§ see supplementary information S1 (box) to read more about targeted and global methods of epigenome mapping

Abbreviations: HGPS: Hutchinson-Gilford Progeria Syndrome; WS: Werner Syndrome; Soma: all body tissues except the germline cells; HSCs: hematopoietic stem cells; MSCs: mesenchymal stem cells; HPLC-MS: high performance liquid chromatography-mass spectrometry; RRBS: reduced representation bisulfite sequencing; WGBS: whole genome bisulfite sequencing; SILAC-MS: Stable isotope labeling by amino acids in cell culture- mass spectrometry; MCAM: methylated CpG island amplification microarrays; ChIP-Seq: Chromatin Immunoprecipitation followed by sequencing; ChIP-chip: Chromatin Immunoprecipitation followed by microarray hybridization; LINE: long interspersed nuclear element; SINE: short interspersed nuclear element; LTR: long tandem repeat.

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Table 2

Modulation of lifespan by components and modifiers of chromatin[¥]

Chromatin modification activity	Protein (complex)	Target histone and/or residue	Chemical inhibitor	Chemical activator	Wild-type impact on health or lifespan	Species
DNA methylation	dDNMT2	5-mC (?)	N/A	N/A	+	*D. melanogaster*
Histone Acetyltransferase	SAS2	H4K16	N/A	N/A	–	*S. cerevisiae*
	GCN5 (SAGA complex)	H3, H3K14	N/A	N/A	+	*S. cerevisiae*
	IKI3, SAS3	H3K9, H3K14, H3K18	spermidine	N/A	–/=	*S. cerevisiae, D. melanogaster, C. elegans*
	CBP	H4K5	N/A	N/A	+	*C. elegans*
Histone Deacetylase (Class I and II)	?	Global effect	TrichostatinA, NaButyrate, PhenylButyrate, SAHA	N/A	+	*D. melanogaster, C. elegans, M. musculus*
	RPD3	H4K5, H4K12	N/A	N/A	–	*S. cerevisiae, D. melanogaster*
Histone Deacetylase (Class III)	SIR2(yeast), SIR2.1(worm), dSIR2 (fly)	H3K9, H3K14, H4K16	Nicotinamide	N/A	+ +/= +/=	*S. cerevisiae, D. melanogaster (?), C. elegans (?)*
	SIRT1	H3K9, H3K14, H4K16	Nicotinamide	SRT1720, SRT2104, SRT3025	+	*M. musculus*
	SIRT6	H3K9, H3K56	Nicotinamide	N/A	+	*M. musculus*
Histone Lysine methyltransferase	SET2	H3K36	N/A	N/A	+/–	*S. cerevisiae*
	MET-1	H3K36 (me3)	N/A	N/A	+	*C. elegans*
	SET-15	?	N/A	N/A	–	*C. elegans*
	SET-9, SET-26	H3K4 (me1/2)	N/A	N/A	–	*C. elegans*
	SET1	H3K4 (me3)	N/A	N/A	+	*S. cerevisiae*
	ASH-2, SET-2, WDR-5 (COMPASS complex)	H3K4 (me3)	N/A	N/A	–	*C. elegans*
	E(Z) (fly), MES-2 (worm) (Polycomb complex)	H3K27	N/A	N/A	–	*D. melanogaster, C. elegans*
Histone Lysine demethylase	RPH1	H3K36 (me3)	N/A	N/A	–	*S. cerevisiae*
	LSD-1 (T08D10.2 gene)	H3K4 (me2)	N/A	N/A	–	*C. elegans*

Chromatin modification activity	Protein (complex)	Target histone and/or residue	Chemical inhibitor	Chemical activator	Wild-type impact on health or lifespan	Species
	RBR-2 (worm), LID (fly)	H3K4 (me3)	N/A	N/A	+/– +/=	*C. elegans, D. melanogaster*
	JMJD-2 (worm), KDM4A(fly)	H3K9	N/A	N/A	– –/=	*C. elegans, D. melanogaster*
	UTX-1	H3K27	N/A	N/A	–	*C. elegans*
Histone O-GlcNac regulation	OGT-1	?	N/A	N/A	+	*C. elegans*
	OGA-1	?	N/A	N/A	–/=	*C. elegans*
Histone monoubiquitinyl-transferase	RAD6, BRE1 (H2B monoubiquitination complex)	H2BK123	N/A	N/A	+	*S. cerevisiae*
Nucleosome remodelers	ISW2 (ISWI complex)	N/A	N/A	N/A	–	*S. cerevisiae, C. elegans*
	SWI/SNF	N/A	N/A	N/A	+	*C. elegans*
	CHD1	N/A	N/A	N/A	–	*S. cerevisiae*
	LET-418 (worm), dMI2 (fly) (NurD complex)	N/A	N/A	N/A	–	*C. elegans, D. melanogaster*
Histone chaperone	ASF1	N/A	N/A	N/A	+	*S. cerevisiae*
	HIR	N/A	N/A	N/A	–	*S. cerevisiae*
Histone expression	H3/H4	N/A	N/A	N/A	+	*S. cerevisiae*

¥ see supplemental information S3 (table) for a fully referenced version of this table.

? indicates where the parameter is unknown or unconfirmed.

N/A:not applicable

The “wild-type impact on health or lifespan” column indicates what role the protein or complexhas on lifespan in physiological conditions based on experimental knock-down, mutation or overexpression results(‘–‘ to indicate that they normally restrict health or lifespan, ‘+’ to indicate that they normally promote health or lifespan, ‘=’ when no clear impact on lifespan was reported).