Epigenetic regulation of ageing: linking environmental inputs to genomic stability

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Abstract | 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 remodelling by environmental stimuli affects several aspects of transcription and genomic stability, with important consequences for longevity, and outline epigenetic differences between the 'mortal soma' and the 'immortal germ line'. Finally, we discuss the inheritance of characteristics of ageing and potential chromatin-based strategies to delay or reverse hallmarks of ageing or age-related diseases.

Lifespan
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 cell can survive; and replicative lifespan, which corresponds to the number of mitotic cell divisions a mother cell has undergone.

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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 function at the cellular, tissue and organismal levels, and with a wide range of diseases, including cardiovascular and neurodegenerative diseases, metabolic disorders and many cancers. Healthspan is the duration of disease-free physiological health (for example, high cognition and mobility) and is highly relevant to human ageing. Understanding the changes that occur during ageing and identifying regulators of lifespan and healthspan should pave the way for interventions that will promote a longer youthful period, increase vigour and potentially reverse some of the 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 approximately 70%[2]. Environmental stimuli, such as dietary 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 of queens and workers (for example, honeybees), in which individuals with similar genomes have large differences in lifespan (for example, the approximately tenfold difference between the lifespans of queens and workers in honeybees)[4]. Such differences can be triggered by early exposure to environmental stimuli and are relatively stable throughout life, although some remodelling is still possible later on[4]. Even in relatively controlled environments, lifespan among isogenic individuals is highly variable, with large differences between the age of the individual at the first and the last death[5]. Although these differences could be purely stochastic, this result suggests that even minute environmental variations may cumulatively influence lifespan. Although a portion of this non-genetic variation may stem from somatic mutations, it is likely that the majority results from other non-genetic, regulated factors that cause stable changes in healthspan and longevity.

In this Review, we examine evidence for an epigenetic component in the regulation of ageing. We use the term 'epigenetic' broadly to refer to changes in genomic regulation, although we note that according to its strictest definition, this term encompasses only heritable phenotypic changes without changes in the underlying gene sequence[6]. Among the various modes of genomic regulation, we particularly focus on chromatin (BOX 1), for several reasons. First, changes in 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 in the chromatin landscape could preserve memory of past environmental exposures, leading to long-lasting phenotypic effects[10,11] that may be particularly relevant to ageing.

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Box 1 | **Chromatin structure and regulation**

Chromatin is the nucleoprotein complex that allows the genome to be packaged inside the nucleus. It can be broadly categorized into two states: euchromatin, which is a loose and transcription-permissive compartment; and heterochromatin, which is a dense and compact compartment that contains repressed DNA. The basic repeating units of chromatin, termed nucleosomes, consist of approximately 150 bp of DNA wrapped around histone protein octamers (which contain two of each of the H2A, H2B, H3 and H4 histone proteins)[176]. Post-translational modifications of histone protein amino-terminal tails are thought to regulate the accessibility and expression potential of underlying genes[177], which is referred to as the 'histone code' hypothesis[177]. Interestingly, in addition to canonical histone proteins, the transcription of which is usually restricted to the S phase of the cell cycle, some histone variants can be expressed at any point during the cell cycle. These specific histone variants have been shown to be involved in regulatory aspects of gene expression (for example, H2A.Z or H3.3) or chromatin structure (for example, H3-like centromeric protein A)[178].

The best-studied core histone modifications include Lys and Arg methylation, Lys acetylation, Ser/Thr phosphorylation and Lys poly- or mono-ubiquitylation (reviewed in REF. 179). These modifications can be added and removed by chromatin modifiers, including histone acetyltransferases, histone deacetylases, histone methyltransferases and histone demethylases.

Another layer of regulation includes the methylation status of cytosines in CpG dinucleotides, and the most frequent type of methylation occurs on carbon 5 of cytosine[180]. In mammals, DNA methylation is generally associated with gene repression, although whether methylation has 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, which are large regions with concentrated CpGs that are found in gene-rich areas[180]. DNA methyltransferases 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 remodellers (for example, SWI/SNF or the imitation switch (ISWI) complex) can affect the chromatin and transcriptional landscape by modifying nucleosome positioning, higher-order structure of chromatin and overall nuclear organization[77].

We 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 for chromatin modifiers in modulating lifespan. We also highlight the use of epigenetic signatures as 'ageing clocks' (BOX 2). Next, we explore the influence of environmental inputs that affect ageing on epigenetic remodelling and the potential consequences of this remodelling on genome stability and transcription. We evaluate the contributions of epigenetics to long-lasting and even heritable longevity phenotypes. Finally, we discuss remaining questions and challenges, including the question of 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 (see Supplementary information S1 (box)), which have revolutionized our knowledge of epigenetic factors and their relationships with gene regulation. Reference epigenomes provided by the National Institutes of Health Roadmap Epigenomics Project[12] and the Encyclopedia of DNA Elements (ENCODE) database[13] should also aid our understanding of the remodelling and persistence of epigenomic patterns that occur 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 of meeting growing health demands.

Healthspan
The duration of disease-free physiological health within the lifespan of an individual. In humans, for instance, this corresponds to the period of 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 that are not directly encoded in DNA. We use it specifically to refer to chromatin-level regulation. According to its strictest definition, epigenetics encompasses strictly heritable changes without changes in the underlying gene sequence.

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 affects lifespan. We present findings from model organisms (yeast, worms, flies and mice) and from cellular models of replicative senescence. We also discuss results from organismal and cellular models of diseases with accelerated signs of ageing (that is, progeroid syndromes) such as Hutchinson–Gilford progeria syndrome (HGPS) and Werner syndrome. In presenting this evidence, we note that the precise role of chromatin in modulating 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 for ones that cannot be modified, and doing so in a locus-specific manner, is technically difficult to achieve. Nevertheless, the evidence that has been 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 remodelling during ageing in various tissues in mice and humans (TABLE 1; see Supplementary information S2 (table)). In human cells, global levels of 5-methylcytosine (5-mC) are reduced in senescent cells compared with actively cycling cells[14,15] (TABLE 1). Studies investigating the methylation status of specific CpG sites across the genome suggest that the methylation of such sites outside promoter CpG islands tends to decrease in various tissues in humans[16]. By contrast, in both mice and humans, CpG islands near promoters are typically hypermethylated with age, most notably on genes that are involved in differentiation or development[16–18]. Although changes in DNA methylation in heterogeneous tissues may partly reflect alterations in cell composition[19], age-dependent changes in DNA methylation are detected in more homogeneous cell populations such as haematopoietic stem cells (HSCs)[20,21]. The consequences of age-dependent changes in DNA methylation on cellular function are not yet completely clear; however, in HSCs, regions with altered DNA methylation overlap with the binding sites of specific sets of transcription factors[21]. This observation raises the possibility that these changes in methylation could influence the targeting of transcription factors and thereby contribute to misregulation of gene expression during ageing[21]. Although many studies have focused on 5-mC, the discovery of other forms of DNA methylation, including 5-hydroxymethylcytosine, cytosine methylation at non-CpG dinucleotides[22] and

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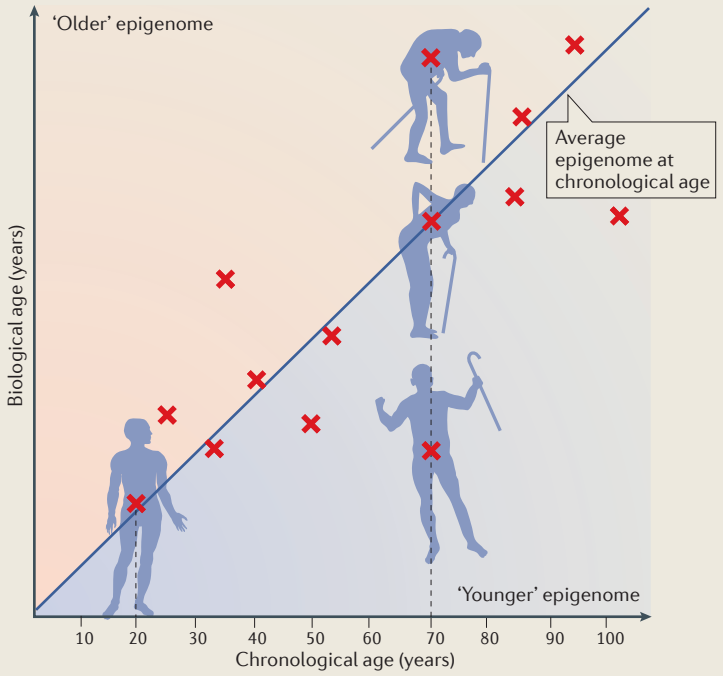
Box 2 | **Epigenetic modifications as biomarkers of age**

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 correlate with chronological age (that is, the age of the individual in time units) or with biological age (that is, the age in years of the population average most similar to the individual)?

Studies have explored the use of changes in DNA methylation (termed DNA methylation age) as a sensor for both chronological age and biological age across species and cell types. On the basis of the methylation status of a defined number of CpGs, highly accurate predictors of chronological age have been constructed in specific tissues such as saliva[181] and peripheral blood cells[26–28]. Surprisingly, such analyses have resulted in accurate estimators of age that seem 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' germ line) is estimated to be far younger than that of somatic cells from the same donor[26,28]. Although DNA methylation allows accurate modelling of chronological age with high correlation coefficients[27,28], there is significant deviation from the linear fit, suggesting that methylation patterns may also reflect biological age (see the figure). Indeed, the use of DNA methylation age may predict the health of various tissues. For example, liver tissue from obese subjects has an increased DNA methylation age compared with 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 anaemia and dyskeratosis congenita[26] or Down syndrome[182]. Moreover, the 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 $p16^{INK4A}$ (REF. 184) and telomere length attrition rate[185]. However, CpG DNA methylation outperforms these molecular markers as a predictor 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 cytosine carbon 5 methylation and other new oxidation derivatives[186] or N^6-methylation of adenine[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].

Chromatin
A complex of DNA and histone proteins that packages genetic information. Chromatin can be broadly stratified in two states: euchromatin, which is a loose and transcription-permissive compartment; and heterochromatin, which is a dense and compact compartment that contains repressed DNA.

Epigenomes
The epigenetic landscapes of cells, 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 for ageing in dividing mammalian cells such as fibroblasts or stem cells.

CpG islands
Clusters of CpG dinucleotides, which are usually found in the promoter regions of some genes. CpG islands are typically defined by a minimum length (~200–500 bp) and an unusual enrichment of CpG (>60%). They are usually unmethylated in the germline or pluripotent state.

Chronological age
The time elapsed since the birth of an individual. Chronological age is often compared to biological age.

Biological age
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, whereas older biological ages correlate with disease onset. Biological age is used as a proxy for individual health.

N^6-methylation of adenine[23–25], deserve exploration in the context of ageing. Notably, 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 in *Caenorhabditis elegans*[30]. However, DNA methylation is present in *Drosophila melanogaster*[29], which is another invertebrate model for ageing. In *D. melanogaster,* overexpression of the putative DNA methyltransferase-encoding gene *dDnmt2* (also known as *Mt2*) increases longevity, whereas flies lacking a functional copy of the gene are short-lived[31] (TABLE 2; see Supplementary information S3 (table)). Whether lifespan changes in *dDnmt2* mutants result from changes in DNA methylation is still subject to debate. Although an initial study identified a potential role for *dDnmt2* in the methylation of CpG sites 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, the mechanism by which CpG methylation affects 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.

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Table 1 | **Epigenetic changes observed during ageing**

Epigenetic mark	Function	Ageing paradigm	Change in epigenetic mark with ageing	Change observed in tissue or cells	Method of detection*	Species
5-mC	Transcriptional repression (?)	Organismal (Werner syndrome)‡	No global change	MSCs	RRBS	*H. sapiens*
		Cellular (senescence)	Global decrease and local increases	Fibroblasts	• Immunostaining • WGBS • Chromatography	• *H. sapiens* • *M. musculus* • *M. auratus*
		Organismal	Minor global increase, local increases and some local decreases	HSCs	• RRBS • WGBS	*M. musculus*
		Organismal	Local increases and some local decreases	Small intestine, colon, lung, liver, spleen, brain, blood, kidney and 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 and LTRs)	Cerebellum	• Immunostaining • Chemical tagging and sequencing	*M. musculus*
		Organismal	Minor global decrease	HSCs	HPLC–MS	*M. musculus*
mCH (where H represents A,C or 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*
H2B§	Core chromatin component	Cellular (senescence)	Decrease	Fibroblasts	Western blot	*H. sapiens*
		Organismal	Decrease	Muscle stem cells	Transcriptional profiling	*M. musculus*
H3§	Core chromatin component	Cellular (senescence)	Decrease	Epidermis and fibroblasts	• Immunostaining • Western blot • SILAC–MS	*H. sapiens*
		Organismal (replicative lifespan)	Decrease and changes in occupancy	Yeast cells	• Western blot • MNase–seq	*S. cerevisiae*
		Organismal	Decrease	Soma‖, whole male flies and 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 and liver	• Immunostaining • Western blot	*M. musculus*

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Table 1 (cont.) | **Epigenetic changes observed during ageing**

Epigenetic mark	Function	Ageing paradigm	Change in epigenetic mark with ageing	Change observed in tissue or cells	Method of detection*	Species
HP1[§]	Component of heterochromatin	Organismal	Remodelling	Head, gut and fat cells	• Immunostaining • ChIP–chip	*D. melanogaster*
HP1α	Component of heterochromatin	Organismal (HGPS[¶] and Werner syndrome)	Decrease	Fibroblasts and 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, involved in transcriptional repression	Cellular (senescence)	Increase	Fibroblasts	• Western blot • SILAC–MS	*H. sapiens*
H3K9me2	Enriched along gene bodies, involved in 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, involved in transcriptional silencing	Organismal (HGPS and Werner syndrome)	Decrease	Fibroblasts and MSCs	• Immunostaining • Western blot • ChIP–seq	*H. sapiens*
		Cellular (senescence)	Decrease	Fibroblasts	• SILAC–MS • Western blot	*H. sapiens*
		Organismal	Increase and remodelling	Head	• Western blot • ChIP–chip	*D. melanogaster*
		Organismal	Decrease	Fibroblasts and soma	• Immunostaining • Western blot	• *H. sapiens* • *C. elegans*
H3K27me3	Enriched in euchromatin along gene bodies, involved in transcriptional repression	Organismal (HGPS and Werner syndrome)	Decrease and remodelling	Fibroblasts and MSCs	• Immunostaining • ChIP–seq	*H. sapiens*
		Cellular (senescence)	Remodelling	Fibroblasts	ChIP–seq	*H. sapiens*
		Organismal	Decrease	Soma	Western blot	*C. elegans*
		Organismal	Increase and remodelling	Brain, muscle stem cells and 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 and kidney	Liquid chromatography	*R. norvegicus*
H3K4me2	Enriched in euchromatin along gene body, involved in transcriptional activation	Organismal	Global increase and remodelling	Cortex	ChIP–seq	*M. mulatta*

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Table 1 (cont.) | **Epigenetic changes observed during ageing**

Epigenetic mark	Function	Ageing paradigm	Change in epigenetic mark with ageing	Change observed in tissue or cells	Method of detection*	Species
H3K4me3	Enriched in euchromatin at promoters, involved in transcriptional activation	Organismal (Werner syndrome)	No global change	MSCs	ChIP–seq	*H. sapiens*
		Cellular (senescence)	Remodelling	Fibroblasts	ChIP–seq	*H. sapiens*
		Organismal	No change	Soma	Western blot	*C. elegans*
		Organismal	Decrease and remodelling	Head	ChIP–chip	*D. melanogaster*
		Organismal	Minor global increase and remodelling	Neurons, HSCs and muscle stem cells	ChIP–seq	• *H. sapiens* • *M. musculus*
H3K36me3	Enriched in euchromatin along gene body, involved in transcriptional elongation	Organismal (replicative lifespan)	No global change and remodelling	Yeast cells	ChIP–seq	*S. cerevisiae*
		Organismal	Minor global decrease, remodelling	Soma and head	• Western blot • ChIP–chip • ChIP–seq	• *C. elegans* • *D. melanogaster*
H3K56ac	DNA replication, DNA damage response and nucleosome assembly	Cellular (senescence)	Decrease	Fibroblasts	• Immunostaining • Western blot	*H. sapiens*
		Organismal (replicative lifespan)	Decrease	Yeast cells	Western blot	*S. cerevisiae*
H4K16ac	Regulation of telomere silencing and regulation of chromatin compaction (?)	Organismal (HGPS)	Decrease	Fibroblasts and 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 and kidney	Western blot	• *H. sapiens* • *M. musculus*
H4K12ac	Enriched in euchromatin along gene body, involved in 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.'?' indicates that the functional role of the epigenetic mark is unclear. 5-hmC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine; *C. elegans*, *Caenorhabditis elegans*; ChIP–chip, chromatin immunoprecipitation followed by microarray hybridization; ChIP–seq, chromatin immunoprecipitation followed by sequencing; *D. melanogaster*, *Drosophila melanogaster*; H3K9me, histone 3 Lys 9 monomethylation; H3K56ac, histone 3 Lys 56 acetylation; *H. sapiens*, *Homo sapiens*; HP1, heterochromatin protein 1; HPLC–MS, high-performance liquid chromatography followed by mass spectrometry; HSCs, haematopoietic stem cells; LINEs, long interspersed nuclear elements; LTRs, long tandem repeats; *M. auratus*, *Melanochromis auratus*; MCAM, methylated CpG island amplification microarrays; *M. mulatta*, *Macaca mulatta*; *M. musculus*, *Mus musculus*; MNase–seq, micrococcal nuclease digestion followed by sequencing; MSCs, mesenchymal stem cells; *N. furzeri*; *Nothobranchius furzeri*; *R. norvegicus*, *Rattus norvegicus*; RRBS, reduced representation bisulfite sequencing; *S. cerevisiae*, *Saccharomyces cerevisiae*; SILAC–MS, stable isotope labelling by amino acids in cell culture followed by mass spectrometry; SINEs, short interspersed nuclear elements; WGBS, whole-genome bisulfite sequencing. *See Supplementary information S1 (box) for more information about targeted and global methods of epigenome mapping. ‡Changes in chromatin reported for Werner syndrome come from MSCs derived from an embryonic stem cell model of Werner syndrome. §Reported studies of core histone changes correspond to all variants of a particular histone family (not assessed separately). ‖Soma refers to all body tissues except the germline cells. ¶Chromatin changes reported for Hutchinson–Gilford progeria syndrome (HGPS) come from patient-derived cells or from a genetic mouse model of the disease.

Core histone expression in ageing and longevity. The folding of cellular genomes into higher-order chromatin structures 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; see 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]. Inactivation of complexes that control the exchange and deposition of histones onto chromatin also influences lifespan in *S. cerevisiae*: the histone chaperone anti-silencing function 1 (Asf1), which promotes histone deposition and stability, is required for normal replicative lifespan, whereas the histone regulation complex HIR, which represses histone expression, limits the replicative lifespan of yeast[40]. Interestingly, increasing the cellular

Histone chaperone
A protein that aids the folding or dimerization of histones, as well as their loading onto the chromatin fibre. Different histones have different chaperones.

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supply of histones H3 and H4, but not of H2A and H2B, increases the replicative lifespan of yeast by up to 50%[40] (TABLE 2; see Supplementary information S3 (table)). The oligomerization of histones into chromatin is initiated by the deposition of H3–H4 dimers, which 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 aberrant activation. To our knowledge, whether histone expression levels limit longevity in Metazoa has not been investigated. However, because histone expression declines in some mammalian cells during ageing[37,38] (TABLE 1), this process may also affect ageing in mammals.

Histone methylation in ageing and longevity. Histone methylation is associated with either 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 REF. 42). The global level or genomic distribution of many histone methylations changes in organismal and cellular models of ageing (TABLE 1). Furthermore, the manipulation of histone methyltransferases and histone demethylases can modulate the longevity of model organisms (TABLE 2).

Data from both physiological and accelerated models of ageing generally support the 'heterochromatin loss model of ageing' (REF. 43) (TABLE 1). According to this model, decreased heterochromatin and/or the inappropriate redistribution of heterochromatin-silencing proteins may cause cellular dysfunction with age. The mechanisms underlying heterochromatin remodelling are not fully understood, but they 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 levels of lamin B1 in proliferating fibroblasts can induce locus-specific remodelling of H3 Lys 4 trimethylation (H3K4me3) and H3K27me3, which mimics alterations observed in senescent cells[39]. Furthermore, widespread alterations in heterochromatin organization are observed in mesenchymal stem cells derived from an embryonic stem cell 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 in which loss of repressive chromatin is detrimental during ageing, manipulation of chromatin regulators to increase repressive histone methylations such as H3K27me3 can result in increased longevity[46–48] (TABLE 2). For example, reduced expression of the H3K27me3 demethylase UTX-1 promotes longevity in *C. elegans*[46,47]. During worm ageing, a global decrease in somatic H3K27me3 (REFS 47,48) and an increase in UTX-1 expression[46] is observed, suggesting that UTX-1 may limit worm longevity. However, results from several organisms indicate that the effect of H3K27me3 on ageing is likely to be more complex. Contrary to what might be expected from the results of altered UTX-1 expression, a reduction in the expression of MES-2, which is the *C. elegans* orthologue of the H3K27 trimethyltransferase component of the *D. melanogaster* Polycomb group protein Enhancer of zeste (E(z)), extends the lifespan of sterile worms[48]. Furthermore, mutation of the gene encoding 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 the brains of old African killifish[50], in contrast to the age-related decrease in H3K27me3 in *C. elegans*[47,48]. The association of H3K27me3 levels with both extension and shortening of lifespan 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 influence on lifespan through other, H3K27me3-independent, effects.

Age-associated loss of heterochromatin is often coupled to the remodelling of histone methylations that are associated with 'active' chromatin. Indeed, redistribution of the active histone modification H3K4me3 (which is 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 the results are normalized to the declining total 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 that of changes in 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 to investigate the effects of histone methyltransferases and demethylases on longevity in flies and in worms have shown that regulators of H3K4me3 can modulate lifespan[46–48,53] (TABLE 2). Specifically, a longevity screen in fertile well-fed *C. elegans* hermaphrodites showed that knockdown or mutation of genes encoding members of the COMPASS (complex proteins associated with SET1) H3K4me3 methyltransferase complex[54] (*ash-2*, *set-2*, and *wdr-5*) increases lifespan, whereas knockdown of the gene encoding the H3K4me3 demethylase RBR-2 shortens lifespan[53]. In addition, overexpressing RBR-2 in the germ line extends lifespan[53], supporting the idea that H3K4me3 demethylation in the germ line promotes somatic maintenance. Consistently, male *D. melanogaster* deficient in Little imaginal discs (Lid), which is the orthologue of RBR-2, also have shortened lifespans[55], although female flies are unaffected. However, regulators of H3K4me3 may have different effects depending on the H3K4me3 complex or the conditions. For example, deficiency in Trithorax, which is part of another type of H3K4me3 methyltransferase complex in *D. melanogaster*[54], does not significantly affect the lifespans of male flies[49]. In addition, several studies indicate that *rbr-2* RNAi can actually extend

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Table 2 | **Modulation of lifespan by components and modifiers of chromatin**

Chromatin modification activity	Protein	Target histone and/or residue	Chemical inhibitor	Chemical activator	Wild-type effect on healthspan or lifespan*	Species
DNA methylation	dDnmt2	5-mC (?)	NA	NA	+	*D. melanogaster*
Histone acetyltransferase	Sas2	H4K16	NA	NA	–	*S. cerevisiae*
	Gcn5 (SAGA complex)	• H3 • H3K14	NA	NA	+	*S. cerevisiae*
	Iki3 and Sas3	• H3K9 • H3K14 • H3K18	Spermidine	NA	– or =	• *S. cerevisiae* • *D. melanogaster* • *C. elegans*
	CBP-1	H4K5	NA	NA	+	*C. elegans*
Histone deacetylase (classes I and II)	?	Global effect	• Trichostatin A • NaButyrate • Phenylbutyrate • SAHA	NA	+	• *D. melanogaster* • *C. elegans* • *M. musculus*
	Rpd3	• H4K5 • H4K12	NA	NA	–	• *S. cerevisiae* • *D. melanogaster*
Histone deacetylase (class III)	Sir2 (yeast), SIR-2.1 (worm), and Sir2 (fly)	• H3K9 • H3K14 • H4K16	Nicotinamide	NA	+ + or = + or =	• *S. cerevisiae* • *D. melanogaster* (?) • *C. elegans* (?)
	SIRT1	• H3K9 • H3K14 • H4K16	Nicotinamide	• SRT1720 • SRT2104 • SRT3025	+	*M. musculus*
	SIRT6	• H3K9 • H3K56	Nicotinamide	NA	+	*M. musculus*
Histone Lys methyltransferase	Set2	H3K36	NA	NA	+ or –	*S. cerevisiae*
	MET-1	H3K36 (me3)	NA	NA	+	*C. elegans*
	SET-15	?	NA	NA	–	*C. elegans*
	SET-9 and SET-26	H3K4 (me1/2)	NA	NA	–	*C. elegans*
	SET1	H3K4 (me3)	NA	NA	+	*S. cerevisiae*
	ASH-2, SET-2 and WDR-5 (COMPASS complex)	H3K4 (me3)	NA	NA	–	*C. elegans*
	E(z) (fly) and MES-2 (worm) (Polycomb complex)	H3K27	NA	NA	–	• *D. melanogaster* • *C. elegans*
Histone Lys demethylase	Rph1	H3K36 (me3)	NA	NA	–	*S. cerevisiae*
	LSD-1 (T08D10.2 gene)	H3K4 (me2)	NA	NA	–	*C. elegans*
	RBR-2 (worm) and Lid (fly)	H3K4 (me3)	NA	NA	+ or – + or =	• *C. elegans* • *D. melanogaster*
	JMJD-2 (worm) and Kdm4a (fly)	H3K9	NA	NA	– – or =	• *C. elegans* • *D. melanogaster*
	UTX-1	H3K27	NA	NA	–	*C. elegans*

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.

Maintenance of 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 DNA damage-responsive transcriptional repressor *RPH1* gene, which encodes a H3K36 demethylase, extends the replicative lifespan in yeast, and yeast with mutant forms of H3 that cannot be methylated on H3K36 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, knockdown or mutation of *met-1*, the gene encoding the putative *C. elegans* enzyme that deposits the H3K36me3 mark, shortens the worm lifespan[51]. These results suggest that the correct maintenance of H3K36me3 levels may be crucial during ageing.

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

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Table 2 (cont.) | **Modulation of lifespan by components and modifiers of chromatin**

Chromatin modification activity	Protein	Target histone and/or residue	Chemical inhibitor	Chemical activator	Wild-type effect on healthspan or lifespan*	Species
Histone *O*-GlcNAc regulation	OGT-1	?	NA	NA	+	*C. elegans*
	OGA-1	?	NA	NA	– or =	*C. elegans*
Histone monoubiquitinyl transferase	Rad6 and Bre1 (H2B monoubiquitylation complex)	H2BK123	NA	NA	+	*S. cerevisiae*
Nucleosome remodellers	Isw2 (ISWI complex)	NA	NA	NA	–	• *S. cerevisiae* • *C. elegans*
	SWI/SNF complex	NA	NA	NA	+	*C. elegans*
	Chd1	NA	NA	NA	–	*S. cerevisiae*
	LET-418 (worm) and dMi2 (fly) (NurD complex)	NA	NA	NA	–	• *C. elegans* • *D. melanogaster*
Histone chaperone	Asf1	NA	NA	NA	+	*S. cerevisiae*
	HIR	NA	NA	NA	–	*S. cerevisiae*
Histone expression	H3 and H4	NA	NA	NA	+	*S. cerevisiae*

See Supplementary information S3 (table) for a fully referenced version of this table.'?' indicates that the parameter is unknown or unconfirmed. 5-mC, 5-methylcytosine; Asf1, anti-silencing function 1; Bre1, brefeldin A-sensitive 1; *C. elegans*, *Caenorhabditis elegans*; Chd1, chromatin organization modifier, helicase, and DNA-binding domains 1; COMPASS, complex proteins associated with SET1; *D. melanogaster*, *Drosophila melanogaster*; E(z), Enhancer of zeste; HIR, histone regulation complex; ISWI, imitation switch; JMJD-2, Jumonji domain protein-2; LET-418, lethal protein 418; *M. musculus*, *Mus musculus*; me1, monomethylation; me2, dimethylation; me3, trimethylation; NA, not applicable; NuRD, nucleosome remodelling and deacetylases; *O*-GlcNAc, *O*-*N*-acetylglucosamine; OGT-1, *O*-GlcNAc transferase 1; Rad6, radiation sensitive 6; *S. cerevisiae*, *Saccharomyces cerevisiae*; SAGA, Spt-Ada-Gcn5 acetyltransferase; SAHA, suberoylanilide hydroxamic acid; Sir2, silent information regulator 2; SIRT, sirtuin; WDR-5, WD-repeat 5. *Effect the protein or complex has on lifespan in physiological conditions based on experimental knockdown, mutation or overexpression results ('–' indicates that the protein or complex normally restricts health or lifespan, '+' indicates that it normally promotes healthspan or lifespan and '=' indicates that no clear impact on lifespan was reported).

Histone acetylation in ageing and lifespan regulation. Histone acetylation directly influences the physical associations between histones and DNA. Histone acetylation is a key, conserved player in longevity, and evidence suggests that its pattern changes during normal ageing (TABLES 1,2). In yeast, global levels of H3K56 acetylation (H3K56ac) decrease during replicative ageing, whereas those of H4K16ac increase, leading to desilencing of telomeric repeats[59]. By 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 (HATs) with the nuclear periphery[60]. Following contextual fear conditioning, older mice fail to upregulate H4K12ac (which is 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 may thus be both a consequence and a cause of the failure of older cells to transduce external stimuli to downstream transcriptional responses, which is particularly detrimental for rapid cell-to-cell signalling in the brain.

Both HATs and histone deacetylases (HDACs) modulate lifespan and metabolic health (TABLE 2). For example, H4K16ac is deacetylated by the sirtuin silent information regulator 2 (Sir2)[63], and increased Sir2 dosage extends yeast lifespan[64] by limiting aberrant recombination at the ribosomal DNA locus. Although Sir2 and its orthologues 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 with an H4 gene that mimics constitutive H4K16ac is not altered by *SIR2* deletion, suggesting that Sir2 regulates lifespan through its activity on acetylated H4K16 (REF. 59).

Although the role of sirtuins (also known as class III HDACs) in lifespan modulation in Metazoa has been challenged[66], new evidence supports a role for Sir2 orthologues — such as NAD-dependent protein deacetylase sirtuin 1 (SIRT1) in mice — in promoting health and lifespan under standard conditions, or at least in the context of longevity and health mediated by dietary restriction[67] (TABLE 2). The role of mammalian SIRT6 in regulating longevity is clearer. SIRT6 can deacetylate H3K9ac[68] and H3K56ac[69] and 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 it may stem from the role of the protein in supporting genomic stability[70,72], partly by recruiting chromatin remodellers 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 below).

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

Sirtuin
A member of the family of class III histone deacetylases (HDACs). The first sirtuin to be discovered was the yeast protein Sir2p. The defining characteristic of these HDACs is their dependency on NAD^+ as a cofactor to catalyse deacetylation or other enzymatic reactions, including ADP-ribosylation.

Dietary restriction
A reduction in food intake without malnutrition. There are many types of dietary restriction regimen, such as intermittent fasting, global caloric restriction or specific reduction of a nutrient type.

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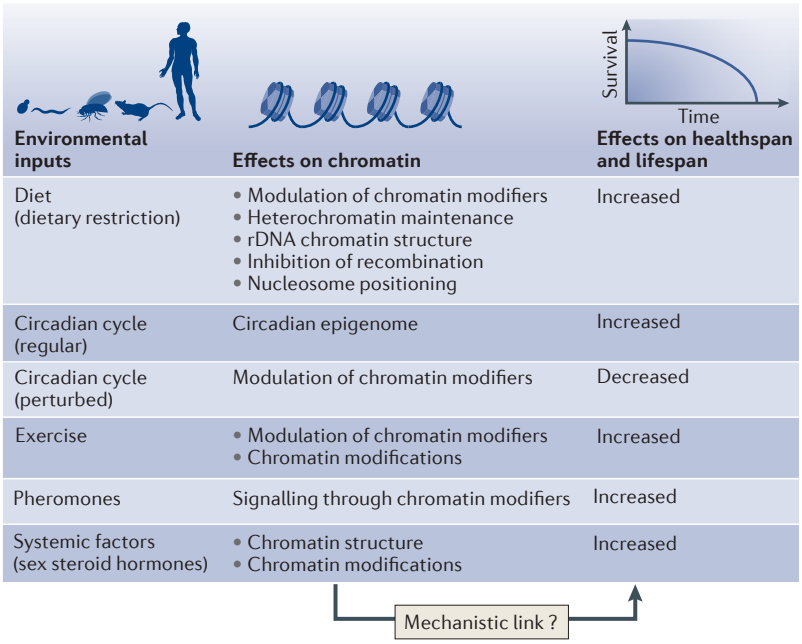

Environmental inputs	Effects on chromatin	Effects on healthspan and lifespan
Diet (dietary restriction)	• Modulation of chromatin modifiers • Heterochromatin maintenance • rDNA chromatin structure • Inhibition of recombination • Nucleosome positioning	Increased
Circadian cycle (regular)	Circadian epigenome	Increased
Circadian cycle (perturbed)	Modulation of chromatin modifiers	Decreased
Exercise	• Modulation of chromatin modifiers • Chromatin modifications	Increased
Pheromones	Signalling through chromatin modifiers	Increased
Systemic factors (sex steroid hormones)	• Chromatin structure • Chromatin modifications	Increased

Figure 1 | **Environmental inputs that affect 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 has also been linked to changes in the chromatin landscape. These include changes in the expression of chromatin modifiers (for example, increased expression of several sirtuins) or increased maintenance of heterochromatin. Robust circadian light cycles also promote healthspan and lifespan and are linked to circadian epigenomic changes (for example, periodic increases in the histone 3 Lys 14 acetylation (H3K14ac) of circadian promoters by the circadian locomoter output cycles protein kaput (CLOCK) protein) and modulation of the activity of chromatin modifiers such as NAD-dependent protein deacetylase sirtuin 1 (SIRT1). Physical activity is also beneficial to healthspan 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 histone deacetylase 4 (HDAC4) and HDAC5 in human skeletal muscle). Recent work has shown that, in *Caenorhabditis elegans*, pheromones may increase lifespan through a mechanism requiring chromatin-modifying enzymes (for example, histone deacetylase SIR-2.1 is required for lifespan extension following exposure to ascaroside 2 or ascaroside 3). Finally, in women, the strong decrease in the production of sex steroid hormones (such as oestrogens) with age contributes to age-related diseases, and oestrogens can directly remodel chromatin at target genes through their receptors. Whether there is a linear pathway from the environmental output to the changes in chromatin and, ultimately, to healthspan and lifespan extension, remains untested. rDNA, ribosomal DNA.

Enzymes regulating other histone modifications. Regulators of other types of histone modification could also affect 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 signalling[75]. Specifically, mutation of *ogt-1*, the gene encoding the enzyme that deposits *O*-GlcNAc, shortens the worm lifespan[75,76], and mutation of *oga-1*, the gene encoding the enzyme that removes *O*-GlcNAc, can extend lifespan in worms, depending on the genetic background[75,76]. However, *O*-GlcNAc can also modify metabolic enzymes, in addition to modifying histones[76]. Thus, the exact mechanism by which cycling of *O*-GlcNAc affects lifespan remains to be explored.

Role of nucleosome remodellers. Nucleosome remodellers also modify chromatin, and their perturbation can regulate lifespan in yeast and in worms (TABLE 2). Chromatin remodellers can modify nucleosome positioning, chromatin higher-order structure and overall nuclear organization (reviewed in REF. 77). Changes in heterochromatin in ageing models have been linked to the activity of nucleosome remodellers such as the nucleosome remodelling and deacetylases (NuRD) complex[78]. The NuRD components histone-binding protein RBBP4 and RBBP7 are downregulated in fibroblasts isolated from patients with HGPS 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 remodelling imitation switch (ISWI) complex extends lifespan in *S. cerevisiae* and in *C. elegans*[79]. Indeed, deletion of *Isw2* (which encodes a subunit of the ISWI complex) in yeast leads to shifts in nucleosome positioning at thousands of stress-response genes and an increased replicative lifespan[79]. Disruption of the orthologous complex in *C. elegans* through another subunit leads to increased longevity[79]. By contrast, SWI/SNF complexes are crucial for promoting longevity in the context of the insulin–forkhead box protein O (FOXO) pathway in *C. elegans*[80]. Indeed, both the transcriptional changes and the lifespan extension promoted by DAF-16 (which is the worm FOXO transcription factor) require functional SWI/SNF[80]. These studies suggest that the regulation of nucleosome positioning and chromatin compaction by remodellers can affect both pro-ageing and 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 remain to be explored.

Environment and age-related epigenetics

Emerging evidence highlights the importance of non-genetic factors in the regulation of ageing and longevity, including diet[3], exercise[81], sexual stimuli[82] and circadian rhythms[83] (FIG. 1). Many proteins that directly or indirectly induce epigenetic remodelling are also modulated by environmental factors. However, causal evidence that can establish linear relationships between external factors and specific chromatin changes, and subsequently 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 extends lifespan and delays signs of ageing in many organisms[3]. Dietary restriction induces changes in gene expression across tissues (reviewed in REF. 10), including changes in the expression of genes that regulate metabolism, stress responses, DNA repair and chromatin structure. These dietary restriction-induced transcriptional changes are consistent with a global preservation of genome integrity and chromatin structure[84]. Thus, the age-delaying effect of dietary restriction may be due to increased genomic stability[10,84]. Dietary restriction can also affect the chromatin landscape (reviewed in REF. 10). For instance, dietary

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restriction induces positional shifts of nucleosomes at thousands of genes in yeast, which may result in 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 dietary restriction and *Isw2* mutation on longevity are epistatic[79]. Thus, nucleosome rearrangements may partly underlie dietary restriction-induced longevity in yeast[79].

Dietary restriction may also affect the global chromatin landscape in Metazoa. A study using a position variegation effect reporter suggests that dietary restriction may delay the age-related loss of facultative heterochromatin in flies[85]. Diet-switch experiments with an unrestricted diet and dietary restriction suggest that diet-regulated changes in heterochromatin may be rapid (occurring within 3 days) and reversible[85], which suggests short-term adaptations. High nutrient intake in humans, which was measured using increased body mass index as a proxy, also seems to induce an 'aged-like' profile of DNA methylation in the liver[86]. Thus, nutrient intake has important connections 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 affects 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 dietary restriction, 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 dietary restriction can occur through systemic sensing of nutrient availability (for example, through signalling pathways) and/or through direct sensing of cellular energy levels that are reflected by changes in NADH/NAD^+ or ATP/ADP/AMP ratios (reviewed in REF. 10). Although these pathways also have well-known non-chromatin substrates, their effects on ageing and longevity may be partly mediated through chromatin.

The insulin and insulin-like growth factor (IGF) signalling pathways are well-characterized nutrient-sensing pathways that regulate lifespan across evolution[1]. FOXO transcription factors are critical effectors of insulin signalling. In high nutrient conditions, FOXOs are phosphorylated and excluded from the nucleus; in low nutrient conditions, they relocalize to the nucleus[87]. Although FOXOs are not classical chromatin modifiers, FOXO1 can directly decrease chromatin compaction at the mouse IGF-binding protein 1 promoter[88]. Furthermore, *C. elegans* DAF-16, the worm FOXO transcription factor, directly recruits the SWI/SNF remodelling 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 dietary restriction-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/SNF subunit SNF2H to DNA break sites and prevent genomic instability through chromatin remodelling and local deacetylation of H3K56ac[69]. Whether, as a rule, sirtuins promote dietary restriction-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 dietary restriction regimens in worms[91] and in flies[92]. In addition, metformin, which is an AMPK activator, extends lifespan in mice[93], although metformin may have additional targets. AMPK can modify several chromatin regulators. In yeast, the AMPK orthologue Snf1 promotes histone acetylation by controlling the genomic occupancy and activity of the HAT Gcn5 (REF. 94) (TABLE 2). In mammals, AMPK can also inhibit histone deacetylation by phosphorylating HDAC4, HDAC5 or HDAC7 (REFS 95,96). Furthermore, AMPK has several other substrates that are directly or indirectly implicated in chromatin regulation and genomic stability, including histone H2B itself[97], SIRT1 (REF. 98) and *O*-GlcNAc transferase[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 glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or hexokinase can, when mutated, extend replicative lifespan in yeast[100,101]. These enzymes can also modulate transcription in the nucleus in a metabolism-dependent manner (reviewed in REF. 10). For instance, GAPDH directly promotes the transcription of H2B genes during the S phase of the cell cycle[102]. The nuclear functions of these enzymes may be important for integrating 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 affect 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 and KDM7 Lys-specific histone demethylases[104]. Thus, perturbation of the Krebs cycle during ageing may induce stochastic or aberrant chromatin remodelling. Interestingly, supplementation with Krebs cycle metabolites such as oxaloacetate, malate, fumarate and α-ketoglutarate can extend the worm lifespan through the genetic pathways that mediate dietary restriction-induced longevity[105–107]. Thus, there are strong molecular associations between dietary restriction, metabolism, longevity and the regulation of chromatin states. Future studies should establish whether these links are fortuitous, or whether dietary restriction requires chromatin remodelling in order to have a lasting effect on longevity.

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) signalling pathways
Important conserved signalling pathways that are sensitive to nutrient levels and that have been linked to longevity and stress resistance across Metazoa. Primary components that are relevant to ageing include the insulin and IGF1 receptors, the activation of which triggers a phosphorylation cascade involving PI3K, AKT and ultimately the forkhead box protein O (FOXO) transcription factors.

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 of age-related diseases such as cancer.

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The circadian clock and ageing. The circadian clock controls many physiological and behavioural systems and is highly linked to energy metabolism[108] and chromatin structure[83]. Disrupting circadian rhythms negatively influences health and longevity across species (reviewed in REF. 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 24 hours 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 programme, which requires the rhythmic recruitment of protein complexes to chromatin[111]. Circadian locomoter output cycles protein kaput (CLOCK), which is a primary molecular component of the circadian clock, has H3K9 and H3K14 HAT activity[112,113]. The circadian epigenome can be influenced by metabolic stimuli, and several energy sensors that mediate dietary restriction-induced longevity also modulate the circadian machinery and the chromatin state of circadian promoters. For instance, SIRT1 deacetylates the circadian clock components period circadian protein homologue 2 (PER2) and brain and muscle ARNT-like 1 (also known as ARNTL)[113,114]. Activation of SIRT1 decreases H3 acetylation, and thus repression of circadian genes, in a time-specific manner[115]. Other energy sensors that modulate the clock and that 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.

Effects 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, although 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, *C. elegans* hermaphrodites are short-lived, with signs of premature ageing, and the expression of *utx-1* is highly upregulated[82]. Interestingly, knockdown 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 remodelling. As a portion of the premature demise induced by the presence of males is dependent on the sensing of male pheromones by hermaphrodites[82], pheromones may influence chromatin states to regulate premature ageing.

Pheromones are not only involved in sexual sensing but also in sensing animal density (termed 'crowding') in *C. elegans*[125]. Notably, exposure to pheromones involved in crowding (ascaroside 2 or ascaroside 3) is sufficient to extend lifespan in *C. elegans*[126]. This pheromone-induced longevity requires the HDAC gene *sir-2.1* but is independent of insulin signalling[126]. Thus, chromatin modifiers may mediate the influence of pheromones, although whether pheromones directly affect chromatin marks or the overall chromatin structure is unknown. Although ascarosides are nematode-specific molecules, their chemical structures resemble those of metabolites, and they are part of a class of molecules termed 'secondary metabolites'. As secondary metabolites are evolutionarily conserved, the potential links between pheromonal signalling, chromatin and ageing deserve further exploration in other species.

Systemic regulation of ageing: an epigenetic connection? Sex steroid hormones (for example, oestrogens and androgens) are systemic factors that decline with age in humans. Indeed, the end of ovarian lifespan (menopause in humans) ends the endocrine activity of the ovary and shuts down the bulk synthesis of female sex steroid hormones. Oestrogens act mainly through nuclear receptors (for example, the oestrogen receptor), which can affect transcription by directly modulating chromatin states[127] and higher-order chromatin structure[128]. Global histone acetylation levels decrease with age in rat brains, and oestradiol promotes histone acetylation in *ex vivo* brain slices from young, but not old, rats[129]. Oestrogens are thought to limit the prevalence or age-of-onset of osteoporosis, sarcopenia, cardiovascular diseases, immune decline and neurodegeneration[130]. In reproductively senescent women, an age-related decrease in oestrogens may induce ageing phenotypes, partly owing to decreased activity of oestrogen receptors on chromatin. More studies are needed to understand the mechanistic effect of sex steroid hormones on human ageing and the chromatin changes that are induced by the age-induced reduction in oestrogen synthesis.

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

Epigenetics, genomic stability and age

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 they may also reduce transcriptional precision. Epigenomic states can themselves be unstable; indeed, the number of epimutations[131] appears to stochastically

Pheromones
Secreted chemical factors that can trigger a response in other members of a given species. Pheromone signalling has been linked to sexual behaviour, feeding and stress.

Epimutations
Aberrant or atypical changes in epigenetic states, which are often stochastic.

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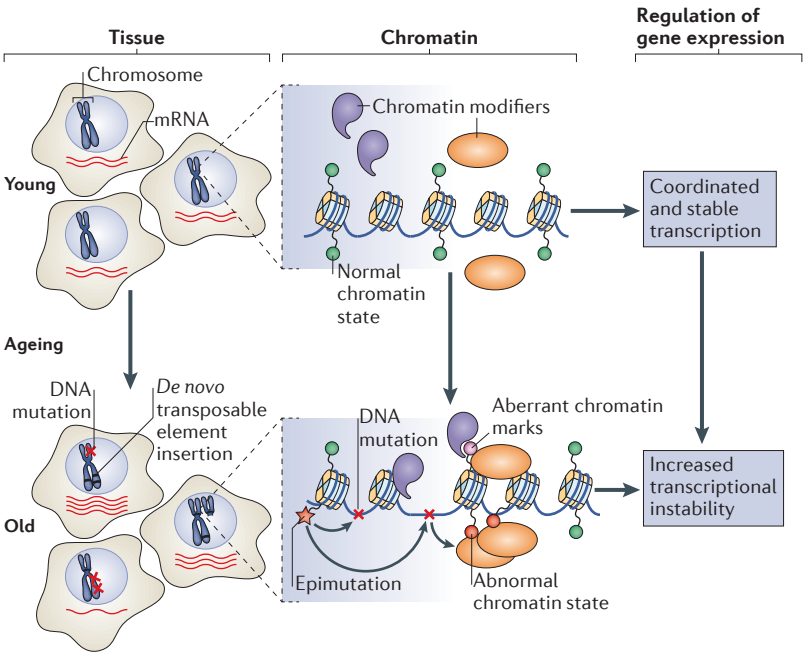

Figure 2 | **A model for the possible crosstalk between chromatin changes and transcriptional and genomic instability during ageing.** A possible model is the following: in cells from young organisms (top left), transcriptional programmes are robustly defined and precise between cells (depicted by consistent mRNA levels). 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 among cells of a tissue (depicted by variable mRNA levels among cells). Genomic instability is also a hallmark of ageing and is increased both at a macro level (for example, aneuploidies, depicted by partial chromosome duplication, or increased transposable element insertions) and more locally by DNA mutations in the form of single-nucleotide mutations or small insertions or deletions. DNA damage can trigger the recruitment of chromatin modifiers and the acquisition of abnormal chromatin states. Thus, genomic instability could modify the epigenetic landscapes 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 that are acquired during ageing could also decrease the transcriptional precision of neighbouring genes.

increase throughout life[132]. Thus, accumulated epimutations during ageing may induce further genomic instability (FIG. 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]. Although the extent and importance of mutations during ageing is debated, persistent DNA-damage signalling during ageing may drive local changes in chromatin structure and epigenetic modifications[132,134]. DNA-damage signalling may also promote specific chromatin alterations, such as recruitment of chromatin modifiers including Polycomb repressive complex 1, SIRT1, SIRT6, and DNA methyltransferases (reviewed in REF. 134). The resulting aberrant chromatin environment may in turn further increase the susceptibility of DNA to damage[134]. Consistent with the idea that DNA damage can globally affect chromatin structure, deficiency in Werner syndrome ATP-dependent 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 REF. 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 that are linked to the regulation of longevity, such as NAD or α-ketoglutarate, modulate the activity of proteins that are involved in both DNA repair and chromatin remodelling[137]. For example, the recruitment of NAD-dependent deacetylases SIRT1 and SIRT6 may directly promote genomic stability[69,70,73,74]. By triggering chromatin remodelling, 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 is the activation of endogenous mobile genetic elements (transposable elements). In eukaryotes, 30–80% of the genome is composed of transposable elements[138], which are transcribed and can regulate the expression of nearby genes. Active transposable elements induce extensive genomic instability[139], and they are normally kept in check, especially in the germ line, by heterochromatin marks such as H3K9me3 (REF. 140). Transposition can increase during ageing in several species[72,139,141–144], and activation of transposable elements correlates with neurodegenerative disorders in humans[145]. Conversely, dietary restriction may counteract the age-linked derepression of transposable elements in the livers and the skeletal muscle of aged mice[144]. Reactivation of transposable elements during ageing may result at least in part from heterochromatin loss. It may also be caused by loss of SIRT6 specifically at transposable elements, as SIRT6 normally promotes heterochromatin formation and transcriptional repression in fibroblasts, heart, liver and brain of young mice[72]. Consistent with the idea that activation of transposable elements may negatively affect organismal lifespan, flies that are deficient in Argonaute 2 (Ago2) have exacerbated transposition and a shortened lifespan[143]. This result suggests that activation of transposable elements may cause certain aspects of ageing, although the shortening of lifespan could also result from defects in small RNA pathways that are due to Ago2 deficiency. Finally, in senescent human fibroblasts, chromatin accessibility increases at retrotransposon insertion sites, leading to increased transcription and transposition of transposable elements[146]. Together, these observations suggest that increased transposition during ageing is partially associated with aberrant chromatin remodelling and may promote ageing phenotypes.

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 function through an RNA intermediate, such as long interspersed nuclear elements or long tandem repeats (copy–paste mechanism). Type II elements function through a DNA intermediate (cut–paste mechanism). They usually occupy a large portion of eukaryotic genomes.

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Box 3 | **Epigenetics of the 'mortal soma' and the 'immortal germ line'**

An emerging question in the field of epigenetics in ageing is whether different epigenetic mechanisms act in somatic cells and in germ cells during ageing. Regulatory mechanisms that maintain germ cells may be more robust[187], and several studies (reviewed in REF. 188) have explored the hypothesis that aged somatic cells could 'rejuvenate' through reprogramming, which is the process by which differentiated cells are converted back to a germ cell or embryonic stem cell (ES cell)-like state through the re-expression of pluripotency regulators[189]. Indeed, both senescent and aged fibroblasts undergo some aspects of chromatin rejuvenation upon reprogramming, including restoration of DNA methylation patterns at the loci of the pluripotency genes octamer-binding protein 4 (*OCT4*) and *NANOG*, and the disappearance of senescence-associated heterochromatin foci[188]. Aged haematopoietic stem cells (HSCs), which normally show persistent age-dependent changes in gene expression, produce induced pluripotent stem cells (iPSCs) with similar transcriptional profiles to those of both normal ES cells and iPSC 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 in embryos, is similar to that of lines derived from young cells[190], which is consistent with a stable resetting of epigenetic states rather than a masking of defects in the pluripotent state. However, the possibility cannot be excluded that the seemingly rejuvenated properties of iPSCs generated from aged donor cells result from the selection of cells with more 'youthful' chromatin states during reprogramming rather than from 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 cells show global hypermethylation at repetitive elements and site-specific CG dinucleotide 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 have disordered chromatin and show 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 showed that several genes encoding chromatin modifiers are downregulated with age, such as Polycomb genes Enhancer of zeste homologue 2 (*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. Determining 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 signs of ageing.

Epigenetics and transcriptional instability. Age-related epimutations could also affect 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 six genes that were assayed, 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 importance and effects of alterations to transcriptional noise during ageing.

Although transcriptional instability may be a by-product 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 gene encoding Rph1 promotes 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 whether 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 germ line 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 aggregation, 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, although 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 *Schizosaccharomyces 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 germ line is normally 'immortal' (BOX 3). However, there are examples of progressive sterility over generations, in species from worms to mammals, a phenomenon called 'germline mortality'. Understanding progressive germline mortality could provide 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 REF. 158). Mutations in *spr-5*, which encodes one of the worm orthologues of the H3K4me2 demethylase LSD1 (also known as KDM1A), increase H3K4me2

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levels in whole worms and in primordial germ cells and induce sterility by generation 20 (REFS 159,160). A predicted null mutation of the *rbr-2* H3K4 demethylase gene, or simultaneous loss of *spr-5* and *rbr-2,* results in progressive sterility at higher temperatures[57], as does loss of function of the gene encoding the H3K4me2–H3K4me3 methyltransferase SET domain-containing 2 (*set-2*)[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 in 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 that are 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 that is extended for up to four generations[166]. This extension can be reverted by transient inactivation of the *rbr-2* demethylase[166]. The mechanisms of this lifespan extension are unclear, as global levels of H3K4me3 are unchanged in worms that are 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 in 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 among genes that are involved in metabolic pathways[166], suggesting that the loss of H3K4 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 descended from grandparents that were starved have increased organismal lifespan up to the third generation, although the extent of lifespan extension varies from 22% to 70% among replicates[167]. Transgenerational inheritance of metabolic states has also been observed in mammals (reviewed in REF. 154); 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].

Stress resistance is a phenotype associated with longevity. In *C. elegans*, exposure of the parental generation to high glucose concentrations 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 WDR-5 and SET-2 (which are components of the COMPASS H3K4 methyltransferase complex), 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 remain derepressed in the absence of the stressor over several 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 remodelled during ageing, and the experimental perturbation of chromatin modifiers can directly affect lifespan. Thus, a compelling hypothesis is that the epigenomic changes that are observed 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 various scales, from cells to organs. These events can have systemic effects on ageing at the organismal level. Although the sequence of events leading to genomic and transcriptional instability during ageing is unclear, genome surveillance and epigenetic remodelling demonstrably influence each other. Thus, we propose that environment-induced epigenomic instability throughout life is an important 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 chromatin states during ageing, notably upon environmental changes or cellular reprogramming, suggests that chromatin-regulating enzymes may be important therapeutic targets in the pursuit of healthy ageing. Various drugs that target chromatin modifiers have been identified[171,172], and several of these increase the lifespan of model organisms (TABLE 2). Improving the specificity of such drugs and testing their efficacy in different cellular contexts could help to treat age-related diseases, as has been proposed for Alzheimer disease[173]. An important goal for drug design will be the specificity of delivery, given the differential effects of the drugs in different 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

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is still in its infancy. This is partly owing to the technical challenge of definitively studying how alterations in chromatin states may cause 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 determine how to direct the activity of an enzyme to a particular histone residue and how to target the inhibition or activation of a mark at a subset of the loci of interest rather than genome-wide. Finally, it will be important to develop techniques to further probe the chromatin landscape at the single-cell and single-molecule levels[174,175]. This will help to distinguish between three biological scenarios: the consistent chromatin remodelling that occurs in all ageing cells within a population; the emergence or disappearance of specific subpopulations during ageing; and the changes in allele-specific epigenetic regulation during ageing in humans or in outbred populations.

Reference epigenomes have transformed our understanding of transcription and have 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 to develop better strategies for delaying or reversing aspects of ageing and age-related diseases.

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Competing interests statement
The authors declare no competing interests.

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