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Histone methylation and aging: Lessons learned from model systems

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Abstract

Aging induces myriad cellular and, ultimately, physiological changes that cause a decline in an organism's functional capabilities. Although the aging process and pathways that regulate it have been extensively studied, only in the last decade have we begun to appreciate that dynamic histone methylation may contribute to this process. In this review, we discuss recent work implicating histone methylation in aging. Loss of certain histone methyltransferases and demethylases changes lifespan in invertebrates, and alterations in histone methylation in aged organisms regulate lifespan and aging phenotypes, including oxidative stress-induced hormesis in yeast, insulin signaling in *Caenorhabditis elegans* and mammals, and the senescence-associated secretory phenotype in mammals. In all cases where histone methylation has been shown to impact aging and aging phenotypes, it does so by regulating transcription, suggesting that this is a major mechanism of its action in this context. Histone methylation additionally regulates or is regulated by other cellular pathways that contribute to or combat aging. Given the numerous processes that regulate aging and histone methylation, and are in turn regulated by them, the role of histone methylation in aging is almost certainly underappreciated.

1. The aging process

1.1. Physiological changes associated with aging

Aging is associated with a number of detrimental physiological effects that impact the health and overall function of an organism. Among humans and other mammals, these include a decline in immune function, increasing susceptibility to diseases, chronic inflammation, reduction of muscle mass (sarcopenia), increased incidence of cancer, and the onset of age-related degenerative disorders such as Alzheimer's and Huntington's diseases [1]. Although these phenotypes are manifest at a systemic or organismal level, they are ultimately caused by changes in cellular functions, and, indeed, molecular pathways that contribute to, or help

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slow, aging have been identified. Many processes, including autophagy, mitochondrial (oxidative phosphorylation) efficiency, and proteasome function, decline with age, while incidence of DNA damage increases; these have been implicated in various aging phenotypes [2–5]. A decrease in mitochondrial efficiency causes increased production of reactive oxygen species (ROS), which can damage macromolecules, including DNA, and also function as second messengers, thus ectopically activating signaling; both processes are thought to contribute to aging pathologies [6–8]. In addition to a potential increase in damaged molecules in aged cells due to accumulation of ROS, there is also lowered turnover of damaged or insoluble proteins by the proteasome and proteins and other macromolecules by autophagy [5,9]. The accumulation of protein aggregates that results from decreases in proteasome function and autophagy contribute to the pathophysiology of Alzheimer's and Huntington's diseases [9,10]. Increased ROS and damaged macromolecules are significant sources of cellular stress, and it is well known that activating stress response pathways can promote longevity and slow the progression of aging [11].

Improperly repaired DNA damage causes mutations, and the accumulation of mutations within one cell's genome can eventually lead to cancer; old cells are, of course, subject to more cumulative DNA damage and mutations than young ones. Cancer, during which cells undergo dysregulated cell divisions and disrupt the organism's physiology, is sufficiently detrimental that a process termed cellular senescence is thought to have evolved to counter it [12]. During cellular senescence, tumor suppressor genes are activated to irreversibly halt progression of the cell cycle [13]. As an organism ages, the number of senescent cells increases. Indeed, a general decline in stem cell function has been reported with age, which is thought to contribute to tissue degeneration [14–16]. This decline in function results from both reduced numbers of stem cells in older animals, possibly as the result of cellular senescence, and a reduction in their multilineage differentiation capacity [17–22]. The complex age-associated phenotypes are thus the result of alterations to cellular processes that occur during and/or as a result of the aging process.

1.2. Model organisms and the study of longevity

Some physiological and many cellular aspects of aging are conserved among eukaryotes, and, indeed, much insight into the molecular mechanisms of aging has come from work done in various eukaryotic models, such as the budding yeast *Saccharomyces cerevisiae*, the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the house mouse *Mus musculus*, as well as mammalian tissue culture and progeria disorders, which cause premature aging, in humans. Like mammals, yeast are subject to the phenomenon of replicative senescence, in which individual cells are only able to undergo a limited number of mitoses [23]. Similarly, age-induced sarcopenia occurs in *C. elegans* and *Drosophila* [24,25], which may indicate that stem cell exhaustion contributes to aging in *Drosophila* as well. Although other physiological aspects of aging, including decreased immune function, chronic inflammation, and increased incidence of cancer, have not been shown to occur in invertebrate models, the molecular pathways and dysfunctions associated with aging are remarkably conserved. The age-associated decline in autophagy was first observed in yeast and only later found in *C. elegans*, *Drosophila*, and mice [2]. Activation of stress response pathways increases lifespan in yeast, *C. elegans*, and *Drosophila*, as well as

delays cellular senescence in mammalian cell culture [26]. Similarly, restricting caloric intake (caloric or dietary restriction), increases lifespan from yeast to humans, was first described in rodents, and has been extensively studied in *C. elegans* [27]. In *C. elegans*, repression of the insulin and insulin-like signaling (IIS) pathway was shown to promote longevity [28], and correlative studies have subsequently suggested that this mechanism may be conserved in mice and humans [29]. Thus, model organisms, particularly invertebrate models, have been the source of much of our knowledge regarding the molecular pathways that drive aging physiology.

1.3. Chromatin and aging

Another more recently discovered molecular phenotype associated with aging is a dysregulation of transcription, as manifested by changes in gene expression. This phenomenon has been observed in many organisms, though it is not clear whether this dysregulation causes pathophysiologies associated with aging or is itself the result of the aging process [30–35]. Along with alterations in the transcriptome, in many organisms, chromatin structure changes with age [36], and, indeed, some of these changes have been shown to cause age-associated phenotypes in yeast [37]. In general, chromatin is thought to take on a more euchromatic and transcriptionally active (“open”) conformation as an organism ages [38]. In particular, an increase in histone acetylation, mediated by decreased Sirtuin levels, contributes to this phenotype in some organisms [37,39,40], though global decreases in histone levels have also been observed [37,41,42]. Another indication that chromatin state impacts the aging process comes from the field of induced pluripotent stem cells (iPS cells). During the induction of pluripotency, terminally differentiated cells from adults are made to take on characteristics of embryonic stem cells [43]; in essence, to become younger. This reversion to a younger state is accompanied by, and depends on, altered chromatin structure [44]. Thus, changes to chromatin structure are likely to contribute to aging phenotypes.

In this review, we discuss the role of histone methylation in the aging process across eukaryotes. Although increased histone acetylation has been shown to cause age-associated phenotypes [37,39,40], to date, there are only a few studies showing a causal relationship between altered histone methylation and longevity or age-associated pathologies (with the notable exception of cellular senescence in mammals). Additional work has revealed changes in global methylation or methylation patterns in old versus young organisms, which suggests the possibility that misregulation of histone methylation may cause aging pathologies. We also discuss the roles of histone methylation in cellular pathways known to cause age-related phenotypes. Taken together, these data suggest many mechanisms by which changes in histone methylation may contribute to aging pathologies.

2. Global changes in histone methylation patterns in aged organisms and progeria models

Several studies in mammalian systems and *Drosophila* have identified age-associated changes in histone methylation states. These findings are summarized in Table 1. In human cells cultured from patients with Hutchinson-Gilford progeria syndrome (HGPS), a genetic

condition that causes premature aging, there is an upregulation of trimethylated lysine 20 on histone H4 (H4K20me3), and a downregulation of both trimethylated lysine 9 on histone H3 (H3K9me3) and trimethylated lysine 27 on histone H3 (H3K27me3). Along with the decrease in H3K9me3, reduced association of the heterochromatin compaction protein HP1a with pericentrosomal DNA was observed [45]. The loss of H3K27me3 in HGPS was recently shown to be largely from gene poor regions [46], which is consistent with the loss of peripheral heterochromatin seen in cells from HGPS patients and invertebrate models of this disorder [47,48]. Thus, accelerated aging in HGPS is associated with a reduction in heterochromatin, though it should be noted that H4K20me3, which increases in cells derived from HGPS patients, also marks heterochromatin. Interestingly, HP1-associated heterochromatin has been shown to be required to slow aging phenotypes in *Drosophila* [49]. While H3K9me3 levels are increased in aging flies [50], a reduction in dimethylated H3K9 (H3K9me2), to which HP1 can also bind [51,52], is seen [49]. This reduction in H3K9me2 is correlated with a loss of HP1 from chromatin, and, indeed, reducing HP1 levels causes loss of muscle integrity and derepression of age-associated recombination of rDNA genes in *Drosophila* [49]. However, it should be noted that the increase in H3K9me3 levels were observed in the heads of female flies, the reduction in H3K9me2 is seen in the whole bodies of mixed male and female flies [49,50]; thus, it is possible that these differing results are due to cell type- or gender-specific alterations in methylation. Taken together, these data support the model of increasingly open chromatin with age.

Histone methylation has also been assessed in brain tissue from SAMP8 mice, a strain of mice bred under selection for accelerated aging that are subject to premature neurodegeneration [53,54]. In this system, both H3K27me3 and mono- and dimethylated lysine 79 on histone H3 (H3K79me1/2) increase, while monomethylated H4K20 (H4K20me1) and trimethylated lysine 36 of histone H3 (H3K36me3) decrease [55]. Thus, in SAMP8 mice, there is an increase in histone modifications associated with heterochromatin and a decrease in marks indicative of active transcription, which is different from the loss of heterochromatin associated with HGPS. The differences between HGPS and SAMP8 could be caused by 1) different mechanisms by which the modifications assessed accelerate aging, 2) different changes in histone methylation in different tissues, or 3) biological differences in the aging process in humans versus mice. Additionally, as observed in HGPS, an increase in H4K20me3 was observed in livers and kidneys in aged rats [56]. In *Drosophila*, decreases in H3K36me3 and trimethylated H3K4 (H3K4me3) were seen during aging [50]. Interestingly, an analysis of dimethylated lysine 4 on histone H3 (H3K4me2), which is associated with transcriptional activation, in macaques found that this modification increases at enhancer regions and transcription start sites of genes involved in stress responses during aging [57]; activation of stress response pathways is thought to promote longevity [11].

Although the different histone methylation events studied and methodologies used hinder comparison, several trends emerge about histone methylation and aging. First, it is clear that there are age-associated changes in histone methylation, which raises the possibility that alteration of histone methylation contributes to aging pathologies. Second, despite sometimes conflicting data from different model systems that clearly need to be resolved, there is a trend of increases in “activating” histone marks (H3K4me2/3, H3K36me3) and

decreases in “repressive” marks (H3K27me3, H3K9me2/3) indicative of a more actively transcribed, euchromatic genome. This is consistent with previous observations of an open chromatin conformation in aging cells and organisms [38]. Intriguingly, given the observation in macaques [57], it is possible that one role of the open chromatin in aged organisms is to promote the transcription of genes that have anti-aging functions, even though such an open chromatin structure has been associated with detrimental increases in gene expression [37,39,40].

3. Direct evidence that histone methylation regulates aging

3.1. Identification of histone methyltransferases and demethylases that impact lifespan

Although the association studies discussed above clearly demonstrate a misregulation of histone methylation during aging, in many cases it remains uncertain whether these changes are a cause or an effect of the aging process. Recent studies in model organisms suggest that histone methylation plays causative roles in aging; these are summarized in Table 2. The first evidence that altered histone methylation drives the aging process came from RNAi screens performed in *C. elegans*. These identified putative histone methyltransferases and demethylases that regulate worm lifespan, including the methyltransferases ASH-2, SET-9, SET-15, and SET-26, as well as the demethylases MES-2, JMJD-2, LSD-1, and UTX-1 [42,58–60]. LSD-1 had previously been implicated as the major effector of LiCl-induced lifespan extension in worms [61]. Although these nine enzymes, when knocked down or mutated, regulate organismal lifespan in *C. elegans*, only UTX-1 levels have been shown to change with age [62]; levels of the other enzymes have not been assessed. The functions of ASH-2 and UTX-1 in regulating lifespan in *C. elegans* have been well characterized and are discussed in detail below. Of the remaining, only SET-9 and SET-26, a closely-related paralog pair, have been further studied. Extension of lifespan by loss of either SET-9 or SET-26 is partially reduced in a *daf-16* mutant background, indicating that both proteins act upstream of the FOXO pathway, though not exclusively [42]. Although no changes in histone methylation were observed in *set-26* RNAi animals, there is a slight reduction in the age-dependent decrease in total histone H3 levels [42]. It should be noted that whether SET-6, SET-15, MES-2, JMJD-2, and LSD-1 affect the methylation state of histones, as opposed to other proteins, and whether their catalytic activity is required for their effects on aging, has not been determined. While the mechanisms by which all the identified histone methyltransferases and demethylases regulate lifespan are unknown, significant progress has been made in understanding the functions of the canonical activating H3K4me3 and repressing H3K27me3 modifications during aging.

3.2. High levels of H3K4 trimethylation reduce lifespan in *C. elegans*

In eukaryotes, trimethylation of H3K4, a classic mark of transcriptionally active chromatin, is catalyzed by the Trithorax complex. In *C. elegans*, loss of any of three Trithorax proteins (WDR-5 and the methyltransferases SET-2 and ASH-2) decreases global levels of H3K4me3 and increases lifespan [58]. Similarly, loss of RBR-2, which demethylates H3K4me3, decreases lifespan and increases H3K4me3 levels in *C. elegans*. Significantly, loss of ASH-2 in *rbr-2* mutant animals restores normal levels of H3K4me3 and rescues the observed decrease in lifespan, suggesting that high levels of H3K4me3 are detrimental to

lifespan in worms [58]. Although it is unknown how increased H3K4me3 decreases longevity, production of mature eggs during adult life is required for loss of ASH-2 to increase lifespan, which led to the idea that ASH-2 may regulate endocrine signaling from germ cells to soma [58]. Further work showed that the lifespan increase caused by loss of ASH-2, SET-2, or WDR-5 is inherited transgenerationally; that is, that wildtype F3 and F4 descendants of outcrossed *ash-2*, *set-2*, or *wdr-5* mutants retained increased longevity, though this phenotype was lost one generation later [63]. This increase in lifespan correlates with global differences in transcriptional profiles, though not with global alterations in H3K4me3 levels; indeed, many of the differentially-expressed genes are linked with longevity [63]. However, the H3K4 methylation status of genes with altered expression levels in mutants and their wildtype descendants versus wildtype worms was not specifically analyzed, and, therefore, many of these effects may be indirect. Nevertheless, this study demonstrates that alterations in histone methylation can affect lifespan transgenerationally, though the mechanism by which this occurs remains unclear.

3.3. Lifespan regulation by the repressive H3K27me3 modification

Aging is also regulated at the level of transcription by trimethylation of H3K27. The H3K27me3 demethylase UTX-1 plays a central role in aging in *C. elegans* through its regulation of the insulin and insulin-like signaling (IIS) pathway. An increase in *utx-1* expression in aging worms correlates with a global reduction of H3K27me3 levels and is required for a loss of this mark at the *daf-2* locus, which encodes the insulin receptor, causing an age-dependent increase in *daf-2* expression [60,62]. Consistent with this, *utx-1* knockdown results in a 30% increase in lifespan, and loss of DAF-16 during *utx-1* RNAi abrogates the increase in lifespan this treatment incurs. As DAF-16 is required for the increase in lifespan upon loss of DAF-2, this suggests that the primary function of UTX-1 in the context of aging is in regulation of IIS [62]. Intriguingly, knockdown of the UTX-1 ortholog KDM6A in human cells also results in decreased expression of an insulin receptor, *IGF1R*, and this locus has a decrease in H3K27me3 in aged tissues from both humans and macaques. Thus, it is possible that an increase in IIS mediated by the activity of the H3K27me3 demethylase KDM6A at the insulin receptor locus is a conserved mechanism that promotes aging in animals [62].

Changes in H3K27me3 levels have also been shown to affect lifespan in *Drosophila*. Mutation of the Polycomb repressive complex 2 (PRC2) components *E(z)* and *Ese* both decreases total H3K27me3 and extends lifespan [64]. Furthermore, hypomorphic alleles of *trx*, which encodes a H3K4 methyltransferase in the Trithorax complex, introduced into *E(z)* and *ese* heterozygotes partially reduced lifespan while increasing levels of H3K27me3 [64]. This later phenotype is thought to be due to decreased recruitment of the H3K27 acetyltransferase CBP, as methylation and acetylation on this residue are mutually exclusive [65]. However, Siebold et al. [64] noted that a previous ChIP screen had revealed that *E(z)* is associated with the *Odc1* gene, which encodes an ornithine decarboxylase [66], and thus suggest that PRC2-mediated deposition of H3K27me3 functions to repress expression of genes that promote stress resistance. Indeed, they found that heterozygotes for both *E(z)* and *esc* had increased resistance to oxidative stress than did wildtype flies [64].

Additionally, changes in H3K27me3 have been linked to loss of function in mammalian mesenchymal stem cells and muscle satellite cells. In human adipose-derived mesenchymal stem cells, promoters of genes that drive adipogenesis and myogenesis are marked by H3K4me3 and H3K27me3, characteristic of bivalent domains, in both early and late passage cells (young and old, respectively). In young cells, induction of adipocyte differentiation causes a loss of H3K27me3 at promoters of genes involved in this process, while H3K27me3 remains associated with these genes in old cells. The retention of H3K27me3 at these loci is correlated with transcriptional downregulation, and thus inhibition of the adipogenic differentiation program in old cells. This may be caused by an increase in EZH2, which deposits this mark, and increased association of both EZH2 and BMI1 (a PRC1 component) at these loci [67]. In contrast, histone methylation is altered prior to induction of differentiation in muscle satellite (stem) cells in old versus young mice. In old mice, an increase in transcription start site-associated H3K27me3 is observed, though a significant portion (30%) of genes that gain this mark are not expressed in muscle satellite cells or their differentiated progeny. H3K27me3 also accumulates in gene poor regions, suggesting a general accumulation heterochromatin and transcriptional silencing with age [18]. Thus, in both muscle and mesenchymal stem cells, aging is associated with increased levels of H3K27me3 and transcriptional repression.

4. Histone methylation is involved in pathways that regulate aging and lifespan

Although only a few studies show that altering histone methylation regulates lifespan, there is accumulating evidence that histone methylation impinges upon pathways known to affect aging in eukaryotes. In this section, we discuss how histone methylation regulates, and is regulated by, these processes; a summary of the literature is provided in Table 3. Most of the work in this area has focused on how histone methylation impinges upon the cognate process, rather than the effects altered histone methylation have on lifespan. However, two recent studies have identified stress-related changes in histone methylation that affect lifespan and aging phenotypes. Thus, it is possible, and indeed likely, that understanding the cross-regulation between stress response pathways and histone methylation will provide insight into the aging process.

4.1. Autophagy

Autophagy is the process by which damaged molecules, macromolecules, and cellular components are degraded into basal metabolites that are reused by the cell to make new ones. A double membrane is formed in the cytoplasm and cellular components are engulfed in this structure, either stochastically or in a directed manner. The autophagosome then fuses with a lysosome, wherein its components are digested. This process is tightly regulated; in particular, the initial fusion of the membrane to form an autophagosome requires the action of the proteins LCB3, DOR, and WIPI1 [68]. During aging, the efficiency of autophagy is decreased, promoting the accumulation of damaged macromolecules and organelles, which contributes to various aging phenotypes. The decrease in autophagy has been linked to an increase in the proportion of damaged mitochondria, and thus to increased ROS, as well as

the accumulation of insoluble protein plaques in Alzheimer's and Huntington's diseases. Indeed, abrogating the age-associated decline in autophagy increases longevity [9].

Expression of *LCB3*, *DOR*, and *WIP1* are regulated at the level of histone methylation in human cell lines [69]. The histone methyltransferase G9a, which mono- and dimethylates lysine 9 on histone H3 (H3K9me1/2), associates with the promoters of these genes and represses their expression. Significantly, induction of autophagy by starvation causes G9a to dissociate from the promoters of these genes, concomitant with a reduction in H3K9 methylation and increased gene expression. Inhibition or knockdown of G9a in cultured cells also promotes formation of autophagosomes, which further suggests that regulation of *LCB3*, *DOR*, and *WIP1*, among others, at the level of transcription by histone methylation is a key mechanism by which autophagy is controlled [69]. It is therefore possible that age-associated changes in histone methylation patterns may regulate cellular pathways that contribute to aging by altering the transcription of genes whose products are necessary parts of the pathway. Interestingly, in human HGPS cells and aged *Drosophila*, H3K9 methylation is reduced [45,49]. Thus, it is possible that particular loci become hypermethylated at H3K9 during aging, despite a global decrease in H3K9 methylation.

4.2. DNA damage response

As organisms age, their cells accumulate DNA damage, both in the form of mutations from improperly repaired DNA and from irreparable lesions, particularly double stranded DNA breaks (DSBs), that continuously elicit the DNA damage response (DDR). Indeed, γ H2A.X foci, which mark DSBs, are more prevalent in old cells and organisms than they are in young [70]. The DNA damage could be caused by an age-dependent decrease in a cell's ability to effect repairs; likewise, continued activation of the DDR pathway may contribute to aging pathologies [71]. In non-homologous end joining (NHEJ), DSBs induce the local phosphorylation of H2A.X, causing formation of γ H2A.X. γ H2A.X recruits Mcd1, which, in turn, promotes the association of the MRN complex with DSBs. MRN serves as a platform for the assembly for ATM kinase and mediator proteins, including 53BP1, which helps activate the DDR pathway downstream of ATM [72,73]. ATM phosphorylates many substrates, including Chk1 and Chk2, which mediate cell cycle arrest and activate damage repair proteins or induce apoptosis, respectively [74]. In alternate NHEJ, PARP1 is recruited to DSBs, where it catalyzes the formation of poly-ADP ribose chains, which recruit various enzymes required for DNA repair [75]. Histone methylation is both required for the recruitment and activation of DDR components and is altered in response to DNA damage (discussed below), suggesting that histone methylation may both regulate efficacy of the DDR and, potentially, mediate its effects on aging.

H4K20me2 or H3K79me2 is required for the recruitment of 53BP1 to DNA lesions in human cells and yeast [76–79]. In cultured cells, the methyltransferase MMSET is recruited to DSBs by MDC1 where it causes a local increase in H4K20me2, thus permitting binding of 53BP1 [80]. However, during the growth phases of the cell cycle, H4K20me1/2 levels are low in mammals [81], and H3K79me2 mediates recruitment of 53BP1 to DSBs, though how prevalent this mechanism is varies between cell types [79]. Indeed, H3K79 methylation is not required for 53BP1 recruitment using a chicken system [82], suggesting divergence in

the role of histone methylation in DNA damage responses among eukaryotes. In budding yeast, in which H4K20me1 was only recently confirmed to exist and regulates telomere silencing [83], recruitment of Rad9, the 53BP1 ortholog, to DSBs occurs through interaction with H3K79me2 [78]. In mammalian cells and yeast, the local increase in H3K79me2 is mediated by the methyltransferase DOT1L (Dot1 in yeast); this enzyme is required for repair of DSBs [77,78]. Thus, methylation of H4K20 and H3K79 are critical for the initiation of the DNA damage response, and any changes in the activities of enzymes regulating these marks may contribute to reduced DDR and thus accelerate the aging process.

H3K9me3, a classic constitutive heterochromatin mark, is also required early during DDR activation in cultured human and mouse cells. H3K9me3 levels increase at the site of DNA damage, though global levels remain unchanged [84,85]. H3K9me3 accessibility may also be regulated during the DDR, as in the context of normal cells, members of the HP1 family of proteins associate with this mark [51,52]. Upon DNA damage, HP1 β is phosphorylated, causing it to dissociate from H3K9me3, exposing H3K9me3 in the vicinity of DSBs [85]. The acetyltransferase Tip60 is then able to bind H3K9me3, which enhances its enzymatic activity [85-87]. When DNA damage occurs, Tip60 acetylates ATM kinase, thus further activating it to promote the DDR [85]. It has also recently been shown that a global reduction in H3K9me3 levels promotes the resolution of DSB repair, consistent with the idea that a more open chromatin structure enhances DNA accessibility to repair factors [88].

Interestingly, both H3K4me3 and H3K27me3 are associated with DSBs. In yeast, H3K4me3 is deposited by Set1, which is recruited to DSBs and is essential for NHEJ in the absence of the yeast MRN ortholog [89]. In cultured cells, H3K4me3 is required to recruit the tumor suppressor ING to DSBs [90], which mediates cell cycle arrest until repair is accomplished or senescence induced [91]. The methyltransferase EZH2, which trimethylates H3K27, is also recruited to DSBs, where it is thought to inhibit transcription in the vicinity of DNA damage [84]. Studies in cultured cells and *C. elegans* confirmed that knockdown or knockout of EZH2 sensitizes cells and worms to DNA damage [92]. It is further demonstrated that the EZH2 is recruited to DSBs independently of γ H2A.X by PARP1 and that it functions to repress transcription in the vicinity of DNA damage [92], consistent with the idea that transcription must be repressed in the presence of DNA lesions [93].

4.3. Environmental stress responses

Cells must respond to numerous exogenous and endogenous stresses, including ROS, heat, osmotic imbalances, unfolded proteins, and damaged organelles [94]. As discussed above, many of these stresses increase during aging, and stress response pathways tend to be activated in aged organisms. Consistent with this, activating stress response pathways promotes longevity [26]. Indeed, hormesis, in which low levels of exposure to a particular stressor extends lifespan and confers resistance to more extreme levels of stress [95], may very well utilize epigenetic changes as a form of cellular memory. Recent work in yeast has shown that altered histone methylation is critical for stress responses, during both acute stress responses and hormesis. Thus, regulation of stress response genes is a powerful mechanism by which histone methylation may impact aging.

Recent work in yeast has suggested that chromatin modifications regulate the dynamics of transcriptional changes in response to oxidative stress [96]. In a high throughput screen, H3K4me3 was found to correlate with repression of ribosomal protein and biogenesis genes under stress conditions. Deletion of *SET1*, which encodes the H3K4 methyltransferase, results in upregulation of ribosomal protein and biogenesis genes in cells exposed to oxidative stress, heat shock, or rapamycin treatment, further confirming this result [96]. In the case of the ribosome biogenesis genes only, deletion of members of the RPD3L histone deacetylase complex causes transcriptional upregulation under oxidative stress conditions. It is suggested that H3K4me3 recruits the RPD3L complex to ribosome biogenesis genes to silence their expression during stress responses, and, further, that a distinct mechanism must be responsible for repression of the ribosomal protein genes [96]. Changes in H3K4me3 levels (discussed in Section 2) or distribution (see Section 4.4.3) during aging may therefore affect lifespan by altering the stress response.

Also in yeast, the H3K36me3 demethylase Rph1 is a critical player in the environmental stress response [97]. Under normal conditions, Rph1 is associated with the promoters of stress response genes, where it functions to repress transcription. However, in response to DNA damage and oxidative stress, the kinase Rad53 (ortholog of mammalian CHK2) phosphorylates Rph1, causing it to dissociate from these promoters, thus increasing transcription of target genes, possibly by the stress response transcription factor Msn2/4 [97]. Approximately 26% of genes upregulated by loss of Rph1 under oxidative stress conditions have increased H3K36me3 levels at their promoters, which suggests that at least part of Rph1's repression of stress response genes is mediated by its demethylase activity. Interestingly, the Rph1 DNA binding site is substantially similar to that of Msn2/4, raising the possibility that this demethylase may also function to repress stress responses under normal conditions by excluding transcriptional activators from their promoters [97]. This mechanism (exclusion of other proteins) may be used by histone methyltransferase and demethylases in other contexts, and, indeed, could explain how SET-9 and SET-26 affect lifespan in *C. elegans* without altering histone methylation [42].

Rph1 has also been implicated in extending chronological lifespan in yeast during hormetic adaptation to low levels of oxidative stress. Transiently increasing mitochondrial ROS production has been shown to promote longevity in yeast [98], and the H3K36me3 demethylase Rph1 is required for this process [99]. As in acute oxidative stress, when yeast are subject to low levels of oxidative stress, Rph1 is phosphorylated by Rad53, causing its dissociation from DNA. However, during hormetic (low level) oxidative stress, Rad53 is activated in the absence of DNA damage by the Tel1 (ATM) kinase [99]. In this context, Rad53 activation causes dissociation of Rph1 from sub-telomeric chromosomal regions, concomitant with an increase in H3K36me3 at these loci. Unlike during acute stress response, H3K36me3 accumulation functions to repress transcription by enhancing the binding of silencing protein Sir3 to these loci. It is suggested that the Rph1 demethylase functions to transmit a signal from stressed mitochondria to the nucleus during oxidative hormesis [99]. Thus, altered histone methylation may be a common mechanism by which hormesis functions to regulate lifespan.

4.4. Cellular senescence in mammals

As mammals age, their cells undergo a process termed cellular senescence, which is characterized by irreversible cell cycle exit and the secretion of various pro-inflammatory cytokines (the senescence-associated secretory phenotype, SASP) [100,101]. Both aspects of senescence contribute to aging phenotypes, and both are thought to be anti-cancer adaptations. Although the SASP can affect most cells, a loss of proliferation (canonical cellular senescence) has a particularly pronounced effect on stem cell function. When stem cells become senescent, they are no longer able to divide, and thus tissue regeneration and homeostasis are lessened, which contributes to tissue degeneration and immune deficiencies [102]. Additionally, cytokines secreted by senescent cells contribute to chronic inflammation and, paradoxically, may even promote cancer [101]. Both cell cycle arrest and the SASP are regulated by histone methylation.

4.4.1. Transcriptional control of the cell cycle—Canonically, cell cycle arrest is mediated by transcription of the *INK4/ARF* locus, which encodes the p15^{INK4b}, ARF, and p16^{INK4a} tumor suppressor genes [13]. Expression of the *INK4/ARF* locus is increased in aged mammalian tissues and senescent cultured cells [13] and was recently shown to be upregulated in old mice [103]. In addition to regulation by histone acetylation [104,105], this locus is subject to Polycomb-mediated repression in young cells, which is relieved in old cells, as seen by a loss of H3K27me3 at the promoter [106–109]. The alteration of H3K27me3 levels is mediated by a decrease in expression of the histone methyltransferase EZH2 and increased JMJD3 demethylase expression [106–109].

The complexity of transcriptional control of cell cycle arrest during senescence is only beginning to be appreciated. Polycomb-mediated repression of *INK4/ARF* expression is regulated by both signaling pathways and histone ubiquitination. In aged mouse pancreas, there is a reduction in PTEN, which inhibits the ATK/PI3K pathway; this correlates with an increase in *EZH2* and *BM11* expression and a decrease in *p16^{INK4a}* expression. Likewise, in MEFs, loss of PTEN causes an increase in *EZH2* and H3K27me3 at the *INK4/ARF* locus [110]. In human cells, expression of *JMJD3*, which encodes an H3K27me3 demethylase, is activated by ERK signaling and the transcription factor AP1 [111]. Thus, repression of *INK4/ARF* is regulated by the opposing activities of ATK and ERK signaling. The displacement of PRC1 and 2 from the *INK4/ARF* locus, and thus transcriptional activation of this locus, is further regulated by ZRF1, which binds monoubiquitinated lysine 119 of histone H2A (H2AK119ub), in human and mouse cell lines [112]. Furthermore, despite the predominance of this locus in studies on cell cycle arrest, the loss of H3K4me3 at additional loci required for cell cycle progression, mediated by JARID1B and Rb, has been implicated in the loss of cell proliferation [113]. Additionally, though the mechanism is unknown, a decrease in H3K79me3 is seen during aging of human cell cultures, and loss of the H3K79 methyltransferase DOT1L can induce senescence in this system [114]. Histone methylation is thus a highly regulated and important regulator of cellular senescence in mammals.

4.4.2. Broad redistribution of H3K4me3 and H3K27me3 in senescent cells—Numerous studies have assessed the status of H3K4me3 and H3K27me3 at specific cell cycle-related loci in cultured mammalian cells. However, until recently, the genome-wide

distribution of these marks has not been examined during senescence. Shah et al. [115] discovered that the distribution of these marks is drastically altered in senescent vs. proliferating human cells. Particularly, broad regions of the genome are enriched for each of these marks specifically in senescent cells (termed mesas), and the H3K4me3 and H3K27me3 mesas significantly overlap. These occur mainly in gene-poor regions that are highly associated with Lamin B1 in proliferating cells. In addition distinct genomic regions are found depleted for H3K27me3 (canyons), which are much less frequently associated with Lamin B1 binding domains. Instead, the H3K27me3 canyons are enriched for gene bodies and enhancers; indeed, a number of genes upregulated during senescence, including many of those in the senescence-associated secretory phenotype (SASP), are located within these canyons. It is further discovered that in HGPS, in which Lamin B1 is mutated, the senescence-associated H3K27me3 canyons are present even in proliferating cells. These results suggest that at least some of the changes in histone methylation observed during human aging result from previous alterations in nuclear architecture [115].

4.4.3. SASP is regulated by changes in histone methylation induced by DNA damage—Unrepaired double stranded DNA breaks, a hallmark of old cells, constantly activate the DNA damage response [116]. One effect of this is the activation of the APC/C ubiquitin ligase by Cdh1. APC/C targets the histone methyltransferases G9a and GLP, which catalyze deposition of H3K9me1/2, for proteasome-mediated degradation [117]. In non-senescent human cells, G9a and GLP are associated with the promoters of *IL-6* and *IL-8/Gro-α*, two cytokines upregulated as part of the SASP. In senescing cells with unrepaired DNA lesions, both G9a and GLP are reduced at the *IL-6* and *IL-8* promoters, which correlates with a decrease in repressive H3K9me1/2 at these loci and transcriptional upregulation of the genes. It is further showed that reduction of G9a/GLP and an increase in *IL-6* and *IL-8/Gro-α* expression occur during DNA damage-induced senescence in skin papillomas and in tissues from aged mice that accumulate DNA damage. This suggests that the reduction of H3K9me1/2 by DNA damage-induced G9a/GLP degradation contributes to the physiology of aging [117], and shows a clear link between aging pathologies, DNA damage, and histone methylation.

5. Concluding remarks

The role of histone methylation in the regulation of lifespan and aging phenotypes is just beginning to be studied. While there is ample evidence for global changes in levels of histone methylation during aging, and even for a genome-wide redistribution of particular methylated histones (summarized in Table 1), the mechanisms by which these are achieved and by which they impact aging are unknown. Similarly, while loss of particular histone demethylases and methyltransferases promote longevity in invertebrate model systems (see Table 2), in most cases, the processes affected by a reduction in these enzymes are unknown. For example, knockdown of UTX-1 in *C. elegans*, which likely globally affects H3K27me3 levels, mediates lifespan extension primarily through its effects at the insulin receptor locus [60,62]. As the field progresses, it will be critical to determine 1) which loci are subject to altered histone methylation during aging, 2) whether and how the change in histone methylation at a particular locus affects lifespan and aging phenotypes, and 3) how altered histone methylation is accomplished, *i.e.*, by changes in levels and activities of

histone methyltransferases/demethylases or redistribution of these enzymes along the chromatin and what drives these processes.

The available evidence strongly implicates histone methylation in the transcriptional regulation of both longevity and pathways that contribute to aging phenotypes. In addition to changes in histone methylation, both globally and at specific loci, during the aging process, the loss of several histone methyltransferases and demethylases increases lifespan in *C. elegans*. As the molecular pathways that underlie aging pathologies are highly conserved, it is thus likely that histone methylation also regulates lifespan in other organisms. Furthermore, expression of genes involved in insulin and insulin-like signaling (IIS); the senescence-associated secretory phenotype (SASP); tumor suppression; autophagy; and, particularly, stress response are regulated by histone methylation (Figure 1A); thus, histone methylation must regulate the aging process, at least insofar as these pathways contribute to it. Indeed, as these processes, and probably many others, are regulated at the level of histone methylation, any changes in histone methylation may result in their misregulation, as is beautifully exemplified by the increase in SASP gene expression induced by chromatin changes that result from chronic DNA damage [117]. Furthermore, as repair of DNA damage requires novel histone methylation, the observed age-associated alterations in histone methylation may affect the ability of aged cells and organisms to execute the DDR (Figure 1B). Thus histone methylation, and alteration thereof, is likely to impact many different facets of the aging process.

Nevertheless, histone methylation is potentially a powerful target for treating, slowing, or reversing age-associated physiological changes. Like other epigenetic modifications, histone methylation is reversible, and thus by altering the activity of enzyme that regulate these modifications it may be possible to revert chromatin in aged patients to a younger state. As at least IIS and the SASP are implicated as being directly controlled by chromatin state, it is theoretically possible to disrupt these aspects of aging by potentiating or inactivating histone methyltransferase/demethylase activity. Indeed, numerous small molecule inhibitors of histone deacetylases (HDACi) have been identified, and several have proven effective in improving the efficacy of cancer treatment regimens [118,119]. Thus, in principle, regulating the function of chromatin modifying enzymes may be a powerful means to combat aging and other diseases to which epigenetic dysregulation contributes. In particular, recent advances in the development of small molecule inhibitors of methyltransferases and demethylases have yielded compounds that inhibit the function of specific enzymes without significant cytotoxicity [120]. Thus, as additional histone methyltransferase and demethylase inhibitors are discovered, and the roles of histone methylation in the aging process are elucidated, it may be possible to develop novel treatments to delay or prevent the onset of age-associated pathologies.

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Highlights

- Review role of histone methylation in aging of various model systems
- Levels and distribution of histone methylation change during aging
- Altered histone methylation may impact repair of DNA damage
- Histone methylation transcriptionally regulates many aging-related processes
- Role of histone methylation in aging is only beginning to be understood

Figure 1. Impact of differential histone methylation on aging processes

(A) A depiction of how histone methylation regulates and is regulated by major pathways that affect aging. Histone modifications are circled, and aging processes shown in large text. Red indicates a process that promotes or is caused by aging, while purple text highlights processes that promote longevity. Arrows indicate positive regulation (promotion) and lines ending in bars signify inhibition of the terminal process. Yeast-specific responses are in blue text. Numbers next to lines indicate from what system this data was derived. 1) yeast, 2) *Drosophila*, 3) *C. elegans*, 4) mammals. The diagram is based on data from references [60,62,64,96,97,99,106-109,117]. (B) Histone methylation necessary for DNA damage

repair may be altered during aging. Numerous chromatin changes occur following DNA damage that promote repair of lesions, as indicated; several of these modifications are known to be altered during aging, highlighted in red. Down arrow, decrease; R, significant redistribution; ?, uncharacterized, though different methylation events at the same residue are known to be altered, *e.g.*, H4K20me3 increases. Supporting data from references [45,115].

Table 1
Age-associated changes in histone methylation

The results from studies that have analyzed changes in global levels of histone methylation are summarized. HGPS, Hutchinson-Guilford progeria syndrome; this indicates tissues derived from human patients.

System	Tissue	Modification	Change	Reference
Mammals	rat kidney and liver	H4K20me3	increase	[56]
	mouse brain	H4K20me1	decrease	[55]
	mouse brain	H3K27me3	increase	[55]
	mouse brain	H3K36me3	decrease	[55]
	mouse brain	H3K79me1/2	increase	[55]
	macaque brain	H3K4me2	increase	[57]
HGPS	fibroblasts	H4K20me3	increase	[45]
	fibroblasts	H3K9me3	decrease	[45]
	fibroblasts	H3K27me3	decrease	[45,46]
<i>Drosophila</i>	whole animal	H3K4me3	decrease	[50]
	whole animal	H3K9me3	increase	[50]
	whole animal	H3K36me3	decrease	[50]
	whole animal	H3K9me2	decrease	[49]

Table 2
Regulation of aging by histone methyltransferases and demethylases

Compilation of the literature demonstrating that changes in histone methylation regulate lifespan and aging phenotypes. In all cases, the effect the proteins have on aging was assessed by loss of function mutation and/or siRNA-based knockdown. Blank entries represent missing data.

System	Protein	Effect	Mark	References
<i>C. elegans</i>	UTX-1*	increase lifespan	H3K27me3	[60,62]
	ASH-2	increase lifespan	H3K4me3	[58]
	SET-2	increase lifespan	H3K4me3	[58]
	WDR-5**	increase lifespan	H3K4me3	[58]
	RPD-2	decrease lifespan	H3K4me3	[58]
	SET-15	increase lifespan		[59]
	SET-9	increase lifespan	none found	[42,59]
	SET-26	increase lifespan	none found	[42]
	LSD-1	increase lifespan		[60,61]
	MES-2	increase lifespan		[42]
	JMJD-2	increase lifespan		[42]
	T26A5	increase lifespan		[60]
<i>Drosophila</i>	E(z)	increase lifespan		[64]
	Esc	increase lifespan		[64]
Mammals	G9a*	inhibit SASP		[117]
	GLP*	inhibit SASP		[117]

* indicates that expression of this enzyme and histone modification are altered during aging; the levels of others have not been assessed.

** signifies that WDR-5 is not a histone methyltransferase or demethylase; instead, this enzyme forms a complex with ASH-2 and SET-2 methyltransferases. SASP, senescence-associated secretory phenotype.

Table 3
Histone methylation contributes to pathways that drive aging

Recent work in yeast and cultured mammalian cells that implicates changes in histone methylation in cellular processes that drive aging are summarized.

Pathway	Modification	Change	Enzyme	Effect	References
Autophagy	H3K9me3	decrease	G9a, GLP	increase transcription of genes involved in autophagy	[69]
	H4K20me2	increase	MMSET	recruit 53BP to DNA lesions	[80]
	H3K79me2	increase	Dot-1	recruit 53BP to DNA lesions	[77-79]
	H3K9me3	increase		enhance Tip60 activity, which activates ATM	[85]
	H3K4me3	increase	Set-1		[89]
	H3K4me3	increase		recruit tumor suppressor ING	[90]
	H3K27me3	increase	EZH2	repress transcription near DNA lesions	[84]
Stress response	H3K4me3		Set-1	repress ribosome protein and biogenesis genes	[96]
	H3K36me3	decrease	Rph-1	activate stress response genes	[97]
	H3K36me3	decrease	Rph-1	repress sub-telomeric gene expression during ROS-induced hormesis	[99]

* indicates that this occurs in yeast, and also in human cells during growth phases of the cell cycle. DDR, DNA damage response.