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Epigenetic mechanisms regulating longevity and aging

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Summary

Aging is an inevitable outcome of life, characterized by progressive decline in tissue and organ function and increased risk of mortality. Accumulating evidence links aging to genetic and epigenetic alterations. Given the reversible nature of epigenetic mechanisms, these pathways provide promising avenues for therapeutics against age-related decline and disease. In this review, we provide a comprehensive overview of epigenetic studies from invertebrate organisms, vertebrate models, tissue and in vitro systems. We establish links between common operative aging pathways and hallmark chromatin signatures that can be used to identify “druggable” targets to counter human aging and age-related disease.

Introduction

“Aging” is the gradual loss of molecular fidelity after reaching sexual maturity, culminating in loss of function and ultimately in disease and death. In most organisms, the rate of aging is inversely proportional to mean lifespan. Increased age is also the largest risk factor for cancer, neurodegeneration and cardiovascular disease. On the other hand, “healthspan” is defined as the duration of time for which an organism remains free of chronic disease. Identifying ways to increase lifespan and healthspan are intriguing areas of biogerontology research.

Various animal models have been critical for uncovering key pathways related to aging. Genetically tractable models such as yeast have been used to investigate both replicative lifespan, measured by the maximum number of mitotic divisions a cell can undergo, and chronological lifespan, measured by the length of time a cell can survive in a post-mitotic state (Kaeberlein et al., 2007). Other studies have taken advantage of the short lifespans of worms and flies (Brandt and Vilcinskis, 2013; Tissenbaum, 2012). Studies in these models have contributed greatly to the field, but cannot fully recapitulate the complex nature of human aging, particularly with respect to age-related diseases and the decline of healthspan. Therefore, vertebrate models such as mice have been utilized, taking advantage of genetic proximity to humans and the availability of gene knockout and premature aging models

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(Quarrie and Riabowol, 2004). Unfortunately, mouse lifespan is too long for efficient laboratory studies of normal aging, creating the need for alternative short-lived vertebrate models such as the African turquoise killifish (*Nothobranchius furzeri*) (Harel et al., 2015) which lives for 4–6 months and recapitulates many of the age-related pathological changes found in humans.

Studies in various models have revealed that genetic differences and somatic mutations underlie longevity, but non-genetic contributions also play a major role (Cournil and Kirkwood, 2001). Calorie restriction (Bordone and Guarente, 2005), lowering of basal metabolic rate (Ruggiero et al., 2008), upregulated stress response (Migliaccio et al., 1999), restoration of mitonuclear protein balance (Houtkooper et al., 2013) and reduced fertility (Westendorp and Kirkwood, 1998) have all been shown to correlate with lifespan extension. These observations illuminate the role of “epi”-genetic mechanisms in modulating longevity pathways.

Chromatin is the polymer of nucleosomes composed of DNA wrapping the histone proteins. Chromatin and epigenetic factors influence gene expression dynamically or over long time scales - even through cell division - by regulating access of transcriptional machinery to DNA. These factors are enzymes that modify DNA directly or the core histones H2A, H2B, H3 and H4, as well as variant histones, including H3.3, macroH2A, and H2A.Z. A large number of epigenetic studies have begun to establish operational models in aging. It is clear that with age there is general loss of histones coupled with local and global chromatin remodeling, an imbalance of activating and repressive histone modifications, and global transcriptional change in all aging models. Additionally, particularly in mammalian systems there is global and local change in DNA methylation, site-specific loss and gain in heterochromatin, and significant nuclear reorganization (Figure 1).

It is as yet unclear whether changes in the activity of epigenetic enzymes influence the expression of critical longevity genes or whether alterations in the longevity genes drive large scale epigenetic changes in the genome. In lower model organisms, single point mutations in epigenetic enzymes dramatically alter lifespan. For complex higher organisms that have many redundant enzymes, it is too simplistic to theorize that a few genes drive age-related changes. Rather, experimental evidence supports large-scale alterations in response to environmental stimuli or nutrient availability.

This review consolidates key findings in multiple systems that support an epigenetic role in aging. In addition to the text, epigenetic mechanisms are summarized for multiple systems in Supplemental Table 1 and general benefits and drawbacks of each model highlighted in Table 1 providing readers with a resource to quickly peruse key points for their own studies. Finally, readers are provided with lists of state-of-the-art methodologies, protocols, repositories tools and other resources that can be used to accelerate aging research (Table 2, Supplemental Table 2). Of particular interest are large-scale epigenomic studies and the recent CRISPR mutagenesis strategies.

Evidence for epigenetic changes in aging

General loss of histones as a mechanism of aging

From yeast to humans, an emerging epigenetic trend detected in aging cells is the general loss of histones (Figure 1A, Supplemental Table 1) (Dang et al., 2009; O'Sullivan et al., 2010). Thus far, the majority of evidence suggests that histone loss is tightly linked to cell division. In replicatively aged yeast, micrococcal nuclease-DNA sequencing (MNase-seq) showed a global nucleosome loss of ~50% (Hu et al., 2014). The remaining nucleosomes had higher “fuzziness” in positioning or mapped to regions with stronger positioning sequences. That proper histone dosage is important in yeast is evident from the extended lifespan of strains overexpressing histones (Feser et al., 2010). In addition to transcriptional downregulation linked to cell cycle exit, loss of histones may also occur through post-translational mechanisms such as autophagy. Piecemeal microautophagy of the nucleus occurs in yeast but whether chromatin components are targeted for degradation by this process is unknown (Kvam and Goldfarb, 2007). In human senescent fibroblasts, nuclear blebs bud off cytoplasmic chromatin fragments (CCFs) that are positive for DNA, γ -H2AX and other repressive histone marks and targeted to lysosome for degradation (Dou et al., 2015; Ivanov et al., 2013). However, it is unclear whether such histone loss occurs in aged human tissues that accumulate senescent cells and tissues such as the brain containing non-renewable, differentiated neurons.

In addition to exploring whether histone loss is a feature of *in vivo* aging in higher organisms, future studies may be directed to reveal “pockets” of histone loss in the genome. This requires advanced single cell genomic analyses to acquire sufficient sequencing depth in a population of cells. Elucidating the signaling pathway (s) triggering histone loss will be a key conceptual advance. An additional area of future investigation is characterizing the alternative pool of canonical histones synthesized exclusively in senescence. These are formed via alternative splicing events from histone genes with two exons that eliminate the stem loop terminator, but allow polyadenylation for stabilization in non-proliferating cells. These alternative histones are then incorporated in the genome by replication independent chaperones to create a dynamic chromatin state (Rai et al., 2014), the further study of which may reveal key epigenetic differences between replicative and non-replicative states.

Imbalance of activating and repressive histone modifications

Histone methylation, specifically Histone 3 lysine 4 trimethylation (H3K4me3) (activating) and H3K27me3 (repressing), are epigenetic modifications with close ties to transcription, and have been directly linked to lifespan regulation in many organisms. In worms, the complex composed of ASH-2, WDR-5 and the H3K4me3 methyltransferase SET-2 affects lifespan. Knockdown of the methyltransferase subunits improves longevity, while knockdown of the demethylase subunit RBR2 shortens lifespan. Conversely, overexpression of RBR2 extends lifespan (Han and Brunet, 2012). Importantly, these complexes positively (methyltransferase) or negatively (demethylase) regulate global H3K4me3 levels, providing compelling evidence for an epigenetic role in lifespan regulation (Greer et al., 2010). These effects require an intact germline and longevity effects are transgenerational (Maures et al., 2011). Knockdown or mRNA suppression of the H3K4 mono- and di-demethylase LSD-1

extends lifespan (McColl et al., 2008). Although the exact mechanism by which H3K4 methylation regulates lifespan is not clear, the authors speculate a role for local and global transcriptional changes.

In contrast to H3K4me3, H3K27me3 is primarily a transcriptionally repressive modification catalyzed by the PRC2 complex and removed by UTX-1. During worm aging, there is global decrease in somatic H3K27me3 and increase in UTX-1 expression (Jin et al., 2011). RNAi depletion or heterozygous mutation of UTX-1 in worms increases global levels of H3K27me3 and extends lifespan in an insulin-dependent, but germline-independent manner (see below for insulin signaling pathway discussion)(Maures et al., 2011). The opposing effects of H3K4me3 and H3K27me3 on worm lifespan reflect an increasingly favored model in the aging field that proposes gain of activating marks and loss of repressive marks as hallmarks of aging. However, H3K27me3 provides confounding phenotypes in other organisms that limits enthusiasm in fully establishing such an encompassing model for aging; heterozygous mutations in the H3K27me3 methyltransferase MES-2 (E (z) in flies and EzH2 in mammals) lengthen lifespan (Ni et al., 2012), contrary to what might be expected. The longer lifespan may be due to altered regulation of specific genes that have a dominant lifespan extension effect, thereby overriding genome-wide effects of reduction of the repressive mark.

In flies, overexpression of Lid, the worm RBR-2 homolog, extends lifespan, while its knockdown shortens lifespan of male flies by 18% (Li et al., 2010). Lid affects lifespan by changing global levels of H3K4me3; however, knockdown of Trx, one of three H3K4 methylases, does not alter lifespan of male flies. The role of the other methylases and effect on female flies has not yet been tested. In flies, H3K27me3 seems to influence lifespan in a manner opposite to that in worms. Mutations in H3K27 methyltransferase (PRC2) subunits E (z) and ESC reduce global levels of H3K27me3 and extend lifespan of male flies by derepressing target genes Abd-B and Obc1. Interestingly, mutations in Trx increase H3K27me3 levels of E (z) mutants and suppress their longevity phenotype. Trx antagonizes polycomb-mediated silencing by interacting with CBP and promoting H3K27 acetylation (H3K27ac). Since acetylation and methylation at this site are mutually exclusive, increased acetylation results in both decreased methylation and loss of silencing at PRC2 target sites (Siebold et al., 2010).

Thus, histone modifications in flies influence lifespan; however, this effect appears to be less global, and more focused on targeted expression changes of specific genes influencing increased stress tolerance or modulating calorie restriction pathways (discussed later). In fact, the hypothetical working model for yeast and worms (increase in activating modifications and decrease in repressive modifications with age) appear to be oppositely regulated in flies. A ChIP-chip study of histone modification changes in young and old female fly heads showed a loss in activating marks like H3K4me3 and H3K36me3 and a gain of repressive marks like H3K9me3 with age (Wood et al., 2010). In aging killifish, there is global upregulation of H3K27me3 echoing a trend similar to that in flies (Baumgart et al., 2014). Additional studies will be required to determine if the observed opposite histone modification trends truly reflect major differences in age-related epigenetic changes within different aging models. Certainly, one technical explanation is the potential inability to

measure global increases in activating modifications in some models due to the preferential loss of histones harboring activating marks, thus complicating the results. In the event that this is not a source of variance, it is also possible that some histone modifications are more dominant in some models than others, and as such, certain local modifications in one model may influence molecular aging pathways as strongly as global changes in other models.

Unlike histone methylation, the link between global histone acetylation and longevity is better understood. The polyamine spermidine, a small organic molecule derived from amino acids inhibits histone acetyltransferase (HAT) activity to generate hypoacetylated chromatin states. The level of spermidine declines from yeast to humans with age and spermidine supplementation increases lifespan in yeast, worms, flies and human cells. The longevity phenotype correlates with improved stress response and upregulated autophagy via increased histone acetylation at these genes (Eisenberg et al., 2009).

Evidence of global changes and imbalance of regional chromatin domains in humans come from in vitro studies in cultured cells. Senescence is a state of cell cycle arrest in response to stress, linked to age-associated tissue decline in vivo (van Deursen, 2014). During senescence, many cultured mammalian cells develop senescence-associated heterochromatin foci (SAHF), regions of highly condensed chromatin associated with heterochromatic histone modifications, heterochromatic proteins, histone variant macroH2A, high-mobility group A (HMGA) proteins and late replicating regions in the genome (Chandra et al., 2012; Zhang et al., 2007). A genome-wide study of senescent chromatin showed that more than 30% of chromatin is dramatically reorganized in senescent cells, including the formation of large scale domains (mesas) of H3K4me3 and H3K27me3 over lamin-associated domains (LADs), as well as large losses (canyons) of H3K27me3 outside of LADs (Shah et al., 2013). These changes are linked to the transcriptional downregulation of lamin B1 in senescence (Freund et al., 2012; Shah et al., 2013; Shimi et al., 2011). The profound destabilization of heterochromatic LADs leads to autophagic degradation of lamin B1 and associated repressive histones (Dou et al., 2015). Additionally, senescent cells show strong enrichment of H4K16ac (acetylation at lysine 16 on H4) at promoter elements of active genes where it overlaps with HIRA and newly synthesized H3.3 peaks (Rai et al., 2014). HIRA, a DNA replication-independent histone chaperone that deposits variant histone H3.3 and H4 is required for the steady state maintenance of H4K16ac. These changes suggest that the senescent state hosts a dynamic and imbalanced chromatin environment that is markedly different from the proliferating state.

The role of ubiquitylation and sumoylation in senescence and aging are less understood due to the multitude of substrates targeted. These modifications involve the covalent addition of small peptides such as ubiquitin or SUMO (small ubiquitin related modifier) to lysine groups on histones and other proteins. In yeast, strains lacking subunits of the SAGA/SLIK deubiquitinase module such as *Sgf73* are exceptionally long lived. *Sgf73* impacts yeast replicative lifespan through multiple related pathways mediated by Sir2, a NAD⁺ dependent histone deacetylase (discussed later); (1) there is a direct physical interaction of *Sgf73* with Sir2 in WT yeast that reduces Sir2 activity, (2) presence of *Sgf73* results in asymmetric segregation of ribosomal DNA (rDNA) circles to mother cells and (3) *sgf73* strains show increased silencing of telomere proximal genes (McCormick et al., 2014). Collectively, these

pathways increase lifespan potentially by reinforcing a compact chromatin structure. The role of deubiquitinases in aging of higher organisms remains unknown. In senescent cells, the levels of many ubiquitylated and sumoylated proteins are upregulated including telomerase, p53, histone H2A and PCNA resulting in proteasomal degradation (Grillari et al., 2010). Ubiquitylation of DAF-16 (discussed later), an important longevity protein causes shorter lifespan in worms (Li et al., 2007). SUMO expression is increased in old rats and senescent fibroblasts and overexpression of SUMO causes premature aging in worms (Rytinki et al., 2011). SUMO-induced senescence acts through an interconnected network of pathways that involve sumoylation of proteins in promyelocytic leukemia (PML) nuclear bodies, p53/pRb and its interacting proteins to impact senescence-specific transcription programs (Ivanschitz et al., 2013). However, more work is required to identify the exact chromatin mechanisms.

Many chromatin-related senescence phenotypes occur in aging tissues from various organisms, underscoring the connection between senescence and longevity. High levels of histone macroH2A and heterochromatin protein HP1b accumulate with age in mouse and primate tissues (Herbig et al., 2006; Kreiling et al., 2011). Additionally, H3K9me3 is reduced in cultured cells from normal aged individuals and H4K20me3 is increased in the lysates of aged rats (Sarg et al., 2002; Scaffidi and Misteli, 2006). ChIP-seq in young and old hematopoietic stem cells (HSCs) show a modest increase in the number of H3K4me3 peaks with age, many of which broaden in coverage, especially over HSC identity and self-renewal genes. H3K27me3 peaks do not appreciably change in number but also broaden with age (Sun et al., 2014).

While many more studies will be required to ultimately determine if there is a key “signature” of aging chromatin, the data observed to date strongly suggest a massive alteration in histone modification patterns with aging in multiple models (Fig. 1B, Supplemental Table 1). An overarching theme of these studies is the loss of repressive marks and gain of activating marks that have profound effects on gene expression. In some cases, these global trends may not be evident due to the preferential loss of histones with active modifications. In other cases, the ultimate effects on lifespan may be driven by local and discrete chromatin changes at specific regulatory elements of the genome, resulting in altered transcription of key longevity genes. For example, increases in repressive marks during aging may lead to site specific gains in heterochromatin over genes whose expression are detrimental to lifespan. Together, these studies propose an altered histone modification profile in aging. The consequences of these regulatory changes are either destructive (as in tissue damage) or protective (as in genome stability), and distinguishing these is a major challenge in aging studies.

Transcriptional deregulation as a consequence of epigenetic change

Transcriptional amplification due to histone loss—A consequence of general histone loss in replicatively aged yeast is global transcriptional amplification (Fig. 1C, Supplemental Table 1). Genes most induced with age have specific chromatin signatures in young cells such as fuzzier nucleosome positioning, lack of a nucleosome depleted region (NDR) at the promoter, and weak nucleosome phasing. These genes also exhibit higher

frequency of TATA elements and higher occupancy of repressive chromatin factors like Asf1 and Tup1. Interestingly, the same genes in old cells show a stronger nucleosome loss at the promoter and therefore the greatest induction in transcript levels. Many of these age-related changes can be ameliorated by increasing the levels of histones, strongly implicating direct transcriptional consequences of histone loss (Hu et al., 2014).

Upregulation of intragenic cryptic transcripts due to reduced histone methylation over gene bodies—In addition to changes in canonical transcripts, a histone substitution screen in replicatively aged yeast uncovered a role for H3K36 methylation in lifespan regulation and an effect on cryptic transcription (Fig. 1C, Supplemental Table 1). The deletion of Set2, the only methyltransferase for H3K36 in yeast, shortened replicative lifespan (Sen et al., 2015). Conversely, deletion of the H3K36 di/tri demethylase Rph1, extended lifespan by ~30%, which was attributed to the ability of the demethylase mutant to suppress spurious cryptic transcripts originating from within gene bodies. Loss of the worm Set2 homolog, MET-1, is known to shorten lifespan whereas loss of the demethylase JMJD-2 has pro-longevity effects suggesting a conserved regulatory pathway (Hamilton et al., 2005). A subset of genes also shows upregulated cryptic transcription in old worms (Sen et al., 2015). H3K36me3 levels in young worms are negatively correlated with gene expression variation during aging (Pu et al., 2015). Overall, H3K36 methylation serves a protective role in preserving transcriptional precision and stability, and its loss in aging leads to an altered transcriptome detrimental to a longer life.

Transcriptional noise due to DNA damage—Age has been linked to increased transcriptional noise, defined as a stochastic deregulation of gene expression. Transcriptional profiling of single cardiomyocytes isolated from old mice showed considerable variation in transcript levels of a panel of heart-specific housekeeping genes compared to young controls. Mouse embryonic fibroblasts treated with the DNA damaging agent hydrogen peroxide also showed a similar increase in transcription noise, suggesting DNA damage as a key player (Bahar et al., 2006); however, the increase in noise after damage is a delayed effect when more permanent genomic changes such as mutations, rearrangements or epigenetic changes occur, rather than immediate changes such as protein or lipid oxidation.

Cardiomyocytes are post-mitotic, and interestingly, a similar experiment conducted using constantly renewing cells of the hematopoietic lineage (stem cells, granulocytes, naïve B cells and naïve T cells) failed to show significant increase in noise. Notably though, old cells exhibited a general trend toward higher transcript levels (Warren et al., 2007). One reason explaining this difference is the disparate somatic mutation rates between different tissues. Thus, age-related deregulation of transcription (among other changes) may contribute to cellular fitness and growth, but may differ vastly across differentiated cell types.

Altered transcriptional programs—The most obvious molecular consequence of age is an altered transcriptional program, a phenomenon that has been best studied in senescent human cells. In addition to upregulating alternative histone transcripts (Rai et al., 2014), senescent cells remodel the epigenetic landscape to induce a subset of cellular defense and inflammatory response genes, many of which are secreted from the cell and are characterized as part of the senescence-associated secretory phenotype (SASP)(Coppe et al.,

2008). SASP has been theorized to promote immune clearance of pre-malignant senescent cells to facilitate tumor suppression; however, chronic SASP induction is also believed to promote tissue aging, underscoring the delicate balance between the “benefits” and “consequences” of senescence (Adams, 2009).

A strong epigenetic correlation was shown between loss of H3K27me3 and upregulation of SASP genes in senescent cells (Shah et al., 2013). Additionally, inhibition of the H3K4 methyltransferase MLL1 was shown to reduce SASP indirectly, via repression of pro-proliferative cell cycle genes and DNA damage response genes that in turn reduce SASP (Capell et al., 2016). Taken together, these lines of evidence indicate that transcriptional reprogramming in senescence occurs through altered activity of specific epigenetic proteins. Mimicking these trends, hematopoietic stem cells (HSCs) isolated from old mice show an upregulated inflammatory response and downregulated chromatin remodeling, DNA repair and transcriptional repression compared to young controls. These changes are concomitant with the inability of the older HSCs to rejuvenate the vascular system (Chambers et al., 2007). RNA-seq analyses of highly purified HSC populations showed upregulated gene ontology (GO) categories related to cell proliferation, cell adhesion and ribosomal protein genes while those related to cell cycle, DNA base excision repair and DNA replication were downregulated. Pathway analyses revealed that TGF β signaling is reduced in aging HSCs, which could explain a myeloid bias that is established with age (Sun et al., 2014).

A final compelling genomic feature of senescent cells and aged mouse somatic tissue is upregulation of transcripts from repeat elements (De Cecco et al., 2013), much like in embryonic stem cells. Interestingly, HSCs also upregulate expression of repeat elements (such as LTRs, LINEs, SINEs and others) with age, although the consequence of this upregulation is unclear (Sun et al., 2014). Selective loss of heterochromatin in aged cells may contribute to the upregulation of these transcripts. Together, these studies implicate epigenetic mechanisms in regulation of transcription with age, and suggest that possible intervention resulting in transcriptional stabilization may result in increased lifespan and cellular fitness.

Site-specific losses and gains of heterochromatin

Heterochromatin domains are established early during embryonic development and were thought to be maintained throughout lifespan. However, loss of constitutive heterochromatin (commonly at telomeres, centromeres, pericentromeres) occurs in senescence and aging and is triggered by telomere shortening, transcription changes at boundaries and breakdown of the nuclear periphery. In contrast to a loss in constitutive heterochromatin, senescent fibroblast cells show focal increases in heterochromatin called SAHFs (discussed below) in otherwise euchromatic regions (Fig. 1D, Supplemental Table 1).

Yeast cells constitutively express telomerase and lack a well-defined nuclear periphery and deregulation of key heterochromatic proteins contributes to loss of silencing at heterochromatic regions such as the rDNA, *MAT* locus and subtelomeric regions. A yeast senescence model (*tlc1*) shows relocalization of telomeric protein Rap1 to new positions in the genome, much like HP1 (Platt et al., 2013). Age-related chromatin deregulation at the rDNA locus results in the formation of extrachromosomal rDNA circles (ERCs) that are

causal to replicative aging (Sinclair and Guarente, 1997). In addition, genes at the subtelomeric X-core and X-repeat elements are also derepressed with age (Dang et al., 2009). Yeast Sir2, the founding member of the Sirtuin family of proteins with NAD⁺ dependent histone deacetylation and ADP ribosylation activity, decreases in old yeast cells compromising silencing at rDNA, *MAT*, and subtelomeric loci, contributing to both aging (Dang et al., 2009) and onset of sterility (Smeal et al., 1996). Thus, loss of Sir2 shortens replicative lifespan while overexpression and small molecule Sir2 activators extend lifespan (Howitz et al., 2003; Kaeberlein et al., 1999). Similarly, a gain of function mutant of *SIR4*, another heterochromatic protein acting at the rDNA locus, extends yeast lifespan (Kennedy et al., 1995). Importantly, the Sir2 effect at subtelomeric genes is mediated through histone H4K16ac, which increases as cells age. It is unknown whether gene derepression at subtelomeric loci is causal to aging, but it is interesting to note that deletion of the H4K16 acetyltransferase Sas2 extends replicative lifespan in normal yeast (Dang et al., 2009) and the yeast senescence model mentioned above (Kozak et al., 2010).

Pathways affecting heterochromatin establishment and maintenance are tightly linked to senescence in mammalian cells, resulting in global and gene-specific changes linked to reduced cellular fitness. Constitutive heterochromatin structures are disorganized in senescence, as evidenced by reduced global DNA methylation (discussed below), H3K9me3 levels and HP1 (Scaffidi and Misteli, 2005, 2006). Senescence is also marked by a deficiency in the polycomb-repressive protein EZH2, a histone methyltransferase, and subsequent decrease of repression-associated H3K27me3, leading to rapid senescence. While likely resulting in broader genome wide effects, EZH2 and H3K27me3 loss also results in gene specific upregulation of p16INK4a (p16), a key upregulated SASP and ubiquitous marker for cellular senescence (Bracken et al., 2007). Conversely, overexpression of EZH2 results in increased cellular lifespan (Bracken et al., 2007), an effect attributed through repression of p16. It is interesting to speculate that global H3K27me3 and heterochromatin maintenance may also be stabilized with EZH2 overexpression.

In addition to a marked global loss of constitutive heterochromatin and general increases in transcription, it seems paradoxical that senescent cells form SAHFs that are foci of γ -H2AX, HP1 γ , H3K9me3, macroH2A, HMGA proteins and hypoacetylated histones in otherwise euchromatic regions (Di Micco et al., 2011; Narita et al., 2003; Zhang et al., 2007). SAHF formation is induced by an initial chromatin condensation step mediated by the HUCA histone chaperone complex, composed of *HIRA*, ubinuclein-1 (*UBN1*), *CABIN1* and histone chaperone *ASF1a* that deposits H3.3 into chromatin (Daniel Ricketts et al., 2015). Consistently, H3.3 expression is dramatically upregulated in senescence (Rai et al., 2014). In a subsequent step, other proteins are recruited to the condensed chromatin including phosphorylated HP1, macroH2A and HMGA proteins (Zhang et al., 2007). Loss of the nuclear lamina (Fig. 1E) triggers a breakdown of heterochromatin organization, relocating heterochromatic proteins to other regions in the genome where it may contribute to formation of region-specific foci like SAHFs. The location of SAHFs is not precisely known, but these regions have been correlated with late-replicating regions of the genome. Although the physiological explanation for the SAHFs is unclear, one speculation is that they contribute to the protective response discussed above, that is, as histones are lost and transcriptional noise increases (see below), then the histone chaperone system attempts to

ameliorate this via H3.3 assembly to “plug the holes” in euchromatin to control gene expression.

Taken together, deregulation of chromatin organizational proteins and reorganization of heterochromatic domains are discernible features of senescence and aging. These changes encompass a broad decrease of heterochromatin, site-specific decrease of heterochromatin, as well as region-specific gains of heterochromatin. Broad changes may ultimately affect overall nuclear organization and cellular fitness, while region and site-specific heterochromatin changes may directly regulate expression-specific longevity genes that drive aging or affect lifespan.

Alteration in DNA methylation levels and patterns with age

In addition to histone modifications, epigenetic changes include chemical modifications directly on DNA. DNA methylation is a well-studied epigenetic modification and occurs mostly on the 5-carbon of cytosine residues in CpG dinucleotides. It is relatively easy to study due to simplicity of sample preparation and availability of cost-effective techniques that permit large-scale investigations, including bisulfite conversion coupled to PCR, pyrosequencing, microarrays and deep sequencing. DNA methylation is correlated with transcriptional repression and is important in X-chromosome silencing, centromeric/ repetitive sequence repression and genomic imprinting.

Many commonly utilized aging models have no (such as yeast) or limited (worms and flies) DNA methylation although there are interesting links to aging. Worms lack DNA cytosine methylation (5mC), but methylation on N6 adenine (6mA) has been recently discovered (Greer et al., 2015). 5mC is also rare in flies (Capuano et al., 2014). Overexpression of dDnmt2, the non-redundant *Drosophila* DNA methyltransferase, increases fly lifespan, whereas dDnmt2 mutant flies are short-lived (Lin et al., 2005). DNA methylation in flies may function to repress transposition and 5mC has been localized to retrotransposons, although this finding is controversial (Oakes et al., 2003; Osorio et al., 2010). Like worms, 6mA was also recently identified in *Drosophila* and shown to mark and suppress transposons (Zhang et al., 2015). Given that transposons are also derepressed in senescent mammalian cells (De Cecco et al., 2013), it will be interesting to investigate this new epigenetic modification in higher organisms, and a possible role of 6mA in aging.

DNA methylation is more prominent in mammalian systems and is achieved by the action of three DNA methyltransferases: DNMT1, which has a maintenance role, and DNMT3a and 3b, which are de novo methylases. The removal of DNA methyl groups is mediated by the ten-eleven-translocation (TET) proteins (Du et al., 2014). With age, mammalian cells undergo global DNA hypomethylation and local DNA hypermethylation (Cruickshanks et al., 2013), a pattern which fits the aging model of global heterochromatin deregulation coupled with focal increase in repressive modifications (Fig. 1F, Supplemental Table 1). Loss of methylation occurs primarily in repetitive regions of the genome that correlate with constitutive heterochromatin while hypermethylation primarily occurs at promoter CpGs. In mice, progressive loss of 5mC in livers is evident as the animals age from 6 to 24 months (Singhal et al., 1987). Studies of livers from long-lived Ames mice (see below) compared to normal mice show lower basal levels of DNMT1 regulated by growth hormone deficiency.

The differential expression of DNMT likely causes DNA methylation differences over repeat elements and key longevity genes impacting lifespan (Armstrong et al., 2014).

The DNA methylation status in a number of human tissues has been investigated at 26,486 autosomal CpGs using an arrayed format. The results revealed that promoter CpGs undergo hypermethylation while those outside undergo hypomethylation with age (Day et al., 2013). Other forms of DNA methylation such as 5-hydroxymethylcytosine, 6mA and non-CG methylation have also been investigated, but to a much lesser extent.

In a large scale study from UCLA, it was shown that DNA methylation can serve as a remarkable predictor of age in normal/non-cancerous tissues. This study analyzed ~8000 samples representing 51 healthy human tissue and cell types including liver, kidney, immune and brain cells and ~6000 cancer samples. It was convincingly identified that DNA methylation at 353 CpGs (termed clock CpGs) and not other epigenetic modifications accurately predicted age (Horvath, 2013, 2015). A parallel study investigated the rate of DNA methylation change by building a predictive model of the aging methylome from the blood of individuals aged 19–101 (Hannum et al., 2013). This study identified a smaller set of 71 CpGs that have a high accuracy of age prediction and occur near genes associated with aging. The prediction from blood and the smaller number of CpGs to probe increases the diagnostic value of this study. To understand the underlying mechanism of these epigenetic clocks, it is imperative to study it in model organisms where mutational analyses will reveal specific regulatory pathways. As of now, the clock has been identified in human cells and tissues and efforts to discover such a clock in model organisms such as mice are under way.

In summary, the overarching pattern of DNA methylation changes in aging encompasses global DNA hypomethylation and local hypermethylation that may activate specific transcriptional programs, and this is congruent with the histone modification changes that occur, as described above. It remains elusive what specific genes are directly affected by the altered DNA methylation.

ATP-dependent chromatin remodeling complexes in aging

ATP-dependent chromatin remodeling complexes use ATP hydrolysis to restructure chromatin either permitting or repressing transcription (Bartholomew, 2014). The role of remodelers in senescence and aging is limited, despite their function in large-scale chromatin organization; and it could be that there is an insufficient understanding of their roles due to functional complexity and redundancy of these enzymes in vivo. In line with the overall aging model presented above, the SWI/SNF class of remodelers associated with gene activation is predicted to promote aging, whereas the repressive ISWI class may promote longevity. However, we note that chromatin remodeling could also impact lifespan via regional or gene-specific effects that may not parallel global changes (Fig. 1G).

In support of gene-specific effects, deleting *ISW2* in yeast robustly extends replicative lifespan. Increased resistance to stress is an emerging concept in lifespan extension, and deletion of *ISW2* derepresses a cohort of stress response genes acting through the homologous recombination DNA repair pathway. In support, *ISW2* deletion improved resistance to DNA damaging agents. Genome-wide analysis revealed that deletion of *ISW2*

partially mimics the transcriptome and nucleosome positioning profile of calorie restricted cells, also showing upregulation of stress response genes (Dang et al., 2014). Interestingly, reduction of the worm ortholog of the ISW2 complex extended lifespan while knockdown of BAZ1A (human homolog of Itc1) increased expression of many stress response genes mimicking findings in an *isw2* yeast strain. These data argue in favor of gene-specific effects and suggest that ISW2 orthologous complexes may regulate longevity through stress response mechanisms.

The Nucleosome Remodeling and Deacetylase (NuRD) chromatin remodeling complex has been linked to aging in mammalian cells. The WD40 domain-containing protein, RBBP4, and the related RBBP7 histone chaperone proteins were identified as specific interactors of the lamin A C-terminal fragment. These subunits, along with two other NuRD-specific subunits, are downregulated in progeroid (HGPS) skin fibroblast cells. (Pegoraro et al., 2009). The same trends are recapitulated in primary fibroblast cells isolated from older patients, suggesting that loss of NuRD components is also relevant in physiological aging. The consequence of NuRD downregulation is loss of senescence heterochromatic features, fitting a model in which loss of global protective chromatin is predicted to promote aging. The exact mechanism of senescence induction through this pathway remains to be investigated, and it is particularly interesting to consider how nucleosome remodeling changes affect global and local changes in histone modifications and DNA methylation during aging.

Nutrient signaling and its effects on the chromatin

Nutrient availability is key to determining lifespan in several tested models (Bordone and Guarente, 2005). Nutrients in the form of growth factors, metabolites, amino acids and carbohydrates are sensed by a multitude of proteins including glucose sensors in yeast, the Tor (target of rapamycin) proteins conserved from yeast to humans, the insulin receptors conserved from worm to humans and sirtuins conserved from yeast to humans. One role of these factors is to relay signals to the nucleus leading to general epigenetic modulation of lifespan. In addition to nutrients, other environmental stimuli (such as circadian cycles, hormones, and exercise) also alter the epigenome to influence lifespan and healthspan. For additional discussion of these signals, we direct the readers to two recent reviews (Benayoun et al., 2015; Booth and Brunet, 2016). The best characterized pathway connecting the environment to the epigenome is nutrient signaling which we discuss in detail below.

Glucose sensing and the Ras/AC/PKA pathway

Glucose sensing in yeast involves the Ras/adenylate cyclase (AC)/protein kinase A (PKA) pathway. Deletion of Ras2 or adenylate cyclase has chronological lifespan benefits partially through the effects on oxidative stress protection (Fabrizio et al., 2003; Longo, 1999). The Ras and Tor (discussed below) pathways converge on the stress regulon *SOD2* and involve the glucose-repressible kinase Rim15, and stress-response transcription factors Msn2/4 and Gis1 (Pedruzzi et al., 2000). Calorie restriction in the long-lived Ras2/Sch9 (of the Tor pathway) mutants further increases lifespan, for a combinatorial longevity increase of 10-fold, suggesting independent pathways in calorie restriction.

The Gis1 related protein Rph1, which is also a H3K36 demethylase, affects yeast replicative lifespan (Sen et al., 2015) and has a role in gene expression in the stationary phase (Orzechowski Westholm et al., 2012). Surprisingly, deletion of *GIS1* shortens lifespan, and this phenotype may result from activation of Acs2 (nuclear acetyl-CoA synthetase), promoting histone acetylation, while deletion of *RPH1* extends lifespan via a mitochondria-to-nucleus signaling via gene repression at subtelomeres (Orzechowski Westholm et al., 2012; Schroeder et al., 2013). All of the mutants mentioned in this section have markedly different gene expression profiles compared to wild type yeast, strongly indicative of large-scale epigenetic changes, although the exact changes driving longevity are undetermined.

Calorie restriction, the TOR pathway and histone acetylation

The yeast Tor protein (a serine/threonine kinase of the phosphatidylinositol kinase related kinase (PIKK) family) assembles into two complexes: (a) rapamycin sensitive TORC1 comprising Tor1 or Tor2 kinases, Lst8, Kog1 and Tco89 and (b) rapamycin insensitive TORC2 comprising Tor2, Avo1, Avo2, Avo3 and Lst8 (Loewith and Hall, 2011). Unlike yeast, higher eukaryotes have only one Tor protein, although the assembly into two complexes is conserved. TORC1 signaling modulates ribosome biogenesis, translation, autophagy, cell growth and proliferation via signal transduction involving Sch9 kinase, whereas TORC2 signaling primarily regulates cytoskeletal changes required for cell growth. Deletion of *TOR1* and *SCH9*, as well as administration of low doses of rapamycin, extend both replicative and chronological lifespan in yeast (Fabrizio et al., 2001; Kaeberlein et al., 2005; Powers et al., 2006). The broader question of interest here is whether TOR signaling modulates the epigenetic landscape to impact longevity.

There are several lines of evidence for TORC1 modulation of chromatin structure and function. Rapamycin treatment downregulates TORC1 signaling and increases genome-wide occupancy of Rsc9, a subunit of the RSC chromatin remodeling complex, ultimately resulting in gene expression changes (Damelin et al., 2002). Furthermore, rapamycin treatment or nutrient starvation reduces nucleoli size and displaces RNA polymerase I (pol I) from rDNA loci, thus inhibiting transcription at these sites. rDNA silencing is facilitated by increased binding of Rpd3-Sin3 HDAC complex (Rpd3(L)) and deacetylation of H4K5/K12 residues. At ribosomal protein (RP) gene promoters, TORC1 signaling favors the occupancy of Esa1-histone acetyltransferase complex, resulting in histone H4 acetylation and active transcription. In response to nutrient starvation or rapamycin treatment, TORC1 signaling is inhibited, releasing the Esa1-containing complex from RP gene promoters and returning chromatin to a condensed state by Rpd3 (L)(Rohde and Cardenas, 2003). These repressive chromatin effects of rapamycin all serve to lower transcription of protein synthesis apparatus, ultimately lowering growth.

A chemical genomics screen of H3/H4 residues sensitive to rapamycin identified H3K56 as crucial for TORC1 signaling (Chen et al., 2012a). In the presence of rapamycin, H3K56ac is reduced globally and locally at rDNA loci where this acetylation functions to make chromatin permissive for pol I transcription and is also required for rRNA processing. In H3K56 substitution mutants, deletion of the acetyltransferase Rtt109 and deacetylases Hst3/4 (both targeting H3K56) all decrease lifespan. Additionally, H3K56 and *rtt109*

mutants are sensitive to DNA damaging agents (Driscoll et al., 2007; Han et al., 2007). This implies that balanced and cycling H3K56ac is important to preserve genome integrity and promote longevity (Dang et al., 2009). Thus, in yeast, TORC1 signaling negatively regulates longevity via histone acetylation, affecting ribosome biogenesis, translation and rDNA regulation.

The worm homolog, CeTOR or let-363, is an amino acid sensor (McCormick et al., 2011); combining mutations of CeTOR substrate RSKS-1 (Sch9 of the Tor pathway in yeast) and DAF-2 (insulin signaling receptor in the insulin signaling pathway as discussed below) lead to a fivefold extension in worm lifespan (Chen et al., 2013). CeTOR associates with accessory proteins to form rapamycin sensitive TORC1 and rapamycin insensitive TORC2 complexes, as in yeast. TORC1 signaling increases protein translation, anabolic processes, and energy expenditure that is detrimental to a longer lifespan (Wullschleger et al., 2006). Depletion of many factors in this pathway leads to lifespan extension (Crawford et al., 2007; Hansen et al., 2007). From an epigenetic perspective, TOR-mediated expression of ribosomal proteins by pol II in worms may involve histone acetyltransferase activity as in yeast, although it has not been directly tested.

In flies, overexpression of TOR pathway proteins including dTsc1, dTsc2, PTEN, FOXO (homologous to worm DAF-16 discussed below), 4E-BP, or dominant negative versions of dTOR and dS6K, all extend lifespan by interrupting nutrient sensing and mimicking calorie restriction. Additionally, TORC1 inhibition by rapamycin also extends lifespan in some strains (Kapahi et al., 2004). The primary tissues where these pathways operate are the nervous system and fat body. Insulin signaling and TOR downregulation in flies improves cardiac function, locomotion and imparts neuroprotection against neurodegenerative diseases like Alzheimer's and Parkinson's (Hirth, 2010; Partridge et al., 2011), also suggesting major epigenetic changes underlying rapamycin treatment.

Calorie restriction and the insulin signaling pathway

One of the most conserved and well-studied pathways in aging is the insulin and insulin-like growth factor 1 (IGF-1) signaling (IIS) pathway first identified in worms. Nutrient sensing by insulin/IGF-1 engages DAF-2, the only known member of the insulin receptor family in worms. This initiates a cascade of phosphorylation events via the activity of phosphatidylinositol-3-kinase (PI3K) and Akt, ultimately inactivating and promoting the cytoplasmic sequestration of fork head (FOXO) transcription factor DAF-16. Under calorie restriction, DAF-16 is instead sequestered in the nucleus where it activates genes that promote longevity (Kenyon, 2005; Kenyon, 2010). In support of this pathway, it was shown that mutations in AGE-1, encoding PI3K, lengthen lifespan; these were the first long-lived mutants discovered in any organism (Friedman and Johnson, 1988). Subsequently, another group showed that mutations in DAF-2 double worm lifespan (Kenyon et al., 1993). It was further shown that DAF-16 mutations suppressed longevity phenotypes of AGE-1 and DAF-2, suggesting they operated in the same pathway (Lin et al., 1997; Ogg et al., 1997). Additionally, disabling DAF-2 arrests larval development in the dauer diapause stage that shows increased lifespan, inhibited reproductive maturity and delayed onset of senescence. Conversely, a high glucose diet decreases rate of dauer formation, shortens lifespan of DAF-2 knockdowns, decreases

DAF-16 activity and shortens lifespan of WT worms (Kenyon et al., 1993; Kimura et al., 1997). DAF-16 may be phosphorylated at sites (not targeted by Akt) by AMPK, a pro-longevity kinase that is activated by low cellular energy states. DAF-16 phosphorylated by AMPK can influence gene expression patterns independent of its subcellular localization (Greer et al., 2007).

Of interest to this review, DAF-16/FOXO identification provided an exciting link to discovery of epigenetic mechanisms operating in the nucleus and ultimately affecting lifespan. DAF-16 co purifies with the ATP-dependent chromatin remodeling complex, SWI/SNF. Further, DAF-16 physically recruits SWI/SNF to coregulated target genes for local chromatin remodeling and activation of genes affecting dauer formation, increased stress resistance and longevity (Riedel et al., 2013). There is considerable cross-talk between SWI/SNF type remodelers and histone acetylation and this could be an interesting area of future study.

Reduced levels of *Drosophila* insulin like peptides (*dilps*), loss of insulin receptor homolog InR (homologous to worm DAF-2) or insulin receptor substrates such as CHICO and Lnk leads to considerable lifespan extension (Clancy et al., 2001; Gronke et al., 2010; Slack et al., 2010; Tatar et al., 2001). Some mutant “dwarf” mice, including the Ames (Bartke, 2000), Snell (Flurkey et al., 2001), Laron (Zhou et al., 1997) and Little mice (Eicher and Beamer, 1976), have impaired insulin signaling and increased lifespan. Their diminished growth is due to impaired development of the pituitary (thus decreasing growth hormone (GH) and other pituitary hormone levels) or mutations that make them GH resistant. A consequence of reduced GH is low circulating levels of IGF-1 and therefore impaired insulin signaling. In mammalian models, there is a strong correlation between growth retardation and duration of life (Sherman and Campbell, 1935), and there have been efforts to induce growth retardation to benefit lifespan, including reducing growth hormone (GH) levels, mutating GH receptors and calorie restriction (McCay et al., 1989). We note that aging studies in dwarf mice should be interpreted with caution since longevity is not necessarily a result of delayed aging but an effect of delayed attainment of sexual maturity. Regardless, these mice provide an excellent model to investigate aging delay due to reduced insulin signaling. A common theme to all the dwarf mice are low levels of IGF-1; it is likely that the downstream effectors of the IGF-1 pathway may in fact impact epigenetic changes much like in worms (Bartke and Brown-Borg, 2004).

In light of these studies, there has been renewed interest in characterizing the lifespan benefit of drugs that target the insulin pathway. Metformin has been prescribed as an anti-diabetic drug for decades and works by decreasing hepatic glucose production, sensitizing insulin, reducing mitochondrial respiratory chain function and activating AMPK (Viollet et al., 2012). Animals studies with metformin showed remarkable lifespan and healthspan benefits mimicking calorie restriction (Martin-Montalvo et al., 2013). Currently, the US Food and Drug Administration has approved a clinical trial (named Targeting Aging With Metformin or TAME) to repurpose the drug as an anti-aging intervention. Thus, it is of immense interest to continue characterizing the epigenetic outcomes of reduced insulin signaling in numerous models, possibly uncovering new pathways for targeted intervention strategies.

The link between metabolism and histone acetylation via sirtuins and other enzymes

The diverse environmental stimuli (including nutrients) alter the metabolic activity and supply of cofactors and substrates within cells. Cells can sense this altered energy status by, for example, assessing NAD⁺ levels that are reduced with age. Sirtuins are NAD-dependent deacetylases implicated in aging in multiple model systems (Longo and Kennedy, 2006). Calorie restriction induces an oxidative metabolic state manifested in high NAD⁺ levels and consequently high Sirtuin activity. Overexpression of yeast Sir2 and Sir2 homologs (SIR-2.1 in worms, dSir2 in flies and SIRT1 in mammals) extend lifespan (Herranz et al., 2010; Kaeberlein et al., 1999; Kim et al., 1999; Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001); however, only yeast Sir2 has been strongly connected to calorie restriction pathways.

Importantly, Sir2 is a histone deacetylase and regulates lifespan in yeast through multiple epigenetic mechanisms impacting heterochromatin, extrachromosomal rDNA circles (ERCs), H4K16ac and subtelomeric gene expression as discussed above (Dang et al., 2009). Worms grown in presence of SirTuin Activating Compounds (STACs) like resveratrol (a plant polyphenol) show a slight (10%), but consistent, increase in lifespan (Wood et al., 2004). Boosting NAD⁺ levels by genetic or pharmacological means also extends worm and mouse lifespan (Mouchiroud et al., 2013; Zhang et al., 2016). Two models for Sir2 action have been found (1) in yeast, where Sir2 may stimulate heterochromatin formation or reduce H4K16 acetylation at subtelomeric loci to increase lifespan, (2) in mammals, where it may directly deacetylate FOXO transcription factors and regulate its target genes (Brunet et al., 2004; Daitoku et al., 2004; Frescas et al., 2005).

The effect of H4K16 deacetylation on fly lifespan is more complicated as different HDACs have disparate effects on lifespan. As in yeast and worms, dSir2 overexpression and STACs such as resveratrol and fisetin extend lifespan in flies acting via the calorie restriction pathway (Wood et al., 2004). However, haploinsufficiency of Rpd3, another HDAC targeting H4K16, also results in lifespan extension (Rogina et al., 2002). Although the Rpd3 findings are similar to yeast, *Drosophila* longevity is not mediated through heterochromatic gene silencing. Together, these data suggest that in flies, different deacetylases (dSir2 and Rpd3) function in opposite ways at euchromatin to affect longevity by influencing specific gene expression patterns (Frankel and Rogina, 2005). The disparity may stem from the multiple substrates targeted by these proteins but nevertheless underscore the importance of specific gene regulation in longevity, which, as discussed above, may be a more dominant mechanism regulating lifespan in some models.

Another metabolite, acetyl-CoA, increases in aged flies contributing to increased acetylation of H4K12 and key metabolic enzymes. Targeted reduction of ATP citrate lyase, an acetyl-CoA producing enzyme and the acetyltransferase for H4K12, Chameau, promotes longevity (Peleg et al., 2016). However, the exact role of acetylation in limiting lifespan in flies is still unclear. For one, different deacetylases, dSir2 and Rpd3, have different effects on lifespan. Second, increased H3K9ac achieved by mutation of Su (var) 2-1 has no effect on lifespan (Frankel and Rogina, 2005). Third, administration of different HDAC inhibitors like sodium butyrate, TSA, PBA and SAHA result in different changes in mean lifespan. The dosage, time of administration and genetic background of flies used are critical to observing lifespan

changes (McDonald et al., 2013). Thus, more work will be required to determine the key epigenetic changes underlying fly lifespan modulation.

The hallmark study that first reported mean and maximum lifespan extension by calorie restriction in a vertebrate model was conducted in rats (McCay et al., 1989). In mice, calorie restriction intervention can be initiated at weaning (3–6 weeks), one year or even up to 19 months to see health benefits. Adult-initiated calorie restriction (~1 year) increases mean but not maximum lifespan (Weindruch and Walford, 1982). Calorie restriction initiated in older mice (19 months) has significant effects on mean and maximum lifespans in addition to reducing cancer incidence (Dhahbi et al., 2004). Late stage benefit occurs at least partially through the mTOR pathway; treatment with rapamycin at 600 days also extended mean and maximum lifespans of male and female mice (Harrison et al., 2009). For a more elaborate discussion of possible calorie restriction effects on the epigenome, we refer the readers to a specialized review (Vaquero and Reinberg, 2009).

The lifespan effect of altering mammalian sirtuins is complex. Mammals have seven Sirtuins (SIRT1-7) with SIRT1 being the closest mammalian homolog of yeast Sir2. SIRT1 localizes to the nucleus and deacetylates H1K26, H4K16, H3K9 and a number of non-histone targets including p53, p300, Suv39h1, FOXO transcription factors and PGC1 α (Chen et al., 2012b). Unfortunately, altering systemic SIRT1 levels has not yet shown any lifespan changes in mice, although there are health benefits linked to SIRT1 expression (Libert and Guarente, 2013). Of note, there is one report of lifespan extension in mice by overexpression of neuronal SIRT1 (Sato et al., 2013).

In 1997, resveratrol was found to be a potential chemotherapeutic agent in mice (Jang et al., 1997). Resveratrol reduced the K_m for binding to both NAD and the acetylated substrate (Howitz et al., 2003; Mitchell et al., 2014). Later, it was shown that resveratrol had beneficial effects on health and survival of mice on a high calorie diet (Baur et al., 2006). SRT1720, another STAC, was capable of modestly extending mouse lifespan (Mitchell et al., 2014). One of the beneficial effects of SIRT1 activation is improved mitochondrial function. With age and declining NAD⁺ levels, mitochondrial (but not nuclear) OXPHOS subunits are downregulated resulting in a pseudohypoxic state. The effect is exaggerated by deleting SIRT1 and attenuated by increasing NAD⁺ levels, in this case by feeding mice NMN, a precursor of NAD⁺ (Gomes et al., 2013).

Surprisingly, the less studied SIRT6 was recently shown to promote longevity in male (but not female) mice by ~16% when overexpressed (Kanfi et al., 2012). SIRT6 is nuclear, deacetylates H3K9 and H3K56 and acts as a PARP1 ADP-ribosylase under oxidative conditions to promote DNA repair.

These observations support the model whereby histone acetylation and regulation via sirtuins and other metabolic enzymes regulates lifespan. Overall, the epigenetic mechanisms of sirtuin action and their precise targets in aging are still unclear; however, the connection to histone acetylation is strong.

Diseases of epigenetics and aging – laminopathies

Laminopathies are heterogeneous diseases characterized by deleterious changes to nuclear organization, resulting from mutations in genes encoding nuclear lamina proteins (Dechat et al., 2008). Hutchinson-Gilford progeria syndrome (HGPS) is a rare premature aging laminopathy disease that has been the subject of intense study. HGPS is caused by mutations in lamin A, with most cases involving the expression of progerin, a truncated dominant negative lamin A protein (De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003). HGPS is a complex disease with multiple phenotypes indicating possible defects in normal aging and underlying epigenetic regulation of aging. Of interest to this review, HGPS cells exhibit severe abnormalities in nuclear morphology (Goldman et al., 2004), compromised DNA damage repair, alterations in chromosome organization, abnormal heterochromatin and accelerated rates of cellular senescence (Burtner and Kennedy, 2010; Kudlow et al., 2007; Merideth et al., 2008; Norton et al., 2011; Prokocimer et al., 2013). Specifically, HGPS exhibits many alterations in heterochromatin factors, namely decreased levels of HP1, H3K9me3 and H3K27me3 and increased levels of H4K20me3 (McCord et al., 2013; Scaffidi and Misteli, 2005; Shumaker et al., 2006). Late passage HGPS cells exhibit loss of peripheral heterochromatin (Goldman et al., 2004), reduction in H3K9me3 and H3K27me3 levels (Scaffidi and Misteli, 2006; Shumaker et al., 2006), and the downregulation of H3K27me3 methylase EZH2 (Shumaker et al., 2006). A recent genome-wide analysis of H3K27me3 in HGPS cells suggested a redistribution of the remaining amount of this modification across the genome (McCord et al., 2013). In addition, pre-senescent H3K4me3 marks have been observed in proliferating HGPS cells, indicative of major chromatin reorganization in progeroid cells prior to senescence (Shah et al., 2013).

Laminopathies are well modeled in mice, including in the *Zmpste24* and *BubR1* models. *Zmpste24* encodes a metalloproteinase involved in maturation of lamin A, one of the major components of the lamin network (Bergo et al., 2002; Corrigan et al., 2005; Pendas et al., 2002). *BubR1* encodes a key protein involved in the mitotic checkpoint response, ensuring accurate mitotic chromosome segregation; levels of BubR1 are reduced in normally aged animals (Baker et al., 2004; Hartman et al., 2007; Matsumoto et al., 2007). Mutations in lamin A, *Zmpste24*, or *BubR1* result in premature aging, including the acceleration onset of various age-related pathologies. *BubR1* deficient mice also accumulate senescent cells, and in the *BubR1* hypomorphic mouse, targeted clearance of senescent cells results in delay of onset for age-related phenotypes and *BubR1* overexpression results in increased healthspan and lifespan (Baker et al., 2011), providing a strong link between onset of senescence and aging.

These models suggest other interesting links between epigenetic regulation and aging. *Zmpste24* mice have been shown to have hypermethylation at rDNA (Osorio et al., 2010), a phenotype associated with normal rodent aging (Oakes et al., 2003). *Zmpste24* mice have also been shown to have global hypoacetylation on core histones H4 and H2B, which has been attributed to changes in expression of key cell cycle and metabolic genes in pathways associated with age-related pathologies (Zhang et al., 2015). In addition, a recent study demonstrated that BubR1 is directly acetylated by the histone acetyltransferase CBP, and this modification targets the protein for degradation; however, acetylated BubR1 is as a

novel deacetylation target of SIRT2 (North et al., 2014). Overexpression of SIRT2 or treatment with a NAD⁺ precursor in *BubR1* hypomorphic mice results in increased BubR1 levels and increased median lifespan (Corrigan et al., 2005). However, SIRT2 does not restore BubR1 levels to WT and cannot reverse many of the age-related phenotypes.

We note that laminopathies are complex diseases and determining causality of aging is particularly challenging in these models. Specifically, it remains unclear whether reduction in lifespan and presence of age-related pathology directly results from the lamin or related mutations, or if the phenotypes are secondary to significant metabolic changes. Despite these challenges, it is of great interest to understand how the lifespan is extended in these premature aging models and uncover other epigenetic mechanisms that may underlie maintenance of normal levels of BubR1 or other key checkpoint factors whose reduction contributes to premature aging phenotypes or genetic instability.

Conclusions and Perspectives

This review provides a comprehensive overview of the compelling epigenetic evidence linked to aging from animal, tissue, and cell-based models that continue to be critical for identifying key longevity pathways. Studies of chromatin changes suggest two recurring themes in aging: (1) global upregulation of activating marks and downregulation of repressive marks and (2) gene-specific changes in chromatin states regulating expression of key longevity genes. These general themes are heavily influenced by environmental stimuli, nutrient signaling and metabolic state. While much has been uncovered in these studies, there remain many future challenges in epigenetic aging research, particularly with regard to characterizing key epigenetic changes that may be causal to aging. These challenges include linking specific chromatin modifications to changes in aging-specific signaling pathways using genetic manipulations, identifying causal epigenetic changes in age-related diseases, uncovering specific enzymes for targeted therapeutic strategies to improve lifespan and healthspan, linking metabolism to epigenetic outcomes in aging, particularly related to mitochondrial aging where a vast amount of literature is already available (Britic and Larsson, 2013) and optimizing epigenomic modification strategies to become a viable option for potential therapy.

A number of recent technological advances provide promising leads towards addressing these challenges (Supplemental Table 2). Single cell/low cell number genomics (Nawy, 2014; Shankaranarayanan et al., 2011) may prove to be critically important in identifying mutations in key epigenetic enzymes and/or aberrant patterns of epigenetic modifications in specific disease cohorts or cell types. Continuing advances in CRISPR (Cong et al., 2013) technology, including use of CRISPR technology for enzyme targeting, will also provide new methods for genetic and epigenetic manipulation. In particular, genomics coupled with direct manipulation of epigenetic factors and targeted epigenetic reprogramming will greatly advance our understanding of the role of epigenetic changes that are causal to aging.

In addition to these advanced tools, new studies in emerging aging models, such as eusocial insects, and in yet-uncharacterized models will provide additional opportunities for insight into key epigenetic mechanisms in aging. In the case of the Indian jumping ant

Harpegnathos saltator, a worker can replace a queen in the colony, resulting in a change in longevity, acquisition of reproductive function, and loss of worker behavior, all of which can ultimately be reversed. The epigenetic mechanisms that underlie this transition are of great interest, including characterization and manipulation of epigenetic patterning during development, which lead to key behavioral differences in these organisms (Simola et al, 2016). Particularly long lifespans have been observed in several types of deepwater fishes, various crustaceans, bow head whales, several turtles and naked mole rats (relative to other rodents) among others. While some may be unfeasible for creation of laboratory models, tissue and cellular studies of these or similar organisms may prove to be insightful. In addition, short-lived model organisms such as yeast, worms and killifish are useful for quick lifespan estimations (Table 1). Together with the technological advances highlighted above, new experimental avenues and models in aging research will provide key insight into the epigenetic pathways that underlie longevity and aging, and will likely identify factors and pathways that can be targeted to improve health and lifespan in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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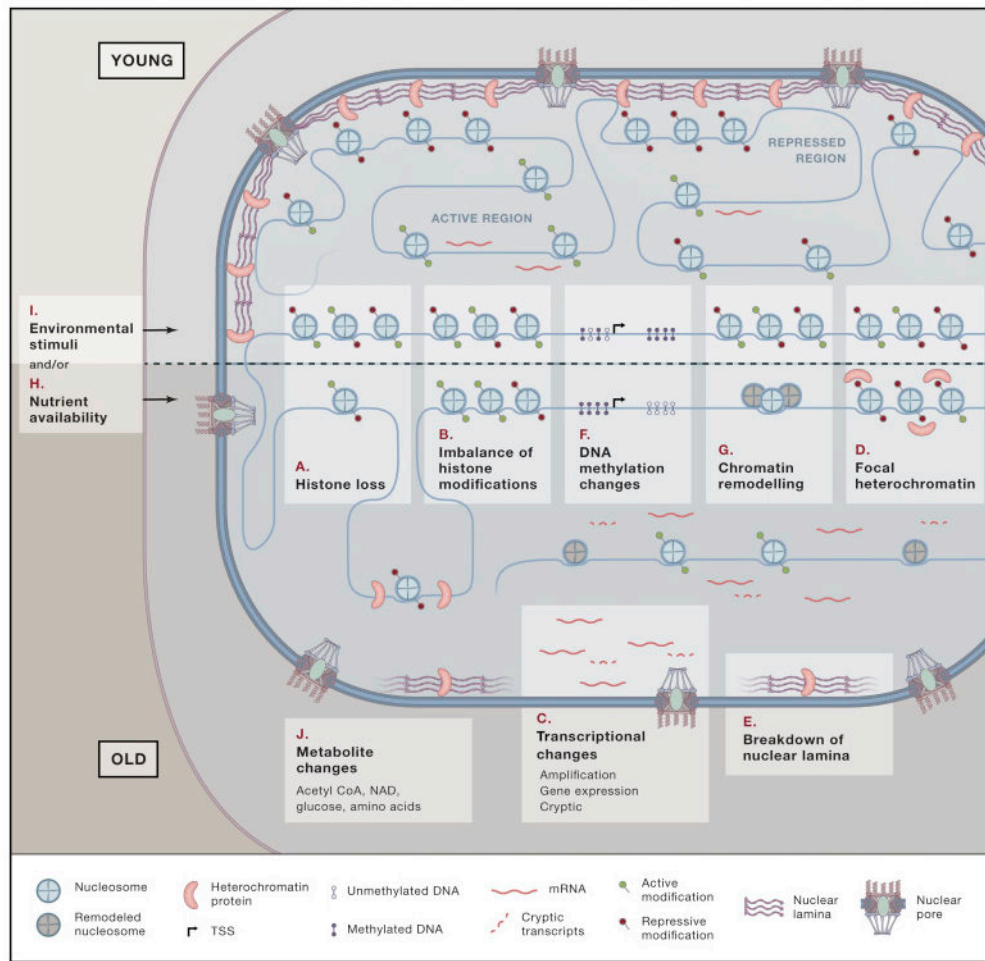


Figure 1. The epigenetic hallmarks of senescence and aging

Senescence and aging are characterized by (A) loss of histones, (B) imbalance of activating and repressive modifications, (C) transcriptional changes, (D) losses and gains in heterochromatin, (E) breakdown of nuclear lamina, (F) global hypomethylation and focal hypermethylation and (G) chromatin remodeling. These changes are heavily dictated by (H) environmental stimuli (I) and nutrient availability that in turn (J) alter intracellular metabolite concentrations.

Table 1

Pros and cons of models used in aging and longevity studies

Models	Pros	Cons
Yeast	Genetically tractable	Cannot model human aging since yeast expresses telomerase, lacks p53, no DNA methylation, no repressive histone methylation, and does not develop age-related diseases
	Non-essential gene deletion collection and essential conditional deletions available	
	Short lifespan	
	Two lifespan arms to investigate mitotic and post-mitotic aging	
Worm	Genetically tractable	Cannot model human aging, no DNA methylation and does not develop age-related diseases
	RNAi	
	Short lifespan	
	Mostly post-mitotic cells to investigate senescent decline and organismal aging	
Fly	Genetically tractable	Cannot model human aging and does not develop age-related diseases
	RNAi	
	UAS-GAL4 system	
	Short lifespan	
	Mostly post-mitotic cells to investigate senescent decline and organismal aging	
	DNA is methylated (very low)	
Killifish	Short-lived vertebrate model	Newly established model
	Shows age-related human pathologies	
	Stable lines can be constructed by CRISPR	
Normal mouse	Vertebrate model, highly orthologous to humans	Most strains used in aging research are inbred to a high degree that might not be representative of a genetically-diverse aging human population
	Shorter lifespan compared to other vertebrates/mammals (2–3 years)	
	Multiple genetic tools, knockout mouse models, inbred strains	
	Stable lines can be constructed by CRISPR	
	Multiple premature aging and long-lived models available	
Premature aging mouse models	Short lifespan	Epigenetic studies confounded by extreme consequences of genetic mutations
Long-lived dwarf mouse models	Useful models to study impaired insulin signaling	Most models show developmental delays rather than aging delays
Senescent cell cultures	Homogenous system	Senescent cells may not be relevant to in vivo tissue aging, unless the tissue accumulates senescent cells with age
	Stable lines can be constructed by CRISPR and RNAi	

Table 2

Repositories and tools for aging research

Models	Description	Link/Reference
Yeast	<i>Saccharomyces</i> genome database	http://www.yeastgenome.org/
	Published lifespan data	http://lifespandb.sageweb.org/ , McCormick et al, Cell Metabolism,2015
	Wilcoxon rank sum test to test significance of lifespan differences	http://data.kaeberleinlab.org/scripts/ranksum.php
	Yeast outgrowth data analyzer (YODA) for chronological lifespan assays	http://yoda.sageweb.org/
	Information about yeast gene deletion collection	http://www-sequence.stanford.edu/group/yeast_deletion_project/deletions3.html
	Library of histone mutants (plasmid)	(Nakanishi et al., 2008)
	Library of histone mutants (integrated)	(Dai et al., 2008)
	Screen for H3 and H4 residues affecting replicative lifespan	(Sen et al., 2015)
	Screen for yeast genes affecting replicative lifespan	(McCormick et al., 2015)
	Screen for yeast genes affecting chronological lifespan	(Powers et al., 2006)
	Worm	<i>C. elegans</i> information resource
http://www.wormbook.org/		
Genome-wide data in <i>C. elegans</i>		http://www.modencode.org/
Published lifespan data		http://lifespandb.sageweb.org/
Worm transcriptome data		http://spell.caltech.edu:3000/ http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/index.html?worm
Fly	Database for <i>Drosophila</i> genetics and molecular biology	http://flybase.org/
		http://www.fruitfly.org/
		http://www.flymine.org/
	Genome wide data in <i>Drosophila</i>	http://www.modencode.org/
Published lifespan data	http://lifespandb.sageweb.org/	
Killifish	Killifish genome browser	http://africanurquoisekillifishbrowser.org/
	Published lifespan data	http://lifespandb.sageweb.org/
Normal mouse	Mouse genome information	http://www.informatics.jax.org/
	Mouse ENCODE data	http://www.mouseencode.org/data
	Published lifespan data	http://lifespandb.sageweb.org/
	Lifespan of commonly used inbred strains	https://www.jax.org/research-and-faculty/research-labs/the-harrison-lab/gerontology/available-data
Premature aging mouse models	Targeted allele detail and overall phenotype	http://www.informatics.jax.org/marker/MGI:1890508https://techtransfer.universityofcalifornia.edu/NCD/20469.html
Long-lived dwarf mouse models	Targeted allele detail and overall phenotype	http://sageke.sciencemag.org/cgi/content/full/2001/1/tg11

Models	Description	Link/Reference
Cell cultures	Information about different cell types in the ENCODE project	http://genome.ucsc.edu/ENCODE/cellTypes.html
	Genome wide data on multiple cell types (ENCODE project)	https://www.encodeproject.org/
	Genome-wide epigenetic studies in human cells and tissues	http://www.roadmapepigenomics.org/
	Resource for Progeria research	http://www.progeriaresearch.org/
		https://catalog.coriell.org/
National Institute on Aging's (NIA) Aged Cell Bank at Coriell	https://catalog.coriell.org/	

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