CHAPTER

AGING EPIGENETICS: CHANGES AND CHALLENGES

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CHAPTER OUTLINE

Epigenetics of Aging and Longevity. https://doi.org/10.1016/B978-0-12-811060-7.00001-2

1. INTRODUCTION

Aging corresponds to the breakdown of cellular and tissue function over time, which is associated with increased prevalence of chronic diseases (e.g., neurodegenerative and metabolic disorders, cancer), ultimately leading to death. Evidence in invertebrate model organisms and human studies support the idea that aging is regulated at the genetic level but also by nongenetic factors [1,2]. Interestingly, even the lifespan of isogenic individuals reveals large differences between the first and last death in controlled environments [3], suggesting that even small environment variations may dramatically impact aging and lifespan. A number of environmental modulators of the aging process include dietary interventions [4], upregulated stress response [5], physical exercise [6], and circadian rhythms [7].

The strictest definition of 'epigenetics' only covers phenotypic changes that are heritable through generations without underlying changes to the genetic material [8]. However, in the broader definition, which will be used hereafter, 'epigenetics' encompasses alterations at the level of chromatin that may play a significant role in regulating gene expression. In eukaryotic cells, chromatin corresponds to a nucleoproteic structural polymer, whose basic units are nucleosomes. Nucleosomes are composed of ~150 bp DNA fragments wrapped around octamers of histone proteins, each unit containing two H2A, H2B, H3, and H4 histone proteins, which can be replaced by functional histone variants at specific loci (e.g., H2A.Z, H3.3, CENP-A) [9]. Chromatin can be found in two main states: euchromatin, a loose compartment permissive to transcription, and heterochromatin, a compact compartment that contains repressed regions of the genome. According to the 'histone code' hypothesis, combinations of histone posttranslational modifications are thought to modulate the accessibility and expression of underlying genes [10]. DNA methylation constitutes another layer of epigenetic regulation, the most well-studied type of which occurs in 'CpG' dinucleotides [11]. A final key layer of epigenetic regulation is attained through modulation of nucleosome positioning by ATP-dependent chromatin remodelers (e.g., SWI/SNF), which impacts regulatory sequence accessibility and higher-order chromatin compaction [12]. Several classes of noncoding RNAs (i.e., miRNAs, circRNAs, and lncRNAs) have been found to be able to modify transcriptional regulation and sometimes impact the chromatin landscape [13–15].

Epigenetic alterations are considered one of the hallmarks (pillars) of the aging process [16,17], a role supported by many changes to chromatin marks throughout life and by the impact of interference with chromatin regulatory complexes on the lifespan of model organisms [18–20,209]. Interestingly, accumulating evidence suggests that age-related epigenomic changes may interact with other hallmarks of aging, such as genome instability or loss of protein homeostasis [19]. Emerging evidence suggests that specific species of these ncRNA may become misregulated with aging [22–25] and may even partially drive aging or age-related diseases phenotypes [22,25,26]. In this review, we will focus on the potential impact and changes in DNA and histone modifications throughout aging.

To this date, most of the knowledge of chromatin regulation remodeling with age has relied on global assessment of changes. Only a few studies have attempted to interrogate genome-wide locus-specific epigenomic changes with aging, with the exception of DNA methylation studies. Understanding the global and locus-specific epigenomic changes that accumulate during aging, identifying corresponding molecular regulators of health and lifespan, will be crucial to eventually increase healthy youthful years of life, and potentially reverse some aspects of aging.

2. EPIGENETIC ALTERATIONS AND THE AGING PROCESS

2.1 THE 'AGING EPIGENOME'

The pervasiveness of age-related alterations in chromatin regulation across cell types and species is now well documented (recently reviewed in Refs. [18–21]). These epigenomic alterations are thought to underlie at least in part accompanying alterations in transcription with aging, ultimately impacting cell and tissue function. In this chapter, we will focus exclusively on studies of chromatin aging throughout organismal lifespan across model organisms (e.g., yeast, worms, flies, mice; Table 1.1).

2.1.1 Histone Deposition and Chromatin Structure in Aging

DNA packaging into higher-order chromatin structure impacts many cellular processes relevant to the aging process (e.g., transcription, DNA repair, and DNA replication [27]). Profound changes in global chromatin organization and structure have been observed during aging, and these changes have been linked to aging phenotypes in model organisms (Table 1.1). Chromatin organization and function can be affected by changes in core histone expression, incorporation of functional histone variants, or the activity of nucleosome remodelers, which are all relevant to the aging process.

The bulk of core histone expression is restricted to the S-phase of the cell cycle [28], and very little de novo synthesis occurs in postmitotic or terminally differentiated cells [29]. The longest-lived proteins in the proteomes of rat brain and liver identified using ^{15}N stable isotope labeling followed by mass-spectrometry include a number of canonical and variant histones proteins (e.g., canonical H2A and H2A.X), with stability in the order of months [30,31]. Interestingly, core histone protein levels decrease during yeast replicative aging [32], and in mammalian models of cellular senescence [33]. Muscle stem cells from old mice have lower transcript levels of histone genes [34]. Substantial histone reduction modulates genome-wide nucleosomal occupancy and global transcriptional outputs. In yeast, decreased histone expression is linked to a decrease in nucleosome occupancy and the aberrant upregulation of corresponding genes [35]. Consistently, reduced amounts of core, linker, and variant histones following deletion of the High Mobility Group Box 1 protein gene *Hmgb1* in mouse fibroblasts or deletion of its ortholog *nhp6* in yeast cells are associated to globally decreased nucleosome occupancy, increased chromatin accessibility, and increased transcription [36]. Interestingly, the experimental modulation of protein complexes controlling the exchange and deposition of histones into chromatin can modulate *Saccharomyces cerevisiae* lifespan: histone chaperone ASF1, which promotes histone deposition and stability, is required for normal replicative lifespan, whereas deletion of the HIR complex, which represses histone expression, increases yeast replicative lifespan [32]. Moreover, overexpression of histone H3 and H4, but not histone H2A and H2B, extends yeast replicative lifespan [32]. Interestingly, alterations of nucleosome occupancy have been observed in aging liver and may facilitate the activation of lipogenesis genes [37]. However, whether the observed remodeling results from changes in core histone expression or deposition is unclear. Future studies will need to evaluate whether total histone expression levels are a limiting factor in metazoan longevity remains unknown.

A number of histone variants (e.g., H3.3, macroH2A) that can replace canonical histones in the chromatin fiber have distinct genomic profiles of incorporation and are thought to have structural or regulatory impact [38]. A variant of histone H3, H3.3, has garnered particular interest in the context of aging [39]. Interestingly, mass spectrometry analyses have shown that H3.3 is highly enriched for 'active' posttranslational modifications (e.g., H3K4me3, H3K79me2) in drosophila and human cells [40,41], suggesting that it may be important for gene expression by modulating chromatin structure or

Table 1.1 Epigenomic Marks, Chromatin-Modifying Enzymes, and Regulation of Aging

Chromatin Modification Type	Mark	Functional Role	Change With Organismal Aging	Change Observed in Cell Type (*Species*)	Depositing Enzymatic Complex, Wild-Type Impact on Lifespan (*Species*)	Removing Enzymatic Complex, Wild-Type Impact on Lifespan (*Species*)	References
DNA methylation	5-mC	Transcriptional repression (?)	Minor global increase, Local increases and some local decreases Local increases and some local decreases Increase (LINEs), local decreases and rare local increases No global change	HSCs (*Mus musculus*) Small intestine, colon, lung, liver, spleen, brain, blood, kidney, muscle, pancreatic beta-cells (*Homo sapiens, M. musculus*) Sperm (*H. sapiens*) Cortex, hippocampus (*H. sapiens, M. musculus*)	dDNMT2 (?) + (*Drosophila melanogaster*)	N/A	[48,115,116,159, 189–195]
	5-hmC	Transcriptional activation (?)	Minor increase (SINEs, LTRs) (Minor) global decrease No global change or increase	Cerebellum (*M. musculus*) HSCs, Liver, T-cells, PBMCs (*M. musculus. H. sapiens*) Hippocampus (*M. musculus*)	N/A	N/A	[115,159,195–199]
Histone methylation	H3K9me2	Transcriptional repression	Decrease	Whole male flies (*D. melanogaster*)	N/A	N/A	[200]
	H3K9me3	Transcriptional silencing	Increase, remodeling Increase No global change Decrease	Head (*D. melanogaster*) Hippocampus (*M. musculus*) Cerebellum (*M. musculus*) Fibroblasts, Soma (*H. sapiens, C. elegans*)	N/A	JMJD-2 −/= (*Caenorhabditis elegans*) KDM4A −/= (*D. melanogaster*)	[201–205]
	H3K27me3	Transcriptional repression	Decrease Increase, remodeling	Soma (*C. elegans*) Brain, MuSCs, HSCs (*Nothobranchius furzeri, M. musculus*)	E(Z)−(*D. melanogaster*) MES-2−(*C. elegans*) (Polycomb complex)	UTX-1−(*C. elegans*) JMJD-1.2 + (*C. elegans*) JMJD-3.1 + (*C. elegans*)	[34,115,204,206–211]
	H4K20me3	Pericentric heterochromatin	Increase	Liver, kidney (*Rattus norvegicus*)	N/A	N/A	[212]

	H3K4me2	Transcriptional activation	Global increase, remodeling	Cortex (*Macaca mulatta*)	SET-9–(*C. elegans*) SET-26–(*C. elegans*)	T08D10.2–(*C. elegans*) SPR-5–(*C. elegans*)	[55,204,206,213–216]
	H3K4me3	Transcriptional activation	No change Decrease, remodeling Minor global increase, remodeling	Soma (*C. elegans*) Head (*D. melanogaster*) Neurons, HSCs, Muscle stem cells (*H. sapiens, M. musculus*)	SET1 + (*Saccharomyces cerevisiae*) ASH-2–(*C. elegans*) SET-2–(*C. elegans*) WDR-5–(*C. elegans*) (COMPASS complex)	RBR-2 ± (*C. elegans*) LID +/= (*D. melanogaster*)	[34,115,118,119,201, 204,206,215,217–220]
	H3K36me3	Transcriptional elongation	No global change, Remodeling Minor global decrease, remodeling	Yeast cells (*S. cerevisiae*) Soma, Head (*C. elegans, D. melanogaster*)	SET2 ± (*S. cerevisiae*) MET-1 + (*C. elegans*)	RPH1–(*S. cerevisiae*)	[78,79,201,204,217]
Histone acetylation	H3K56ac	DNA replication and DNA-damage response	Decrease	Yeast cells (*S. cerevisiae*)	N/A	SIRT6 + (*M. musculus*)	[64,90,152,221,222]
	H3K14ac	Transcriptional activation	N/A	N/A	GCN5 + (*S. cerevisiae*) (SAGA complex) IKI3 –/= (*S. cerevisiae, D. melanogaster, C. elegans*) SAS3 –/= (*S. cerevisiae, D. melanogaster, C. elegans*)	SIR2 +/= (*S. cerevisiae*) SIR2.1 +/= (*C. elegans?*) dSIR2 +/= (*D. melanogaster ?*) SIRT1+/= (*M. musculus*)	[62,63,147,223–233]
	H3K9ac	Transcriptional activation	N/A	N/A	IKI3 –/= (*S. cerevisiae, D. melanogaster, C. elegans*) SAS3 –/= (*S. cerevisiae, D. melanogaster, C. elegans*)	SIR2 +/= (*S. cerevisiae*) SIR2.1 +/= (*C. elegans?*) dSIR2 +/= (*D. melanogaster ?*) SIRT1+/= (*M. musculus*) SIRT6 + (*M. musculus*)	[62,63,90,147,152, 221–233]
	H3K18ac	Transcriptional activation	N/A	N/A	IKI3 –/= (*S. cerevisiae, D. melanogaster, C. elegans*) SAS3 –/= (*S. cerevisiae, D. melanogaster, C. elegans*)	N/A	[224]

continued

Table 1.1 Epigenomic Marks, Chromatin-Modifying Enzymes, and Regulation of Aging—cont'd

Chromatin Modification Type	Mark	Functional Role	Change With Organismal Aging	Change Observed in Cell Type (*Species*)	Depositing Enzymatic Complex, Wild-Type Impact on Lifespan (*Species*)	Removing Enzymatic Complex, Wild-Type Impact on Lifespan (*Species*)	References
	H4K16ac	Chromatin Compaction regulation	Increase Decrease	Yeast cells (*S. cerevisiae*) Liver, kidney (*H. sapiens, M. musculus*)	SAS2, -, *S. cerevisiae*	SIR2 + (*S. cerevisiae*) SIR2.1 +/= (*C. elegans*) dSIR2 +/= (*D. melanogaster*) SIRT1+ (*M. musculus*)	[62–64,147,223,224, 229–233]
	H4K12ac	Transcriptional elongation	Decrease Upon contextual fear conditioning	Hippocampus (*M. musculus*)	N/A	RPD3−(*S. cerevisiae, D. melanogaster*)	[143,234–236]
	H4K5ac	Transcriptional activation?			CBP + (*C. elegans*)	RPD3−(*S. cerevisiae, D. melanogaster*)	[234–237]
Other chromatin modification	H2BK123Ub	Transcriptional activation?			RAD6 + (*S. cerevisiae*) BRE1 + (*S. cerevisiae*) (H2B ubiquitination complex)	N/A	[238,239]
Histone expression	H2A[a]	Core histone	Decrease	Yeast cells (*S. cerevisiae*)	= (*S. cerevisiae*)	N/A	[32]
	H2A.1	Canonical histone	Decrease No change	Cortical neurons (*R. norvegicus*) Heart, liver, kidney (*Gallus gallus*)	N/A	N/A	[44]
	H2A.2	Canonical histone	Increase No change	Cortical neurons (*R. norvegicus*) Heart, liver, kidney (*G. gallus*)	N/A	N/A	[44]

	H2A.X	Histone variant	Increase	Cortical neurons (*R. norvegicus*)	N/A	N/A	[44]
	H2A.Z	Histone variant	No change	Cortical neurons (*R. norvegicus*)	N/A	N/A	[44]
	macroH2A	Histone variant, heterochromatin	Increase	Lung, liver (*M. musculus*)	N/A	N/A	[240]
	H2B[a]	Core histone	Decrease	MuSCs (*M. musculus*)	= (*S. cerevisiae*)	N/A	[32,34]
	H3[a]	Core histone	Decrease, occupancy changes Decrease No Change	Yeast cells (*S. cerevisiae*) Soma, Whole male flies, MuSCs, (*C. elegans, D. melanogaster, M. musculus*) Head (*D. melanogaster*)	+ (*S. cerevisiae*)	N/A	[32,34,200,201,204, 241]
	H3.1/2	Canonical histone	Decrease	Cortical neurons (*R. norvegicus*)	N/A	N/A	[44]
	H3.3	Active and telomeric chromatin	Increase	Quiescent T lymphocytes, heart, liver, kidney, cortical neurons (*H. sapiens, G. gallus*, *R. norvegicus*)	+ (*C. elegans*)	N/A	[42–45]
	H4	Core histone			+ (*S. cerevisiae*)	N/A	[32]
Chromatin structure/ accessibility			Remodeling	Liver (*M. musculus*)	ISW2−(*S. cerevisiae, C. elegans*) (ISWI complex)	N/A	[37,47]
					SWI/SNF+(*C. elegans*)	N/A	[46]
					CHD1−(*S. cerevisiae*)	N/A	[47]
					LET-418−(*C. elegans*) dMI2−(*D. melanogaster*) (NurD complex)	N/A	[242]

HSCs, *hematopoeitic stem cells;* PBMCs, *peripheral blood mononuclear cells;* MUSCs, *muscle stem cells;* LTRs, *long-terminal reports;* LINEs, *long-interspersed nuclear elements;* SINEs, *short-interspersed nuclear elements.*

Note that only chromatin changes occurring during physiological organismal aging are reported in this table.

[a]*Reported studies of core histone changes noted assessed all variants of a particular histone family together; ? indicates where the parameter is unknown or unconfirmed. The wild-type impact on health or lifespan corresponds to the role of the enzymatic complex on lifespan in physiological conditions based on experimental knock-down, mutation, or overexpression results ('−' to indicate that they normally restrict health or lifespan, '+' to indicate that they normally promote health/lifespan, '=' when no clear impact on lifespan was reported).*

function. Consistent with its expression being cell cycle independent (unlike its canonical counterparts), the H3.3 histone variant progressively accumulates with age in cells and tissues from *Caenorhabditis elegans* [42], chicken [43], rat [44], and human [45] (Table 1.1). The age-associated accumulation of H3.3 may lead to the incorporation of the variant into nucleosomes at aberrant loci and impact heterochromatin maintenance or gene expression during aging [39]. Interestingly, a recent study suggests that H3.3 accumulation improves stress resistance and is required for longevity mediated by the Insulin-FOXO signaling pathway in *C. elegans* [42]. The role, if any, of histone variants during human aging deserves further investigation.

ATP-dependent chromatin remodelers may also influence nucleosome positioning, higher-order chromatin structure and overall nuclear organization [12], and may impact organismal lifespan (Table 1.1). For instance, the SWI/SNF complex is required for longevity promotion by the Insulin-FOXO pathway in *C. elegans* [46]. Disruption of the ISWI complex extends *S. cerevisiae* and *C. elegans* longevity [47]. In addition to increased replicative lifespan, yeast that lack *ISW2*, a gene encoding a subunit of the ISWI complex, also display shifts in nucleosome positioning at thousands of stress-response genes [47]. Although these studies support the notion that chromatin remodelers can impact metazoan aging, their importance in the physiological regulation of aging is still unclear.

2.1.2 DNA Methylation

Another core mode of epigenomic regulation is attained through direct modification of the DNA molecule, such as DNA methylation. Many studies have focused on methylation of DNA on carbon 5 of Cytosines (i.e., 5-methylcytosine, or 5-mC) in 'CpG' dinucleotides (cytosine followed by guanine in the 5′ → 3′ direction; see Glossary), which is usually associated with heterochromatin and gene repression [11]. While 5-mC is the best studied form of DNA methylation, other types of DNA methylation have been described, such as cytosine methylation at non-CpG dinucleotides [48], 5-hydroxymethylcytosine (5-hmC) [48], and, more recently, N6-methylation of adenines (6-mA) [49–51]. Interestingly, human studies suggest that 5-mC DNA methylation can reflect chronological age, or to some extent 'biological' age, and DNA methylation profiles can be used to build a molecular 'aging clock' [52–54]. The precise impact of CpG methylation on longevity remains an open question [21]. Interestingly, recent work has suggested that DAMT-1, the putative 6-mA DNA methyltransferase in *C. elegans*, is involved in a paradigm of transgenerational inheritance of lifespan extension [55]. Thus, determining the biological significance and functional relevance of DNA methylation in mammalian aging will require further study.

2.1.3 Posttranslation Modifications of Histone Proteins

Histone proteins are subject to extensive posttranslational modifications, which associate differential accessibility and expression of underlying genes [10,56]. Some histone marks, such as H3K4me3 or H3K36me3, have been associated to an active or open chromatin environment, whereas other marks, such as H3K9me3 or H3K27me3, are linked to regions of repressed chromatin [56]. Extensive changes in global levels of specific posttranslational histone modification have been reported across cell types and species (Table 1.1) and underlie the proposed status of epigenetic alterations as hallmarks or pillars of aging [16,17].

It is interesting to note that opposing trends with aging have been observed for the same histone modification (e.g., H3K27me3) depending on the cell type, or species under study (Table 1.1). Consistently, chromatin-modifying enzymes with the same activity (e.g., H3K27me3 demethylases

UTX-1 vs. JMJD-2) have been observed to have opposing effects on lifespan. These apparently contradicting results suggest that, rather than the global assessment of changes, measuring changes at specific loci targeted by specific enzymes may be more relevant to understand the epigenomic changes associated with aging and longevity. Thus, it will be crucial to investigate genome-wide patterns of epigenomic changes with age in specific cell types to understand the biological significance of the aging epigenome (see Section 4).

2.2 IMPACT OF ENVIRONMENTAL STIMULI ON THE AGING EPIGENOME

Nongenetic or environmental factors, such as dietary intake, physical exercise, or circadian rhythms, can influence aging and longevity dramatically [4,7]. Although causal evidence linking the environmental cues to aging and longevity through specific chromatin changes is still missing, emerging evidence suggests that these factors can also impact the chromatin landscape [21].

Modulation of nutrient intake is an environmental cue whose impact on aging and longevity has been extensively studied. Indeed, dietary restriction (DR), which corresponds to a reduced dietary intake without malnutrition, has been associated to longevity and to decreased signs of aging across many organisms [4]. DR induces profound changes in gene expression across tissues and cell types and can also impact the chromatin landscape [57,58]. For instance, thousands of nucleosomes are repositioned upon DR in yeast [47], and shifted positions partially overlap with nucleosomes that are remodeled in the long-lived *ISW2* deletion mutant [47]. DR was also associated to a delay in the age-linked loss of facultative heterochromatin in Drosophila [59]. Interestingly, high body mass index (suggestive of high food intake), results in 'older' DNA methylation profiles in human liver [60]. The Class III NAD-dependent histone deacetylases sirtuins are important mediators of DR-induced longevity across species [61–63]. Interestingly, the impact of yeast sirtuin SIR2 on lifespan requires intact H4K16, SIR2's target deacetylation residue [64]. Interestingly, modulation of nutrient intake may also lead to epigenetic transmission of longevity phenotype in its strictest definition. Indeed, worms whose grandparents were starved display a 22% to 70% increase in organismal lifespan compared to the control worm group up to the third generation [65]. Though its final impact on aging and longevity is unclear, the transgenerational epigenetic inheritance of metabolic states has also been described in mammals [66]. Thus, nutrient intake modulation has important ties to the regulation of both longevity and chromatin states.

3. SIGNIFICANCE OF THE AGING EPIGENOME

Though it is clear that many epigenomic changes occur with aging, how these changes may ultimately impact the tissue and cell biology is less clear. Because of the potential role of chromatin as a regulatory platform, age-related epigenomic changes may foster biological instability. First, changes to the chromatin landscape throughout life may lead to decreased transcriptional precision and decreased cell and tissue function. Second, accumulating evidence suggests that the chromatin landscape is key to promote genome stability, a feature that may be impacted by age-related epigenomic remodeling. Thus, 'epimutations' (i.e., aberrant or atypical changes in epigenetic states), which seem to increase stochastically with age [67,68], may themselves promote further genomic instability.

3.1 AGE-RELATED LOSS OF TRANSCRIPTIONAL PRECISION

Many age-related changes in gene expression have been described across cell types and species [69,70], and stereotypical epigenomic changes accumulated throughout life may drive the aging transcripome at least partly. Other aspects of transcription may also be regulated by the chromatin landscape (e.g., pre-mRNA splicing and H3K36me3) [71]. In addition to transcriptional levels, age-related remodeling of cellular epigenomes could also adversely impact other aspects of transcription precision. The resulting loss of robustness of transcriptional networks may be responsible, at least in part, for the functional decline that is also associated with aging.

The robustness and integrity of transcriptional networks has been observed to decay during aging in *C. elegans* [72] and in mice tissues [73,74]. Whether aging is also associated to increased cell-to-cell transcriptional noise, another aspect of transcription precision, is still an open question. Indeed, whereas increased transcriptional noise has been observed for 11/15 tested genes in cardiomyocytes with aging [75], there were no detectable changes in transcriptional noise in hematopoeitic stem cells (HSCs) from old mice for any of the six assayed genes [76]. It is important to note that, because of technical limitations, these pioneering studies were limited to few genes and cell types. Recent advances in single-cell profiling techniques [77] now allow high-resolution genome-wide analyses of single-cell transcription across diverse cell types and will be key to understand the significance of transcriptional noise regulation during aging.

Age-dependent changes in chromatin modification may impact the aspects of transcriptional precision. Recent studies support an important function for the H3K36me3 mark in promoting transcriptional precision during the aging process. Sustained H3K36me3 levels throughout life leads to decreased gene expression fluctuations with age and promotes *C. elegans* longevity [78]. Similarly during yeast aging, loss of H3K36me3 levels is associated to increased cryptic transcription, and deletion of the H3K36me3 demethylase gene *Rph1* leads to extended lifespan [79]. Other chromatin features or regulators, such as HDACs [80] or changes in H3K4me3 breadth [81] may also influence specific aspects of transcriptional precision. Interestingly, *C. elegans* individuals treated with mianserin or carrying the longevity promoting *daf-2* mutation (i.e., with activated insulin/FOXO signaling pathway) display both a suppression of transcriptional drift with aging and increased lifespan [82]. Further work will be needed to disentangle the relationship between chromatin and transcriptional precision during aging.

3.2 LINKS BETWEEN EPIGENETIC AND GENOMIC INSTABILITY WITH AGE

Emerging evidence suggests that accumulated errors in DNA repair and genome replication may partially drive the age-related accumulation of mutations but also that of 'epimutations' [83,84]. Indeed, aging is accompanied by a progressive failure of DNA repair pathways [85], which may result from a growing burden of genomic instability events (e.g., single nucleotide mutations, aneuploidy, transposon insertions) [83]. Though it is unclear whether increased DNA repair activity is protective against aging phenotypes, an important role for DNA damage reparation during aging is supported by the progeroid phenotype associated to mutations in genes encoding the DNA repair machinery [86]. Aging is also associated to elevated levels of persistent DNA-damage signaling [68,84], which can foster local changes in chromatin structure and epigenetic modifications [84,87]. DNA-damage signaling can promote the recruitment of chromatin-modifying enzymes (e.g., SIRT1, SIRT6, Polycomb repressor complex) to repair sites [84]. The SIRT1 and SIRT6 enzymes are thought to locally promote genomic stability and telomere integrity [88–91].

In eukaryotes, endogenous mobile genetic elements, or 'transposable elements' (TEs) represent 30%–80% of the genome [92], which usually heterochromatinized in young healthy cells [93]. TE activity leads to extensive genomic instability [94]. Interestingly, increased TE activity has been reported in several species with age [94–100] and is associated with neurodegenerative diseases in humans [101]. Conversely, DR is associated to an attenuation of age-related TE derepression in the liver and skeletal muscle of aged mice [98] and in *Drosophila* [100]. TE derepression is thought to result at least partly from age-related heterochromatin loss, and sirtuin SIRT6 could play an important role in this process, since its activation leads to enhanced heterochromatin and transcriptional repression in fibroblasts, heart, liver, and brain from young mice [99]. Accumulating evidence supports the idea that TE activation has a deleterious impact on organismal lifespan. Flies that lack the Argonaute gene *Ago2* exhibit exacerbated transposition and a shortened lifespan [97], whereas flies with additional copies of the *Dicer2* or *Su(var)3–9* genes display sustained repression of TEs throughout life and extended longevity [100]. Thus, aberrant chromatin remodeling may underlie increased transposition during aging and ultimately promote age-related dysfunction.

4. THE POWER OF GENOMICS: GLOBAL VERSUS GENOME-WIDE LOCUS-SPECIFIC AGE-RELATED CHANGES

In eukaryotes, regulation of gene expression occurs at multiple levels resulting from a complex interaction between noncoding *cis*-acting sequences (e.g., enhancers) and transcription factors (TFs) that together determine if a particular gene will be active or silent. Growing evidence indicates that chromatin modifications and organization (i.e., the epigenome) play a critical role in regulating gene expression at multiple layers [102–104], such as by facilitating or preventing the access of TFs to regulatory sites and by organizing three-dimensional (3D) genome structure. Disruption of the epigenomic landscape—chromatin accessibility and structure—triggers failures in precise transcriptional regulatory programs and ultimately leads to cellular dysfunction and pathologies [105]. As outlined previously, aging impacts various features of the chromatin, including chromatin accessibility and interactions [27]. However, very little is known about which specific loci of the mammalian (or human) genome go through chromatin changes with aging. To date, most studies focused on assessing the aging-related epigenomic changes at the global level, mostly by profiling histone modification levels using global quantification methods such as Western blotting or mass spectrometry (reviewed in Ref. [21]). Although informative these studies failed to capture which genomic loci undergo epigenomic changes with aging (i.e., locus-specific changes). To precisely uncover these changes, epigenomes of many mammalian cell types have yet to be profiled and compared across young and elderly samples. Profiling these cells and uncovering aging-related epigenomic changes genome-wide will give us an opportunity to describe transcriptional programs that are activated or repressed with aging in diverse cell types and tissues.

4.1 DNA-METHYLATION PROFILING IN AGED HUMAN CELLS

Previous studies using DNA methylation microarrays measured the methylation status across a large set of CpG sites in blood cells and revealed aging-induced methylation changes in human immune cells [106–109], which may be linked to immune function declines and even disease incidence and mortality [110]. Moreover, it has been shown that aging-associated methylation patterns take place prematurely

in certain diseases, such as Down's syndrome [111] and HIV [112]. Although leading to highly predictive computational models, these assays do not provide a genome-wide view of epigenomic changes since they only profile the methylation status of the probes available on the microarrays and cannot uncover the full complexity of genome-wide epigenetic landscapes [113]. More recent technologies, such as whole genome bisulfite sequencing or the more targeted reduced representation bisulfite sequencing [114] will likely deepen our understanding of genome-wide 5-mC DNA methylation changes and the biological significance of these changes throughout lifespan.

4.2 LESSONS FROM GENOME-WIDE PROFILING OF CHROMATIN LANDSCAPE WITH AGING

To date, only a handful studies have profiled and compared histone modification and/or chromatin landscapes genome-wide in mammalian cells with aging. Among these, several studies reported genome-wide histone modification changes in purified mouse cells with aging. Liu et al. [34] isolated quiescent and activated skeletal adult muscle stem cells, also known as satellite cells, (i.e., qMuSCs and aMuSCs) from young and old mice to assess gene expression profiles, as well as H3K4me3, H3K27me3, and H3K36me3 genomic patterns. Interestingly, aging of qMuSCs was associated with the accumulation of repressed chromatin domains, potentially explaining their functional decline with age. In another study, Sun et al. [115] studied aging-associated changes in gene expression, DNA methylation, and histone modifications (i.e., H3K4me3, H3K27me3, and H3K36me3) in purified mouse adult HSCs. They observed an increase in the number of loci marked with H3K4me3 (i.e., H3K4me3 peaks), especially encompassing gene promoters associated with HSC identity and self-renewal, suggesting that these aging-related epigenetic changes may contribute to increased stem cell self-renewal and decreased differentiation ability with aging. In a recent study, Avrahami et al. [116] profiled gene expression, DNA methylation, and several histone modification marks (e.g., H3K4me1, H3K27ac) in fluorescence-activated cell sorting (FACS)–purified pancreatic β-cells in young (4–6 weeks) and old (16–20 months) mice. They observed a global drift in DNA methylation in aged cells, with highly differential methylated regions becoming more 'leveled' with age (i.e., displaying less extreme differences). Surprisingly, the genome-wide analysis also revealed an upregulation of key pancreatic islet TFs Pdx1 and NeuroD1 with aging, suggesting that aging is not always coupled with a functional decline in mammalian cells [116].

A major hurdle in analyzing genome-wide changes in chromatin profiles during aging is the ability to profile epigenomes of low cell numbers. To address this challenge, Zheng et al. [117] recently developed novel Chromatin Immunoprecipitation followed by high-throughput sequencing (i.e., ChIP-seq) based assays that allow sensitive profiling of histone modification marks from as few as 500 cells without increasing polymerase chain reaction amplification cycles (i.e., 'Recovery via protection ChIP' or 'RP-ChIP-seq' and 'Favored Amplification RP-ChIP-seq' or 'FARP-ChIP-seq'). The authors took advantage of RP-ChIP to map H3K4me3 from single lenses dissected from young (30-day-old) and old (>800-day-old) mice. They identified 613 gene promoters that exhibit age-related changes in H3K4me3 levels [117]. Interestingly, a significant aging-related increase in H3K4me3 peak height and width was observed in two loci associated with cataract [117]. Moving past age-related changes in histone modifications, Bochkis et al. [37] profiled gene expression, nucleosome occupancy profiles, as well as TF Foxa2 and histone deacetylase Hdac3 in liver samples of young (3 months) and old (21 months) mice. They observed that regions that lose nucleosome occupancy with aging are enriched in putative Forkhead DNA-binding motifs, which is consistent with the increase binding in Forkhead factor Foxa2

that they observed at these sites with age [37]. Genome-wide binding patterns of Foxa2 and Hdac3 during aging provided a potential mechanistic explanation for gene expression alterations that lead to age-associated liver steatosis (i.e., fatty liver disease) [37].

Though patterns of DNA methylation with aging have been relatively well studied in humans, few studies have investigated changes in histone modifications with human aging. A series of studies in particular have investigated changes in the chromatin of neuronal and nonneuronal nuclei collected from postmortem human prefrontal cortex samples. In a pioneering 2010 study, Cheung et al. [118] profiled H3K4me3 histone mark in prefrontal cortex cells from 11 postmortem individuals' ages ranging from 0.5 to 69 years. Though they observed developmental decrease in H3K4me3 levels at approximately 600 developmental gene promoters during the first year after birth, remodeling in the H3K4me3 profiles was less extensive in the elderly (>60 years) prefrontal cortex neuron samples. In a follow-up study, they increased the cohort size to 36 human prefrontal cortex specimens (ages from 34 gestational week to 81 years old) and identified 1157 genomic loci that show developmental changes in H3K4me3 intensity levels [119]. In agreement with their previous study, most of these changes were defined by a rapid gain or loss of the H3K4me3 mark during the late prenatal period and the first year after birth. They observed slower changes during early and later childhood and minimal changes in adulthood.

Together, these studies reveal the power of genome-wide mapping of histone modification marks and chromatin states to uncover age-related epigenome remodeling in mammalian cells and to understand biological significance and implications of these remodeling events.

4.3 ADVANCES IN EPIGENOME PROFILING IN HUMAN CELLS

Until recently, a major obstacle in front of profiling young and aged human cells have been the abundant input material required by existing protocols for genome-wide epigenome profiling. For example, chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq) is a technology that produces high resolution, genome-wide profiles of histone marks and DNA–protein interactions. However, standard protocols require abundant starting material (>1 million cells). This barrier has been particularly difficult to overcome in clinical samples, owing to challenges in obtaining the cell numbers necessary for high data quality with these experiments.

In recent years, mainly driven by big consortia efforts, vast amounts of epigenomic data have been generated in human cell lines and primary cell types. The ENCODE [103] and Roadmap Epigenomics [104] consortia provided the research community with reference epigenomes (histone modifications, chromatin interactions, chromatin accessibility, and DNA methylation profiles) as well as computationally inferred functional annotations (e.g., enhancers, insulators) in 111 human cell lines and types [120–123]. These reference epigenomes have revolutionized our understanding of transcriptional programs in human cells by providing multifaceted and genome-wide epigenomic data along with experimental and computational advances in generating and analyzing genomic data. Notably, analyses of these reference epigenomes have revealed the importance of noncoding regulatory elements for governing cell-specific functions and how the functions of these elements become disrupted in human pathologies. Preliminary studies suggest that aging is also associated with epigenomic changes that reside in noncoding enhancer sequences, likely altering gene regulation programs and not gene sequences themselves. Recent advances in epigenome profiling techniques enable generating various genomic maps from small cell numbers (e.g., clinical samples) and even from single cells. These powerful breakthroughs will help us precisely define aging-associated epigenomic changes at coding and

noncoding loci in diverse human cells and uncover their implications for transcriptional regulatory programs. Among the recent advances in epigenome profiling, The Assay for Transposase Accessible Chromatin (ATAC-seq) technology was developed to interrogate chromatin accessibility from small cell numbers [124,125], and even from single cells [125,126]. ATAC-seq surmounted a major technical barrier and enabled profiling chromatin accessibility of clinical samples with high accuracy and reproducibility [127–129]. Application of this recent technology on human cells of young and elderly individuals hold the promise to uncover which regions of the human genome is going through chromatin accessibility changes with aging, and what are the implications of these changes on cell functions.

Other chromatin features also change with aging including the chromatin structure and interactions [27]. Advances in genomic technologies have revealed information regarding 3D chromatin conformation and have shown that many regulatory elements that are distal on the linear genome map are actually in close physical proximity with each other as a result of the 3D chromatin structure. Current technologies for capturing this 3D structure and chromatin interactions between active regulatory elements include Chromosome Conformation Capture–based methods (3C) [130], 4C [131], 5C [132], Hi-C [133], and Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET) [134]. Among these methods, ChIA-PET technology genome-wide maps long-range interactions mediated by a protein, such as promoter-enhancer interactions mediated by RNA polymerase 2 (Pol2)—an information essential to understand gene regulatory programs [134]. However, a major drawback of ChIA-PET in the context of aging epigenomics is its requirement for very high cell numbers (~100 million cells). A recent technology, namely Hi-CHIP [135], has increased the sensitivity of chromatin interaction profiling while lowering the required cell numbers. With Hi-CHIP, protein-mediated chromatin interactions can be captured from as little as 1 million cells, which is a 100-fold improvement over the ChIA-PET technology.

Reference epigenomes in human and mouse cells and tissues have transformed our ability to understand transcriptional regulation and highlighted the key differences in chromatin states between healthy young tissues. The recent advances in profiling the chromatin accessibility and chromatin structure will accelerate our ability to map and contrast the epigenomes of young and aged cells. These epigenomic profiles hold the key to uncovering how transcriptional programs are established in diverse human cells, and how they are disrupted throughout aging.

5. EMERGING CHALLENGES IN THE FIELD OF AGING EPIGENOMICS

In spite of our growing understanding of the role of chromatin in aging and technical progress in our ability to map the remodeling of many aspects of the chromatin landscape, a number of outstanding challenges are emerging. In this section, we highlight three biological challenges and two analytical challenges that we believe need to be taken into account at this juncture. First, we will discuss so-called ‘epigenetic drugs’ and their potential efficacy to slow down or reverse aspects of aging. Second, the importance of sex-dimorphism in the regulation of aging in general, and in epigenomic aging in particular, needs to be further explored. Third, the importance of epigenomic drift in immune decline needs to be assessed, as this will have tremendous impact on improving the health of the elderly individuals. Next, with the accumulation of various genomic data sets throughout aging, the risk is to lose the ability to synthesize information and identify major aging-related trends, which highlights the importance of developing powerful data-integration methods. Finally, a major caveat is that many aging studies conducted on tissues likely discover both cell intrinsic changes and changes due to altered cellular composition of the profiled tissue.

An important research avenue moving forward is to assess the relative importance of cell-intrinsic versus cell-compositional changes potentially with experiments conducted on sorted cells. However, reliable markers for cell sorting are not available for all cell types, and the expression of such markers may itself be influenced by aging; thus, we also highlight the need to address this question.

5.1 TOWARD EPIGENETIC LONGEVITY DRUGS?

The plasticity of chromatin states in general, and of aging chromatin states upon environmental changes in particular, suggests that chromatin itself could be important therapeutic target to promote healthy aging in human.

A growing number of studies have explored the hypothesis that aged somatic cells could be 'rejuvenated' through in vitro reprogramming to an induced pluripotent stem cells (iPSCs) state [136]. Indeed, iPSCs derived from old donors have been previously associated with improved hallmarks of cellular aging [136], in particular with resetting to a more youthful state of the telomere size, gene expression patterns, and oxidative stress levels. In a recent study, it has been shown that short-term induction of reprogramming in vivo by transient overexpression of the Yamanaka factors (Oct4, Sox2, Klf4, and c-Myc) improves the hallmarks of aging and extends the lifespan in a mouse model of premature aging and in human cells [137], suggesting that in vivo reprogramming has the capacity to rejuvenate mammalian cells and reverse symptoms of aging. These studies also highlight the significance of epigenetic changes as potential drivers of aging-related cellular deterioration and the plasticity of the aging process. Future studies are needed to uncover mechanisms behind reprogramming-related cellular rejuvenation and to establish whether this phenomenon can be safely used to rejuvenate human cells.

Chromatin-modifying enzymes themselves could constitute therapeutic targets for healthy aging in human. Indeed, small molecular inhibitors of chromatin modifiers have been identified and have been successfully used in anticancer therapies [138,139]. Interestingly, treatment with class I and II HDAC inhibitors (e.g., TrichostatinA, Sodium Butyrate) have been shown to increase the lifespan of model organisms [140–142] or to improve cognitive aging in mice [143]. An array of specific inhibitors (e.g., SRT1720) for class III HDACs (i.e., Sirtuins) has been developed. Consistent with a role of sirtuins in aging regulation, treatment of mice with these inhibitors was associated to increased lifespan [144–146], and improvement of several health span parameters, including neuroprotection [147], metabolic health [144], or preservation of bone density [145,148]. There has been less focus on drugs targeting histone methylation. Interestingly, a recent study showed that treatment with inhibitors of H3K79 methyltransferase DOT1L (i.e., epz-4777, epz-5676) improved the lifespan and the accelerated aging phenotype of *Zmpste24*−/−progeroid mice [149]. Improving the specificity of epigenetic drugs and testing their efficacy in different contexts could be instrumental to treat age-related diseases. A major hurdle for epigenetic drug design for human health and longevity will be to minimize potential undesired and potentially deleterious pleiotropic effects.

5.2 SEX-DIMORPHISM AND IMPLICATIONS

Despite the progress of modern medicine, human longevity remains sex-dimorphic, with the life expectancy of women systematically exceeding that of men [150]. Though laboratory mice do not display consistent sex-dimorphism in lifespan [150], many experimental interventions that successfully extend

the life and health span of mice display sex-dimorphic responses [4,151,152]. For instance, rapamycin treatment preferentially extends female lifespan, whereas acarbose treatment preferentially extends male lifespan in mice [4]. In control conditions, thousands of genes can display sexual dimorphic expression across a range of tissues in mice and humans [153–156]. Interestingly, DR, a regimen typically associated to increased longevity and health, leads to a feminization of the gene expression profile of male mouse livers (i.e., renders the gene expression profile more similar to that of female mice) [157]. This observation raises the intriguing possibility that sexual dimorphic gene expression may indeed play an important role in aging and longevity. However, the molecular mechanisms that underlie gender differences in aging and lifespan regulation are still poorly understood.

Recent studies comparing male and female epigenomic profiles across various tissues have revealed sex-dimorphic chromatin features, specifically chromatin accessibility in human T-cells [128], a panel of histone modifications in Roadmap Epigenomics tissues [158], 5-mC and 5-hmC DNA methylation in mice hippocampi [159]. Though a large part of these sex differences likely directly stems from differential sex chromosome ploidy, a number of these differences were also identified on autosomes, suggesting that chromatin may be regulated differently in male versus female cells [128,158,159]. Part of the explanation could be that several genes encoding important chromatin regulators, such as methyl-CpG-binding protein *MeCP2* and H3K27me3 demethylases *UTX* and *UTY*, are situated on the sex chromosomes in humans, and thus expressed in a sexual-dimorphic manner [156]. Despite the potential significance of sex-dimorphism in the aging epigenome and its implications for human health, there is remarkably little known on differential molecular regulation of the female versus male lifespan, and specifically on how epigenetic mechanisms may play a part in this difference.

A key difference in the male and female body environment resides in circulating sexual hormones. Sex steroid hormones (e.g., estrogens like estradiol, or androgens like testosterone) are systemic endocrine factors that decline during aging in humans of both sexes. For instance, in humans, menopause marks the end of ovarian endocrine activity and is associated to a shutdown of female sex steroid hormones synthesis. These hormonal changes are likely to impact gene expression in woman tissues, as estrogens are known to act through nuclear receptors, which can directly modulate chromatin organization and downstream transcriptional activity [160,161]. Interestingly, treatment with estradiol leads to increased histone acetylation in ex-vivo brain slices from young rats but not in slices from old rats [162]. In postmenopausal women, estrogens deprivation is thought to promote the onset of osteoporosis, cardiovascular diseases, immunity decline, and neurodegeneration [163]. Consistently, a recent study showed menopause is associated to accelerate aging according to the DNA methylation epigenetic clock [164]. Future studies will need to examine the mechanistic underpinnings of sex-dimorphic aging in mammals, disentangle the role of sex-steroid hormones in this process, notably through the aging epigenome.

5.3 EPIGENOMICS OF IMMUNE SYSTEM AGING

Aging impacts all of body's cell types and tissues, which leads to changes in cellular functions. Among different cells and tissues, aging-related functional decline in immune cells plays a significant role on our health. The immune system is gaining increasing attention nowadays because of its potential impact on targeted cancer therapies, and on human lifespan and health span extension. The immune system goes through significant changes with aging, including the two 'hallmarks' of immune system aging: (1) functional decline of the adaptive immune system (i.e., immunosenescence), and (2) an increased

permanent systemic inflammation state, also known as "inflamm-aging" [165–167]. These changes decrease the immune system's ability to generate protective responses to immunological threats in the elderly and lead to increases in diseases and infections [168–170]. Transcriptome profiling studies have revealed changes in gene expression during aging in human peripheral blood mononuclear cells (PBMCs) and purified immune cells [171–173]. Moreover, array-based DNA methylation mapping revealed that immune aging is also associated with methylation changes at specific CpG sites that could be highly predictive of organismal chronological and biological age [54,106,108–110,174,175]. Beyond these DNA methylation studies, which are restricted to CpG sites profiled on arrays, we know little about the chromatin signatures of immunosenescence or inflamm-aging that could be predictive of reduced immune responsiveness at the individual level. Moving forward, revealing the aging-associated chromatin signatures in immune cells by leveraging recent advances in next generation sequencing (NGS) profiling [125,126,135,176] will be critical to identify and target individuals most at risk of complications from poor immune responses with age.

5.4 THE CHALLENGES OF MULTIOMIC DATA INTEGRATION AND INTERPRETATION

A major challenge in genomic data analyses for aging research lies in the complexity of the transcriptional regulation of gene expression. Transcriptional regulation occurs at many levels; for a complete view of gene regulation in a biological system, sophisticated computational methods are required to integrate diverse data capturing (1) chromatin accessibility; (2) histone modifications; (3) chromatin interactions; (4) protein binding to DNA; and (5) gene expression—bulk or single-cell profiling. Machine learning algorithms that can capture discriminative patterns from example data and use these patterns for prediction hold promise to extract actionable information via data integration [177]. Similarly, modeling genomics data in the form of interaction networks facilitates data integration and visualization. Another major challenge lies in the interpretation of clinical genomics data in the light of an ever increasing amount and diversity of public functional data repositories (e.g., NHGRI GWAS (National Human Genome Research Institute Genome-wide association study) catalog [178]) and reference data sets (e.g., Roadmap [104]). User-friendly and publically available software tools are needed for easy integration of genomic data with functional genomic repositories and for breaking the exclusivity of data analyses to computational scientists.

5.5 ACCOUNTING FOR CELL INTRINSIC VERSUS CELL COMPOSITION–DERIVED CHANGES WITH EPIGENOMIC AGING

A major challenge in uncovering the regulatory implications of epigenomic changes associated with aging lies in the need to dissect signals from mixed cell populations. For example, PBMCs are frequently profiled in DNA methylation and transcriptome studies, since they are easy to isolate from human blood samples and contain major immune cell types. Profiling of PBMCs has been proven to be effective in assessing one's global immune health and responses [179,180]. However, with age, the composition of PBMCs significantly changes with shrinkage of naïve B and T cell populations—due to age-related decline in thymus and bone marrow activity—and subsequently an increase in myeloid/lymphoid cell ratios. Therefore aging-related epigenomic remodeling in PBMCs is likely to be a combination of cell compositional (i.e., changes in cell frequency in the mixture) and cell-intrinsic (i.e., omic changes in specific cells) changes, as was also suggested by a recent study of single-cell transcriptome profiling in aging HSCs in mice [181]. Dissecting to what extent cell-intrinsic and

cell-compositional changes contribute to the remodeling observed from the profiles of cell mixtures is not a straightforward process. A powerful and user-friendly paradigm has been recently implemented to solve this question on the transcriptomic front [182]. A recent computational study has reinforced the importance of taking into consideration changes in cell-composition in the analysis of DNA methylation profiles and provided a framework for such analyses [183]. However, taking into consideration cell-composition changes while studying the DNA methylation—and other omics—profiles of cell mixtures still requires detailed profiling of subset cell frequencies within the mixture using FACS. Though great advances have been made in addressing the role and importance of cell composition changes during tissue aging, the potential impact of these changes on the epigenome is still largely unclear and will deserve further investigation.

6. CONCLUSIONS

Demographic projections indicate that the elderly population (aged 65 and over) will double and constitute more than 20% of the US population by 2030 [184]. The elderly are at higher risk for infectious diseases (e.g., influenza), autoimmunity (e.g., rheumatoid arthritis), cancer, type 2 diabetes, and cardiovascular diseases. Thus, the demographic increase in this population poses a significant economic burden on society—both in direct medical costs and lost productivity. In light of this, it is becoming critical to improve our understanding of cellular and molecular mechanisms that regulate human aging so as to definite prophylactic or therapeutic strategies to promote healthy aging and to cope with the burden of an aging nation.

Studies in model organisms and human samples have established that aging is associated with significant epigenomic remodeling (Table 1.1). However, the field of aging epigenomics is still in its infancy, and significant challenges need to be addressed in coming years. First, by taking advantage of recent advances in NGS technologies, age-associated changes in chromatin interactions, chromatin accessibility, and histone modification landscapes need to be studied across diverse cell types. Second, the downstream functional consequences of these changes will need to be dissected: by revealing genes, pathways, and TFs that are activated or inactivated with aging in response to these epigenomic changes and may be responsible for aspects of age-associated functional decline of cells and tissues. Third, there is still much to understand about the mechanisms that lead epigenomic changes during aging, for example, are specific chromatin modifiers aberrantly driving these changes? The use of targeted genome and epigenome-editing technologies based on the CRISPR/CAS9 or TALEN technologies [185,186] will be invaluable in addressing this question and will facilitate our progress from correlation to causation. Finally, the impact of pro-longevity or pro-healthy aging interventions on these epigenomic signatures will be essential to establish. For example, can the aging epigenome be reset or reversed through DR or exercise? Studies in model organisms, particularly in mice, will be fundamental in answering these questions.

Despite recent progress, how epigenetic information integrates environmental inputs in the context of a set genetic background throughout life remains poorly understood. Propelled by efforts to attain ‘precision medicine’, the genomics field is rapidly advancing from reference to personal genomes. Just as no two people with the same disease have acquired the disease in the same way, no two individuals are likely to age in quite the same way. Genomic patterns of aging at the individual level are likely

driven by both genotypic and lifestyle differences among individuals. Indeed, recent studies have demonstrated that specific genotypes may drive individual-specific epigenomic patterns (e.g., histone modification and DNA methylation) [187,188]. Future studies will need to take individual genotypes into consideration to uncover the mechanisms that underlie age-associated trajectories in gene expression or in chromatin accessibility of individuals.

LIST OF ACRONYMS AND ABBREVIATIONS

HDAC Histone Deacetylase
TE Transposable element
TF Transcription factor

GLOSSARY

CpG islands Genomic regions of CpG dinucleotides clustering, usually concentrated in the promoter regions of specific genes. They are defined by a minimum length (≥200 bp) and a high enrichment of CG nucleotides (≥60%).

Dietary restriction (DR) Regimen characterized by a reduction in food intake without malnutrition. A state of DR can be achieved through various protocols, such as intermittent-fasting, a fasting-mimicking diet, global caloric reduction, or restriction of a specific nutrient type (e.g., methionine).

Epigenetics The most commonly used definition of the words describes to modes of genomic regulation not directly encoded in DNA, which includes regulatory mechanisms like chromatin modification or noncoding RNA. The strict definition of epigenetics encompasses only strictly *heritable* changes without changes to the underlying gene sequence throughout generations.

Epigenetic drift/Epimutations Aberrant or atypical changes in epigenetic states, driven by stochastic events or external stimuli.

Genomic instability Stochastic loss of genome integrity. Modes of genomic instability encompass large-scale rearrangements (e.g., translocations, inversions, deletions), site-specific alterations (e.g., single nucleotide mutations or indels), or transposable element insertions/deletions.

Lifespan Defined as the time elapsed between the birth and death of an organism. It is typically expressed as time units for 'chronological' aging (i.e., for multicellular organisms) but can also be measured in number of cell divisions for 'replicative' aging i.e., for unicellular organisms such as yeast).

Transposon/Transposable Element (TE) Mobile endogenous DNA elements. TEs can modify their genomic position or increase copy number within a host genome. They are categorized in two main families: Type I transposons function through an RNA intermediate (i.e., LINEs, LTRs; 'copy and paste' propagation), whereas Type II transposons use a DNA intermediate (i.e., 'cut and paste' propagation). They typically constitute >30% of eukaryotic genomes.

ACKNOWLEDGMENTS

The authors apologize to those whose work could not be cited owing to space constraints. D.U. and B.A.B. wrote the review together. The authors also thank Dr. Anne Brunet for helpful comments on the manuscript. D.U. is supported by R35 GM124922. B.A.B. is supported by K99 AG049934.

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