Review

The great unravelling: chromatin as a modulator of the aging process

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During embryogenesis, the establishment of chromatin states permits the implementation of genetic programs that allow the faithful development of the organism. However, these states are not fixed and there is much evidence that stochastic or chronic deterioration of chromatin organization, as correlated by transcriptional alterations and the accumulation of DNA damage in cells, occurs during the lifespan of the individual. Whether causal or simply a byproduct of macromolecular decay, these changes in chromatin states have emerged as potentially central conduits of mammalian aging. This review explores the current state of our understanding of the links between chromatin organization and aging.

Chromatin as a potential modulator of aging

One of the hallmarks of aging is the loss of homeostatic mechanisms that offset the macromolecular wear and tear that occurs during a lifetime of exposure to low doses of extrinsic (ultraviolet and γ-irradiation, nutritional input) and intrinsic (reactive oxygen species, telomere shortening, protein synthesis errors, and replication/transcription-coupled DNA damage) stimuli [1]. Like other macromolecules in the cell, chromatin is subjected to these stresses that can impinge on its structural integrity and function (Figure 1). The nucleosome is the repeat unit of chromatin and consists of a dimer of each core histone (H2A, H2B, H3, and H4) [2]. The physical addition and removal of the linker histone H1 and additional nonhistone chromatin proteins facilitates transitions between higher-order chromatin states during the cell cycle. These transitions are important for rendering DNA accessible for transcription, replication, and packaging it into highly condensed metaphase chromosomes for transmission during mitosis. Chromatin is the template of epigenetic mechanisms such as DNA methylation and histone modifications including lysine methylation, lysine acetylation, lysine ubiquitylation, and serine/threonine phosphorylation. By definition, epigenetic modifications do not involve changes in the DNA sequence and should be heritable but reversible.

It has been proposed that the aging phenotype is, at least in part, due to the progressive divergence from a youthful chromatin configuration to one that contributes to the molecular signatures of aging [3]. In humans, analyses of longevity data on Danish twins and other populations of related individuals indicate that 20–25% of the variation in adult lifespan can be attributed to genetic shifts between identical individuals [4]. This might be due to an increased impact of stochastic somatic mutations as survival extends into old age, including their influence on cognitive and physical ability. However, chromatin modification patterns increasingly diverge with age in monozygotic twins [5]. These changes in chromatin might underlie those subtle phenotypic variations that become more pronounced with extended survival of twins and other closely related individuals. This seminal observation lent additional support to the speculation that aging might be largely the remit of chromatin-based epigenetic regulatory mechanisms. How chromatin is altered during aging has been extensively investigated; perhaps most notably with respect to the reshaping of chromatin during the diverse contexts of cellular senescence. Cellular senescence is an irreversible state of cell cycle arrest and is thought to reflect aging. In this review, we discuss recent developments that elaborate the functional links between chromatin, cellular senescence, and longevity.

Age-associated deregulation of chromatin modifiers

Perhaps the most commonly invoked feature of aging is the increase in stochastic cell-to-cell variation in gene expression [6]. Additionally, a wide range of changes in the expression of chromatin modifiers has been identified in senescence and during aging. This can alter the levels and distribution of chromatin modifications throughout the nucleus and at aging-associated loci, causing the activation of physiological responses that promote aging (Table 1). The following candidates might represent paradigms as to how chromatin and epigenetic mechanisms contribute to the wear and tear of tissues with age.

Epigenetic regulation of p16^{INK4A} expression

p16^{INK4A} (also known as CDKN2a) is an important regulator in the retinoblastoma protein, pRb, tumor suppressor pathway [7]. The primary function of p16^{INK4A} is to stifle proliferation by attenuating cyclin-dependent kinase CDK4/CDK6-mediated phosphorylation of pRb. Increased expression of p16^{INK4a} maintains pRb in its active hypophosphorylated state, sequestering E2F transcription factors to maintain cell cycle arrest. The expression of p16^{INK4A} increases in mammalian cells with senescence and age [8,9] and correlates with increased levels of cellular stress. As a result, p16^{INK4A} has become indelibly associated with cellular senescence and has been suggested as a biomarker of aging [9]. The link was recently

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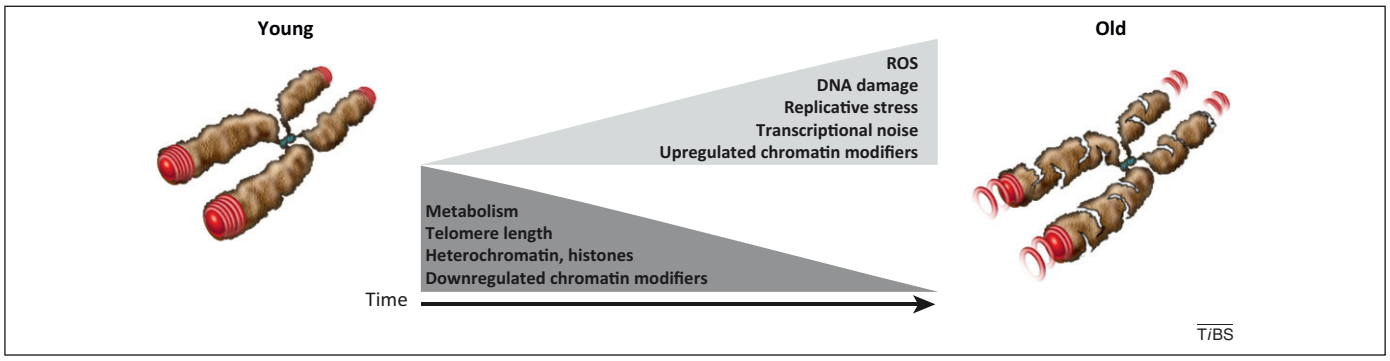

Figure 1. Aging is due to an increased disequilibrium in cellular homeostasis. The decline in metabolic rates and telomere shortening over time can contribute to structural and gene expression changes that are associated with aging. By contrast, chronic exposure to reactive oxygen species (ROS), DNA damage, and replicative stress can cooperatively cause elevated stochastic transcriptional noise. Structural changes in chromatin and the regulation of chromatin modifiers might be common denominators that underlie how these factors affect chromosomal stability and the cellular processes that drive the aging process.

bolstered in a study in which $p16^{INK4A}$-positive cells were specifically eliminated by directed programmed cell death (apoptosis) in the murine BubR1 haploinsufficiency model of premature aging [10]. Purging $p16^{INK4A}$-expressing senescent cells profoundly affected aging and healthspan. Eliminating $p16^{INK4A}$-positive cells throughout life delayed the onset of age-related tissue degeneration and led to lifespan extension, but doing so only in aged adult mice also delayed and improved the manifest traits of aging [10]. These experiments strongly support the idea that $p16^{INK4A}$-expressing cells reflect aging *in vivo*.

Epigenetic chromatin modification at the $p16^{INK4A}$ gene participates in the regulation of senescence and aging. Particularly, the polycomb (PcG) and trithorax (TrxG) proteins maintain chromatin in 'off' or 'on' states, thereby preventing or promoting expression, respectively [2]. In young cells, the polycomb repressive complexes PRC1 and PRC2 establish repressive chromatin throughout the $p16^{INK4A}$ locus [11]. The PRC2 complex consists of embryonic ectoderm development (EED), suppressor of zeste 12 homolog (SUZ12), and enhancer of zeste 2 homolog (EZH2), catalyzing trimethylation of H3 at lysine 27 (H3K27me3) [12] and trimethylation of H1 at lysine 26 (H1K26me3) [13]. This in turn recruits the PRC1 complex, comprising polycomb group proteins BMI1, RING1A, RING1B, MEL18 also known as PcG ring finger protein 2 (PCGF2) and chromobox homolog CBX7 [14], which monoubiquitylates histone H2A at lysine 119 (K119) and silences gene expression [15] (Figure 2a). In fact, ectopic overexpression of BMI1 and CBX7 extends the proliferative lifespan of cells *in vitro* by suppression of $p16^{INK4A}$ [16,17].

This repressive chromatin domain might be reinforced by chromatin looping [18] and by retention of the long intergenic noncoding RNA (lincRNA) ANRIL [19]. The initial recruitment of PRC2 can occur by various means. It could involve tethering of PRC2 to ANRIL [20], or alternatively, BMI1 could recruit PRC2 to the 5′ regulatory domain ($RD^{INK/ARF}$) from where PRC2 could then spread downstream to create the repressive chromatin domain [21]. $RD^{INK/ARF}$ has been identified as a master transcriptional regulator of the $p16^{INK4A}$ locus [22], and recruitment of BMI1 via cell cycle division regulator 6 homolog (CDC6) to $RD^{INK/ARF}$ also appears to regulate the replication timing of the locus, implying a dual role for PcG in DNA replication and transcription at the $p16^{INK4A}$ locus [21]. In senescent and aged cells, PRC1 and PRC2 dissociate from chromatin. This might be due to pRb inactivation and the imposition of the G1 checkpoint that negatively regulates E2F-driven expression of EZH2 and BMI1 [23]. As the expression of EZH2 and BMI1 subsides, the expression of the histone lysine demethylase (KDM) Jumonji D3 (JMJD3) rapidly increases and removes $p16^{INK4A}$-associated H3K27me3 [24]. This enables the association of the TrxG protein mixed-lineage leukemia (MLL)1, which catalyzes trimethyl lysine 4 of histone H3 (H3K4me3) to promote gene expression [25] (Figure 2a). The switch from PcG-dependent H3K27me3 to TrxG-mediated H3K4me3 at the $p16^{INK4A}$ gene could be a central node of a pRb-dependent regulatory loop that drives constitutive $p16^{INK4A}$ expression and senescence.

It has emerged that deviations in the epigenetic regulation of $p16^{INK4A}$ expression might influence the aging process by constraining the self-renewal capacity of certain stem-cell compartments. Elevated $p16^{INK4A}$ has been shown to limit the proliferation and self-renewal of pancreatic β-islet cells, hematopoietic stem cells (HSCs), and neuronal progenitor cells (NPCs) in mice [26–28]. Likewise, the absence of PcG-enforced gene repression though BMI1 deficiency severely compromises HSC and NPC self-renewal [29,29,30]. Reduced expression of EZH2 and BMI1 and diminished levels of H3K27me3 are evident in pancreatic β-islets [31], coinciding with elevated $p16^{INK4A}$ expression. The inhibitory effect of $p16^{INK4A}$ on stem cell function is further supported by the observation that senescent $p16^{INK4A}$-expressing fibroblasts have proved incompatible for induced pluripotent stem (IPS) cell reprogramming, and that genetic ablation of $p16^{INK4A}$ significantly improves this process [32]. Furthermore, inhibition of the core components of the PRC1 and PRC2, including EZH2, markedly reduces reprogramming efficiency [33]. These findings not only illustrate the role of chromatin modifiers in aging but also suggest that gradual changes in their expression and activity could influence a given tissues tolerance of stress and capacity for renewal and regeneration that could diminish healthspan earlier in life.

SIRT1 and SIRT6 in DNA repair and aging

The sirtuin family of NAD^+-dependent lysine deacetylases has a long and controversial relation with aging [34]. Originally identified in yeast, silent information