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Histone Modifications in Ageing and Lifespan Regulation

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Abstract Ageing has been associated with structural changes in chromatin. At the molecular level, multiple histone modifications with established epigenetic mechanisms have been connected to the regulation of lifespan. Here, we review the changes in histone modification profiles during ageing and their possible functional contribution to ageing and lifespan regulation. We brief the state of the knowledge on marks associated with both repressive (H3K9me3, H3K9me2, H3K27me3) and active chromatin (H3K4me3, H3K36me3, H3K56ac, H4K16ac, H2Bub). We further explore new histone modifications that emerged as lifespan-regulating candidates from a recent screen in yeast. Next, we comment on protein arginine methylation and GlcNAcylation, exploring their potential links to ageing. Finally, we provide a perspective on integrative approaches and methodological advances that might aid our pursuit of the epigenetic mechanisms of ageing.

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Introduction

Chromatin provides the scaffold for the packaging of the entire genome. The basic functional unit of chromatin is the nucleosome, which contains 147 base pairs of DNA wrapped around a histone octamer made up of two copies of each of the histone proteins H2A, H2B, H3, and H4.

Research over the last two decades has revealed that covalent modifications of both the histone proteins, and the underlying DNA, can alter the organization of chromatin. This opened the doors for researchers trying to understand the meaning of specific modifications, their role in controlling gene expression, and thereby cellular phenotypes.

In the last few years, the role of histone modifications in the process of ageing has emerged, providing insights into epigenetic mechanisms of ageing and lifespan regulation. Excellent reviews exist on the epigenetics of senescence and the roles of sirtuins or DNA-methylation in ageing [1–5]. In this review, we will focus on histone modifications, review the current state of knowledge, and provide perspective on their role in the regulation of organismal ageing and lifespan.

Histone Modifications Involved in Ageing and Lifespan Regulation

H3K9me3 and H3K9me2

Heterochromatin, the best-known form of repressed chromatin, is characterized by dense structure and absent or strongly diminished transcription. Histone-3 lysine-9 tri-methylation (H3K9me3) is the hallmark of constitutive heterochromatin,

present in pericentromeric and telomeric regions [6]. Its dimethylated form, H3K9me2, besides heterochromatin can also mark inactive euchromatin, which is important in embryonic development [7–9]. Both H3K9me2 and H3K9me3 are able to recruit heterochromatin protein-1 (HP1), which is believed to advance heterochromatin formation [10–12].

Ageing associates with a decrease or redistribution of H3K9me3/2, disruption of HP1 localization, and restructuring of heterochromatin, with loss of repression over constitutive heterochromatin *loci* and concomitant gain in facultative heterochromatin in other genomic regions [13]. A "loss of heterochromatin" model of ageing has been proposed and corroborated by studies linking changes in heterochromatin to ageing phenotypes in *Caenorhabditis elegans*, *Drosophila*, and humans [13–17]. Studies in the models of premature ageing-related diseases also implicate the connection between changes in heterochromatin and ageing [17–19]. The debate on whether the mechanisms of premature ageing are equivalent to the mechanisms of natural ageing is still ongoing. Nonetheless, loss of H3K9me3, HP1, and the H3K9 methyltransferase, SUV39H1, has been observed in both Werner syndrome model cells and cells derived from old individuals, suggesting that these models may indeed provide clues relevant to the mechanism of physiological ageing [17].

The levels of H3K9me3 were reported to decrease with ageing in *C. elegans*, while the levels of H3K9me2 and HP1 decreased in aged *Drosophila* [16, 20]. Interestingly, Wood et al. observed an increase in total H3K9me3 levels, and no change in HP1 expression, in aged flies [21]. They showed instead that the H3K9me3 mark seems to be redistributed, leading to even distribution of H3K9me3 (and HP1) levels between heterochromatin and euchromatin [21]. This redistribution within different chromatin regions coincided with altered nuclear localization of H3K9me3 and HP1 [21]. This study therefore corroborates the idea that ageing is accompanied by restructuring of heterochromatin and redistribution of H3K9me3/2 and HP1, rather than by simple change in their abundance.

H3K27me3

While H3K9me3 is strongly associated with the constitutive heterochromatin repressed in all cell types (e.g. pericentromeric regions), H3K27me3 is found in facultative heterochromatin regions whose repression is more cell-type-specific. H3K27me3 seems to be more dynamically modified to allow repression or derepression of groups of genes during development or differentiation [22].

Levels of H3K27me3 were reported to decrease with age in *C. elegans* [20, 23]. Knockdown of the H3K27me3 demethylase UTX-1 (homologous to the mammalian UTX/KDM6A) increased worms' H3K27me3 levels and extended their lifespan [23]. UTX-1 expression normally increases with age in worms and in human brain [24]. Low UTX-1 expression corresponds to the higher levels of H3K27me3 at the promoter of *Igf1r*, member of the highly conserved ageing-related insulin/IGF-1 pathway. Repression of this pathway by the UTX-1/H3K27me3 axis might therefore also be conserved between species [24]. Interestingly, another known H3K27me3 demethylase, JMJD3/KDM6B, has not yet been connected to ageing, although it is known to control expression from the senescence-associated $p16^{INK4A}$ locus [25, 26]. Surprisingly, histone methyltransferase set-26 that normally deposits methylation marks on H3K27 and H3K9 was necessary for the age-related loss of H3K27me3 in *C. elegans* [20]. Set-26 knockdown prevented the age-related decrease in H3K27me3 (and H3K9me3) and increased worm's longevity [20]. Overall, the studies in *C. elegans* link the increase in H3K27me3 levels to increased longevity.

In contrast, in *Drosophila*, an increase in longevity was coupled to a decrease in H3K27me3 levels [27]. H3K27me3 is classically deposited and maintained by the Polycomb complexes. Polycomb repressive complex-2 (PRC2) contains one of the methyltransferases, enhancer of zeste homolog-1 or -2 (EZH1 or EZH2, respectively) [22]. Mutation of the *Drosophila*'s homologue of EZH1/2, E(Z), as well as mutation of its H3-targeting partner protein ESC, led to the decrease in H3K27me3 levels and concomitant increase in longevity [27]. Consistently, Graffman et al. reported the increase in the expression of a Polycomb protein EED and increase of H3K27me3 levels in human early hematopoietic progenitors with age [28]. Recent meta-analysis of ENCODE and Roadmap Epigenomics data further supports the increase in H3K27me3 levels during ageing by showing the increase in Polycomb proteins and H3K27me3 in age-associated genomic regions [29]. Additionally, in *Drosophila*, mutations to known Polycomb's antagonist Trithorax group proteins restored the H3K27me3 levels and abolished the increase in longevity [27].

The discrepancy between H3K27me3 levels and longevity between *C. elegans* and other models might be the result of inter-species differences. Also, different components of the H3K27me3-maintenance system were investigated in these studies. Differing targets of these maintenance systems, besides H3K27, might contribute to lifespan regulation and account for the differences observed. EZH2, the Polycomb's H3K27me3 methyltransferase, was reported to act independently from PRC2 complex in cancer cells [30]. Furthermore, the cross-talk between H3K27me3 methyltransferases/demethylases and other regulators could help to explain the interspecies differences. For instance, the SIRT1 deacetylase was shown to affect the methylation status of Polycomb target genes [31].

Finally, it is not the global levels of histone marks, but rather their localization and regulation of specific groups of genes, that plays a functional role in the regulation of cellular

fate. Recent findings showed that the expression of Polycomb's *Ezh1*, *Ezh2*, and *Cbx2*, and of its co-regulators *Aurka* and *Aurkb*, decreased with ageing in murine hematopoietic stem cells (HSCs) [32••]. This was accompanied by redistribution of the ChIP-seq-profiled H3K27me3, whose total peak counts did not change, but whose abundance decreased at certain promoters to increase at others [32••].

H3K4me3

H3K4me3 histone mark is the most abundant in close proximity of transcription start sites (TSSs) [33]. It is associated with, though not indispensable for, active transcription [34]. H3K4me3 is also present along with the repressive H3K27me3 mark in so-called bivalent domains that associate with developmentally important genes, allowing them to be activated or repressed as the differentiation proceeds [35].

High H3K4me3 levels seem to promote ageing, as its disruption leads to increase in lifespan: knockdown of each of the H3K4me3 methyltrasferases ASH-2, WDR5, and SET-2 in *C. elegans* led to decrease in H3K4me3 levels and concomitant increase in lifespan [36]. Consistently, knockdown or inhibition of H3K4me3 demethylases caused an increase in H3K4me3 levels and a decrease in the lifespan of *C. elegans* and *Drosophila* [36–39].

Another argument for the functional role of H3K4me3 in promoting ageing is its correlation with gene expression. Sun et al. reported a significant increase of H4K4me3 levels at 267 gene promoters in aged hematopoietic stem cells (HSCs), which overall positively correlated with gene expression [32••]. This was accompanied by a loss of H3K4me3 at 73 promoters, which suggests that not only global levels but also the localization of H3K4me3 to specific genes is important. Consistent with this notion, besides the increase in the number of H3K4me3 ChIP-seq peaks with age, Sun et al. reported concomitant increase in the breadth of ~50 % of the H3K4me3 peaks [32••]. These broadened peaks tended to associate with genes that determine HSC identity, consistently with the recent findings that the broadest H3K4me3 domains control the cell-identity-related genes by increasing their transcriptional consistency [40•].

H3K36me3

H3K36me3, similarly to H3K4me3, is associated with active transcription but located in gene bodies rather than at promoters [41]. It plays a role in sustaining transcriptional elongation by RNA polymerase II and preventing cryptic transcription [42, 43••, 44]. Only very recently has the H3K36me3 mark been associated with regulation of ageing.

A histone mutant screen in *Saccharomyces cerevisiae* revealed H3K36me3 as a mark responsible for lifespan regulation [43••]. Different amino acid substitutions of H3K36 all resulted in shortened lifespan, and deletion of the H3K36 demethylase, Rph1, increased the H3K36me3 levels and extended lifespan of wild type yeast, but not H3K36 mutant yeast, showing that indeed the presence of the methylation at H3K36 is required for the lifespan extension [43••]. The age-dependent loss of H3K36me3 at specific *loci* associated with emergence of cryptic transcription in ageing yeast, a phenomenon that also occurred in aged *C. elegans*, suggesting that H3K36me3-mediated regulation of ageing might be conserved from yeast to worms [43••].

Indeed, another recent study confirmed that H3K36me3 is required for regulation of lifespan in *C. elegans* [45•]. Interference with the *met-1* methyltransferase resulted in decreased H3K36me3 levels and shortened lifespan [45•]. While no obvious age-dependent changes occurred in the distribution of H3K36me3, there was a negative correlation between the initial H3K36me3 levels and the change in gene expression upon ageing, once more suggesting a role for H3K36me3 in maintaining transcriptional consistency [45•]. Additional analysis of data from *Drosophila melanogaster* revealed similar correlation between H3K36me3 and age-dependent transcriptional changes, further supporting the conserved nature of H3K36me3-mediated regulation in ageing [21, 45•].

H3K56ac

The acetylation of lysine 56 on histone H3 (H3K56ac) is evolutionarily conserved from yeast to man [46]. In *S. cerevisiae*, it promotes de novo nucleosome assembly, genomic stability, transcription, and formation of heterochromatin/euchromatin boundaries [47–52]. In mammals, the role of H3K56ac remains elusive, but recent evidence indicates that it also promotes genomic stability [46].

H3K56 can be deacetylated by a variety of NAD^+-dependent deacetylases collectively known as Sirtuins. In yeast, the Sirtuin proteins Hst3, Hst4, and Sir2 deacetylate H3K56, while in mammals, the Sir2 orthologues Sirt1, Sirt2, Sirt3, and Sirt6 carry out this reaction [46, 53–55]. Importantly, the activity of Sirtuin proteins has been strongly linked to increased lifespan [56]. Indeed, Sir2 levels are known to decrease in yeast with increasing age [57]. Furthermore, deletions of Sir2, Hst3, and Hst4 shorten lifespan in yeast, while overexpression of Sir2 lengthens it [57, 58].

The Sir2-mediated lifespan extension in yeast appears to work through the maintenance of genomic integrity of ribosomal DNA (rDNA) [59]. In yeast, the rDNA is thought to play a very important role in lifespan regulation, but mutations that abolish H3K56ac do not mimic the effects of Sir2 overexpression, suggesting that the H3K56ac levels at rDNA are not the sole regulator of lifespan [58, 60]. However, mutations in *rtt109* and *asf* result in hyperamplification of rDNA repeats, which has been linked to decreased lifespan [61, 62]. In accordance with this, an H3K56R mutant that mimics a

constitutively unacetylated H3K56 also displays rDNA hyperamplification and is short-lived [57, 61]. Paradoxically, also an H3K56 acetyl-mimic (H3K56Q) led to increased hyperamplification of rDNA and decrease in lifespan [57, 61]. This suggests that the right balance of H3K56 acetylation is required to maintain cellular function. Nonetheless, H3K56ac levels have been shown to decrease in yeast with increasing age, and it was recently shown in yeast that the downregulation of ribosome biogenesis by TOR signaling, known to be a conserved mechanism for lifespan extension in many organisms, is partly mediated by the downregulation of H3K56ac specifically at the rDNA [63]. These contradictory data make it difficult to determine the degree of involvement of H3K56ac in regulating lifespan in yeast. In mammals, the role of H3K56ac in lifespan extension remains to be investigated.

H4K16ac

H4K16ac is another histone target of sirtuin deacetylases. In yeast, the opposing activities of sir2 deacetylase and Sas2 acetyltransferase establish an H4K16ac gradient in close proximity of telomeres. This gradient defines a heterochromatin/euchromatin boundary and functions to promote transcription by preventing the spreading of telomeric heterochromatin [64, 65]. In *Drosophila*, hyper-acetylation of H4K16 on chromosome X is required for upregulation of gene expression during dosage compensation and the general transcriptional program. Next to its role in transcription, in mammals, H4K16ac is also involved in promoting DNA repair [66, 67].

In yeast, levels of H4K16ac, unlike H3K56ac, increase during ageing [57]. This might be due to the age-related decrease in sir2 [57]. However, as seen with H3K56ac, mutations of H4K16 that mimic its constitutively acetylated and unacetylated forms (H4K16Q and H4K16R, respectively) both result in a reduction of lifespan [57].

In mammals, a study of an in vitro progeria mouse model, which displays a premature ageing phenotype, showed that H4K16 was hypoacetylated in Zmpste-24-null fibroblasts due to a mutant prelamin A (progerin)-mediated mislocalization of the H4K16 acetyltransferase MOF [68]. This was shown to result in deficient recruitment of DNA repair factors to DNA damage. Knockdown of *Mof* in late passage wild-type mouse fibroblasts resulted in a decrease in H4K16ac and an increase in cellular senescence, recapitulating what was observed in the progeria model of Zmpste-24-null fibroblasts [68]. Furthermore, Zmpste-24-null mice whose diets were supplemented with histone deacetylase inhibitors demonstrated a small but significant extension of lifespan [68]. These data suggest that part of the accelerated ageing phenotype observed in progeria syndromes could be a direct consequence of reduced H4K16ac. However, further studies would be needed to clarify the exact extent to which H4K16ac prevents the accelerated ageing seen in progeria syndromes.

H2B monoubiquitylation

Another active mark, histone H2B monoubiquitylation (H2Bub), is required for the trimethylation of H3K4 and H3K79, a role which seems to be conserved between species [69–74]. This suggests that H2Bub could indirectly affect ageing through regulation of H3K4me3 levels.

Indeed, the role of H2Bub itself in the regulation of lifespan has recently been shown in yeast [75]. H2Bub accumulated in heterochromatic regions during cellular ageing, accompanied by an increase in H3K4me3, H3K79me3, and H4K16ac [75]. Disruption of H2B ubiquitylation led to a reduction in yeast lifespan; however, it had no additional effect over a Sir2 mutant, suggesting that the role of H2Bub in yeast ageing might be coupled to the Sir2-H4K16ac axis described above, rather than to H3K4me3 regulation [75].

Potential Role of Other Histone Modifications in the Regulation of Ageing

Up to this point, we have described histone modifications that have already been implicated to influence ageing (see sections above). Intriguingly, a recent screen using a yeast histone mutant library implies that many more histone modifications might regulate lifespan, as summarized in Table 1 [43••]. Of 38 uniquely mutated sites within histones H3 and H4 that change lifespan by over 20 %, 15 sites are residues previously reported to be post-translationally modified. It is tempting to speculate that the identified mutations point toward so far unknown ageing-related histone modifications and thus might prove useful as a resource for researchers interested in the involvement of epigenetics in ageing. Mutations of the majority of the 38 newly identified sites decrease lifespan. Nevertheless, six of the mutations increase yeast lifespan over 25 %. These are K14Q, K64A, K115A, K122A, and R128A in histone H3, and K77A in histone H4.

H3R128 was shown to be methylated in brains of a 12-month-old progeria model mice [86]. However, it remains to be seen whether its levels are affected over the course of the organismal lifespan. Furthermore, the function of this modification is unknown to date. H4K77 is the site of acetylation and ubiquitination [91]. It has been demonstrated at least in vitro that acetylation of H4K77 enhances the instability of nucleosomes, an effect that can also be observed in ageing yeast cells [92]. H3K64, 155, and 122 all map to the lateral surface of the nucleosome where the DNA-protein interaction is at its maximum strength [93]. All three residues can also be acetylated, and this weakens histone-DNA contacts [85, 94, 95]. Interestingly, H3K64 can also be tri-methylated, a modification that localizes to repressive chromatin [81]. It would be

Table 1 Novel histone modification sites with putative function in ageing and lifespan regulation

Histone	Mutation	PTM	Lifespan	Function	Ref.
H3	K14Q	ac	Long	Transcription, repair	[76, 77]
	K18Q	ac/me	Short	Transcription	[78, 79]
	K36A,E,R,Q	me	Short	Transcription	[41]
	K42A,Q	me	Short	?	[80]
	K56Q	ac/me	Short	Transcription, replication, repair	[47–49]
	K64A	ac/me	Long	Transcription, silencing	[81]
	K79E	me	Short	Transcription	[82]
	T80D	p	Short	Mitosis	[83]
	K115A	ac	Long	?	[84]
	K122A	ac	Long	Transcription	[85]
	R128A	me	Long	?	[86]
H4	R19E	me	Short	Transcription	[87]
	K31A	ub/me	Short	?	[88, 89]
	K44A	ac	Short	Meiosis	[90]
	K77A	ac/ub	Long	?	[84]

Lifespan reported if changes are greater than 20 %

PTM post-translational modification

highly interesting to investigate how this interplay between methylation and acetylation would change with age. Finally, H3K14Q is the only lifespan-extending mutation that harbors the acetylation-mimicking glutamine, while the unmodifiable mimic arginine does not alter lifespan. This is particularly interesting as H3K14 acetylation has been correlated with the regulation of stress response genes and the induction and coordination of the DNA damage response pathways that are well known to enhance lifespan [76, 77, 96–98].

Besides the novel targets revealed in the screen, there are two classes of important protein modifications, arginine methylation and GlcNAcylation, that have been associated with ageing and lifespan regulation; however, it remains unclear if their effects depend on epigenetic mechanisms [99–103].

Arginine residues can be methylated by enzymes that belong to the mammalian protein arginine methyltransferases (PRMTs) or to its homologous *Drosophila* arginine methyltransferases (DARTs) [104, 105]. PRMTs were reported to change their expression in an age-dependent manner in rat tissues [99]. Interestingly, PRMT6$^{-/-}$ MEF cells exhibited a senescence-like phenotype, associated with decreased levels of H3R2 methylation, a modification suggested to be involved in the maintenance of euchromatin [100]. However, as PRMTs methylate a variety of target proteins, it remains to be seen whether histone-arginine methylation is involved in regulation of ageing.

The target proteins of O-GlcNAcylation are also diverse, ranging from signaling pathway mediators to epigenetic regulators [106]. However, all core histones can be modified with O-GlcNAc [106–111].

Using anti-GlcNAc antibody, Love et al. identified over 800 genes, whose promoters were enriched in GlcNAc-modified proteins in *C. elegans* [102]. GlcNAcylation localized in proximity of promoters of genes associated with ageing, among other processes [102]. GlcNAcylation is carried out by O-GlcNAc transferase (OGT), while its removal by O-GlcNAcase (OGA) [106]. Deletion of *ogt-1* led to decrease in lifespan, while deletion of *oga-1* to increase in lifespan in *C. elegans* [102, 103]. This effect of the GlcNAc cycling enzymes seemed to be at least partly dependent on the insulin-signaling pathway and its downstream DAF16/FOXO transcription factor, a known regulator of ageing in *C. elegans* and other organisms [102, 103]. As Love et al. investigated DNA-associated protein GlcNAcylation, it seems plausible that the modification was present, among others, at histone proteins. Along with other modifications described in this section, it will be highly interesting to see whether and how GlcNacylation of histones plays a role in regulation of organismal lifespan. However, the detectability, and hence the existence, of histone GlcNAcylation in mammalian cells are currently under dispute, questioning its putative role in ageing in higher organisms [112].

Perspectives

Along with the investigation of novel histone marks in ageing, the challenge for the field will be the integration of the information on single histone marks, to better understand their interplay. A well-known example of interaction between two histone marks occurs in bivalent domains that contain both

active H3K4me3 and repressive H3K27me3. As described by Sun et al., in aged HSCs, 355 bivalent domains were lost and 1245 were gained, either through gain in both H3K4me3 and H3K27me3, or only in H3K27me3 [32••]. This suggests that monitoring of single histone marks in ageing, while important, may only yield partial information. Knowledge of the individual functions of modifications as well as of their interplay will be crucial for complete understanding of their role in ageing. Furthermore, it might be important to put less stress on monitoring the global levels of modifications and pay more attention to their redistribution patterns towards different sets of genomic regions during ageing.

A next step forward should be further integration of the data on histone modifications with data on DNA methylation, cellular signaling, and metabolism. The most recent reports seem to weigh in this direction, raising hopes for development of a complete, systems biology model that would integrate epigenetic, genetic, metabolic, and signaling mechanisms of ageing [17, 32••, 113•].

While integrative approaches will most likely gain in importance in the coming years, thanks to increasing computational expertise and computational power in the field, another challenge will be to uncouple the likely different epigenetic mechanisms of ageing in different tissues or even cell types. Current approaches often still focus on a specific tissue, if not a whole organ. However, the epigenetic patterns of histone modifications vary greatly between specific cell types. The bivalent domains mentioned above, or the enhancers, present a drastically different make-up in different cell types. This suggests that also the changes acquired during ageing might be of different nature. Profiling of whole organs or tissues, although important, might only monitor the average of the cell populations within the organ and thus miss subtle, cell type-specific changes. While it seems obvious in mammals, it is also important in small model organisms: Pu et al. and others used germlineless worms in their ageing research, because of the dramatic changes that occur in the germline during adulthood, which could mask the somatic epigenetic changes [45•]. Therefore, it will be of high importance in the coming years to investigate the mechanisms of ageing in specific, selected cellular populations.

While methods are in place to achieve the population-level cellular resolution, e.g., fluorescence-activated cell sorting (FACS), there remains a challenge of working with very low cell numbers those populations might consist of. Traditionally, many methods, especially ChIP, required very high inputs. Methods have now been developed to perform DNA methylation, RNA-seq, and even ChIP-seq experiments with cellular inputs reaching down to a single cell, accompanied by development of computational approaches and tools enabling the analysis [114–120]. The establishment of the single-cell techniques will also permit the next step forward: investigating the dynamics of epigenetic modifications of single cells in seemingly uniform cellular populations [114, 119].

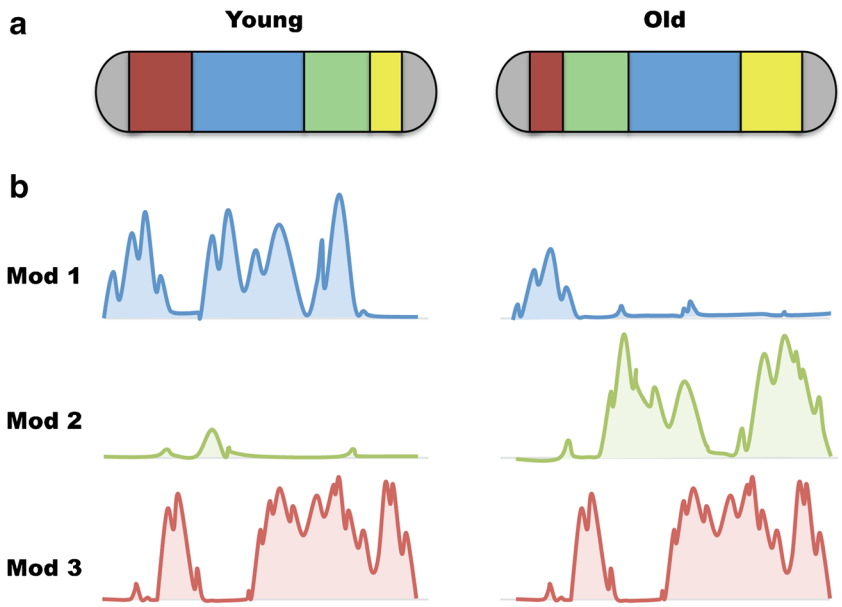

Fig. 1 Redistribution of histone modifications during ageing. Simplified representations of: **a** a chromosome, illustrating how changes in histone modifications (represented by different *colors*) may occur during ageing, with or without affecting the global levels of the modifications. Such age-dependent alterations in chromatin structure might affect DNA-templated processes resulting in the ageing phenotype; **b** a single, ageing-associated *locus* in which ageing-related changes in histone modifications occur. Modification 1 (*blue*) is lost upon ageing, while modification 2 (*green*) becomes enriched. Even if the levels of modification 3 (*red*) remain the same, the new epigenetic state due to changes in modifications 1 and 2 will likely alter DNA-templated processes occurring in this genomic region, contributing to ageing phenotype. The peaks represent putative ChIP-seq profiles

Conclusions

The research of recent years has established links between histone modifications and ageing or lifespan regulation. However, the pool of histone mark candidates involved in ageing is growing and future investigation of the new ageing-associated histone marks, as well as integration of epigenetic data with other areas of cellular activity, will be important to reveal a systems biology mechanism of ageing.

The current understanding of the role that the changes in histone modifications play in ageing is not complete. In many cases, conflicting reports are present in regard to the presence or direction of change in the levels of particular histone marks during ageing. However, their functional role in promoting or restraining longevity is often clearly indicated by studies in mutant models.

It seems that the net changes in amounts of specific histone marks are not as important as their localization. It is rather the redistribution of marks and reorganization of chromatin that may regulate ageing (Fig. 1). Histone modifications are reshuffled to control different sets of genes. This redistribution of modifications, and their net effect on transcription of specific sets of genes, is therefore the most likely explanation for the impact of histone marks on ageing.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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