Review

# Histone methylation makes its mark on longevity

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**How long organisms live is not entirely written in their genes. Recent findings reveal that epigenetic factors that regulate histone methylation, a type of chromatin modification, can affect lifespan. The reversible nature of chromatin modifications suggests that therapeutic targeting of chromatin regulators could be used to extend lifespan and healthspan. This review describes the epigenetic regulation of lifespan in diverse model organisms, focusing on the role and mode of action of chromatin regulators that affect two epigenetic marks, trimethylated lysine 4 of histone H3 (H3K4me3) and trimethylated lysine 27 of histone H3 (H3K27me3), in longevity.**

## Regulation of longevity

Longevity is regulated by both genetic and environmental factors. Genetic mutations in a variety of pathways, including the insulin/IGF-1 pathway, can extend lifespan in various organisms (Table 1) [1,2]. Environmental stimuli can also affect lifespan, either negatively or positively. Stress stimuli (e.g. DNA damage) are known to accelerate aging. Dietary restriction (DR), restriction in food intake without malnutrition, extends lifespan and delays age-related diseases in a wide range of species [3]. In *Caenorhabditis elegans*, lifespan extension by different DR regimens is mediated by signaling pathways (e.g. TOR, AMPK) and downstream transcription factors (e.g. FOXA, FOXO, NRF2), which culminate in changes in gene expression profiles (Table 1) [3–8]. Signaling pathways and transcription factors regulate longevity in a conserved manner (Table 1), and recent evidence in mammalian cells indicates that several of these conserved longevity pathways can modulate chromatin states in mammalian cells [9–12]. These observations raise the exciting possibility that environmental stimuli that affect lifespan may do so by modulating chromatin states. Changes in chromatin state are likely to be more persistent than transient alterations in signaling pathways and transcription factor activity, which could have important implications for how environmental stimuli, dietary regimens or specific drugs may affect lifespan even when applied transiently.

## Chromatin state and longevity

Chromatin state is governed by a series of modifications that include DNA methylation and histone modification [13–15]. These modifications are often termed 'epigenetic' [13], as they do not affect the genetic sequence *per se*. Post-translational modifications of the core histones, also known as 'histone marks', include acetylation, methylation, phosphorylation and ubiquitylation. Specific histone marks are associated with different chromatin states [13]. For example, H3K4me3 is generally associated with gene expression and H3K27me3 is generally associated with transcriptional repression [15]. Histone marks may represent a key intermediary step between signaling pathways that extend lifespan and the expression of specific genes involved in longevity. Histone modifications are reversibly catalyzed by specific enzymes, such as acetyltransferases, deacetylases, methyltransferases and demethylases [16,17]. Thus, the enzymes that reversibly regulate histone marks may affect the expression of specific genes involved in longevity and, as such, represent possible therapeutic targets to help prevent or treat age-dependent diseases, including neurodegenerative diseases and cancer.

Until recently, studies on chromatin regulators and lifespan have focused mostly on histone acetylases and deacetylases, in particular the SIR2 deacetylase family (Table 1) [18–20]. In the past few years, a series of studies has revealed that histone methyltransferases and demethylases also affect lifespan. This review synthesizes these recent findings and describes the role and mode of action of regulators of histone methylation in longevity in diverse model systems. The main focus is on H3K4me3 regulators, but we also examine the regulation and importance of other methylation marks in longevity.

## H3K4me3 modifiers affect longevity in *C. elegans*

Histone methylation is catalyzed by protein complexes composed of a methyltransferase and other proteins that are important for the recruitment of the complex to specific genomic loci. Three major complexes are responsible for generating H3K4me3 in mammals: the COMPASS complex, the Trithorax complex and the Trithorax-related complex [21–27]. These H3K4me3 regulatory complexes are known to be important for development and stem cell function [24,28,29], but until recently their role in longevity had not been studied. Recent work indicates that the complex composed of ASH-2, WDR-5 and the H3K4me3 methyltransferase SET-2 affects lifespan in *C. elegans*. Worm SET-2 is phylogenetically related to methytransferases in the COMPASS complex (Figure 1). Knockdown of ASH-2, WDR-5 or SET-2 in fertile worms significantly extends worm lifespan (Table 2) [30]. Knockdown or mutation of ASH-2, WDR-5 or SET-2 reduces global H3K4me3 levels at the larval L3 stage [30], consistent with other

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 0962-8924/$ – see front matter  doi:10.1016/j.tcb.2011.11.001 Trends in Cell Biology, January 2012, Vol. 22, No. 1

**Table 1. Major pathways that regulate longevity**

| Pathway | Characteristics | Change that extends lifespan | Conservation |
|---|---|---|---|
| Insulin/IGF-1 | Endocrine signaling | Inhibition | Worms, flies, mice, humans |
| TOR | Nutrient/amino acid sensing | Inhibition | Yeast, worms, flies, mice |
| AMPK | Nutrient/energy sensing | Overexpression | Worms, mice (indirect study) |
| SIR2 | $NAD^+$-dependent histone deacetylase | Overexpression | Yeast, worms (in some cases), flies (in some cases) |
| Mitochondrial electron transport chain | Respiration | Inhibition | Worms, flies, mice |
| Germline stem cells | Reproduction | Inhibition | Worms, flies |

Examples of conserved pathways that are known to regulate lifespan in different model organisms (see [1] for a comprehensive review). TOR, target of rapamycin; AMPK, AMP-activated protein kinase; SIR2, silent information regulator 2.

studies [31–34], suggesting that ASH-2, WDR-5 and SET-2 affect lifespan by modulating H3K4me3 levels. ASH-2, WDR-5 and SET-2 function in the same genetic pathway to impact lifespan [30], although whether they interact physically remains to be established. Deficiency in RBR-2 – an H3K4me3 demethylase with homology to human JARID1A/KDM5A [35] – increases H3K4me3 levels [30,35], and reduces lifespan in either wild-type worms or worms that are deficient for ASH-2, SET-2 or WDR-5 (Table 2) [30]. Importantly, overexpression of RBR-2 is sufficient to extend lifespan in *C. elegans* (Table 2) [30]. Thus, RBR-2 counteracts the ASH-2 complex in longevity and H3K4me3 levels (Figure 2). Overall, these studies suggest that limiting the H3K4me3 level is beneficial for longevity in *C. elegans*, perhaps because it reduces the transcription of genes that would normally lead to aging. The ASH-2/WDR-5/SET-2 complex might also affect longevity by acting via as-yet unidentified non-histone targets.

The H3K4 demethylase LSD1 has also been found to affect worm lifespan (Table 2) [36]. Trimethylation of H3K4 first requires mono (me1)- and di (me2)-methylation [15]. LSD1 demethylates H3K4me1/me2 and triggers transcriptional repression of downstream target genes [37]. Knockdown of *lsd-1* or suppression of *lsd-1* mRNA by LiCl treatment extends lifespan in worms by 25% and 46%, respectively [36,38]. It might appear surprising that deficiencies in LSD-1, an H3K4me1/me2 demethylase, extend longevity [36] whereas deficiencies in RBR-2, an H3K4me3 demethylase, shorten lifespan [30]. RBR-2 and LSD-1 regulate lifespan in an opposite manner, possibly because they have non-overlapping age-related gene targets, act in distinct tissues or function at different times of life. Exploring the combined effects of the ASH-2 complex, RBR-2 and LSD-1 on worm lifespan will provide insight into the respective contribution of H3K4me1, H3K4me2 and H3K4me3 to lifespan regulation. Overall, these results indicate that regulators of H3K4 methylation affect lifespan in *C. elegans*, which raises the question of where they act in the organism to control longevity.

## H3K4me3 modifiers in the germline

The ASH-2 and RBR-2 H3K4me3 regulatory complexes appear to promote somatic longevity by acting mostly in the germline [30]. Both the ASH-2 protein and the H3K4me3 mark are highly enriched in the germline and newly fertilized eggs [30,33,34,39]. ASH-2 deficiency no longer extends lifespan in worm mutants with an underdeveloped germline or in mutants that cannot produce fertilized eggs [30]. Thus, the presence of an intact germline is necessary for the ASH-2 complex to regulate lifespan. Furthermore, RNAi knockdown of ASH-2 or SET-2 still extends lifespan in a worm mutant strain that is mostly insensitive to RNAi in the soma [30]. Overexpression of RBR-2 specifically in the germline is sufficient to prolong lifespan [30]. These findings suggest that the ASH-2 complex and RBR-2 regulate lifespan by acting in the germline.

Do H3K4me3 regulatory complex deficiencies extend lifespan because they decrease fertility? Theories of aging have indeed postulated that resources are allocated to either reproduction or maintenance of the soma. Impairing reproduction may thus lead to better maintenance of the soma and lifespan extension [40,41]. This does not seem to be the case for lifespan extension by deficiencies in

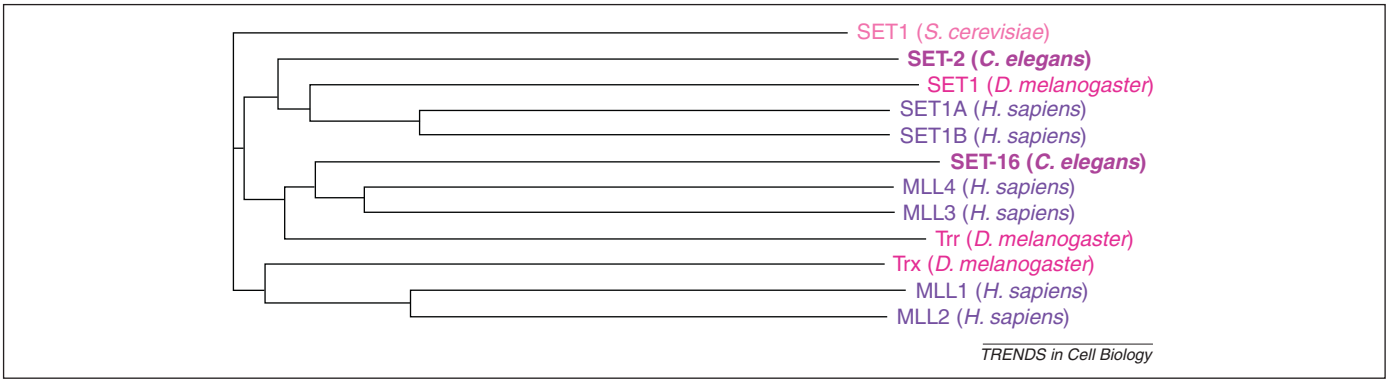


**Figure 1. Phylogenetic analysis of *Caenorhabditis elegans* SET-2 and its homologs in yeast (*Saccharomyces cerevisiae*), flies (*Drosophila melanogaster*) and humans (*Homo sapiens*).** Worm SET-2 is phylogenetically closer to SET1 in flies and SET1A/SET1B in humans than it is to Trithorax (MLL1/2) or Trithorax-related (MLL3/4) families. The phylogenetic tree was constructed with ClustalW2 using full-length protein sequences.

**Table 2. Histone methylation regulators in aging and longevity**

| | *C. elegans* | *Drosophila* | Mammals |
|---|---|---|---|
| **H3K4me3** | | | |
| Change with age? | NT | Decrease ♀ (in the brain) | Change in landscape (in the brain) |
| KD or mutation of methyltransferase | Increase lifespan ⚥ (COMPASS complex) | No effect on lifespan (Trx complex) ♂ | NT |
| OE of methyltransferase | NT | NT | NT |
| KD or mutation of demethylase | Decrease lifespan ⚥ | Decrease lifespan ♂ | NT |
| OE of demethylase | Increase lifespan ⚥ | NT | NT |
| **H3K4me1/me2** | | | |
| Change with age? | NT | NT | NT |
| KD or mutation of methyltransferase | NT | NT | NT |
| OE of methyltransferase | NT | NT | NT |
| KD or mutation of demethylase | Increase lifespan ⚥ | NT | NT |
| OE of demethylase | NT | NT | NT |
| **H3K27me3** | | | |
| Change with age? | Decrease ⚥ | NT | NT |
| KD or mutation of methyltransferase | NT | Increase lifespan ♂ | NT |
| OE of methyltransferase | NT | NT | NT |
| KD or mutation of demethylase | Increase lifespan ⚥ | NT | NT |
| OE of demethylase | NT | NT | NT |

Specific histone methylation marks are altered during aging, and regulators of some of these marks affect lifespan. NT, not tested; KD, knockdown; OE, overexpression.

H3K4me3 regulators, however. In the conditions used for lifespan assays, ASH-2 knockdown does not affect *C. elegans* fertility, as measured by germline morphology, germline cell number, number of eggs laid and brood size [30]. Furthermore, at the temperature used in lifespan assays (20°C), *wdr-5* mutants develop normally and do not display a statistically significant increase in sterility [30], although these mutants do exhibit a slight increase in producing dead eggs [34]. Similarly, neither *set-2* mutants nor *rbr-2* mutants show significant fertility defects at 20°C [30,34]. Thus, longevity due to deficiencies in ASH-2 complex members is unlikely to be caused by a trade-off of resource allocation to reproduction versus maintenance of the soma. Nevertheless, it should be noted that, under more drastic conditions, these H3K4me3 regulators do show fertility defects. At 25°C, *wdr-5* mutants exhibit brood size reduction, endomitotic oocytes and abnormal DNA morphology in their germ cells [34], and *ash-2* genetic mutants have reduced fertility at 20°C and are sterile at 25°C [33]. Thus, there might still be an unidentified connection between the regulation of lifespan and germline stability by members of the ASH-2 complex.

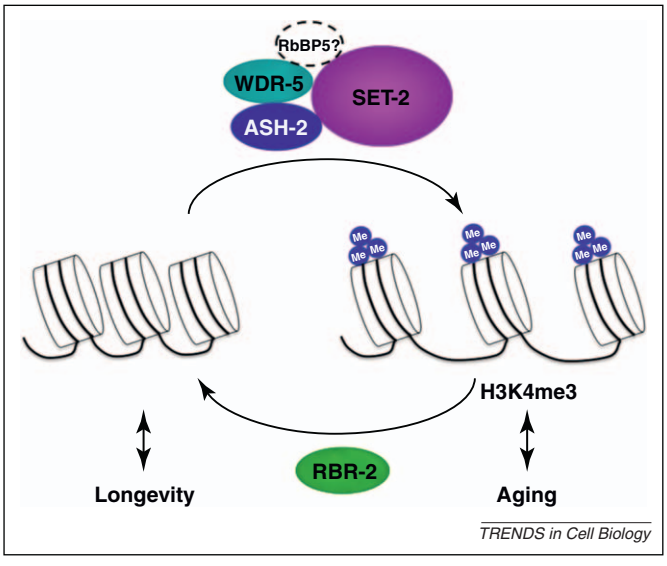


**Figure 2. Regulation of longevity by modifiers of trimethylated lysine 4 of histone H3 (H3K4me3) in *C. elegans*.** The ASH-2 H3K4me3 complex, consisting of ASH-2, SET-2, WDR-5 and possibly RbBp5, may promote aging by trimethylating H3K4. Note that the knockdown of the worm homolog of RbBP5, F21H12.1, has been shown to extend lifespan [84], although its impact on H3K4me3 is unknown. Conversely, the RBR-2 demethylase may promote longevity by demethylating H3K4me3.

## Timing of H3K4me3 regulation

Knowing that the ASH-2 complex plays an important role in longevity regulation, when does it serve to control H3K4me3 levels? Determining the time of action of H3K4me3 regulatory complexes is an important step in deciphering the mechanisms underlying lifespan extension. Recent studies show that ASH-2, WDR-5 and SET-2 are necessary for H3K4 trimethylation in both the developing and the adult germline in *C. elegans* [30,33,34]. For example, *wdr-5* and *set-2* mutant worms exhibit marked decreases in H3K4me2 and H3K4me3 in the developing and adult germline [30,33,34]. The decrease in H3K4me2/me3 marks in *wdr-5* and *set-2* mutant worms is particularly pronounced in the germline stem cell (GSC) region in adult worms [33,34]. Although genetic evidence suggests a role for the H3K4me3 regulatory complex in fertilized eggs rather than in GSCs [30], the regulation of H3K4me3 in the GSC could participate indirectly in lifespan regulation by affecting germline stability.

Surprisingly, the importance of ASH-2 for H3K4me3 regulation differs in development versus adulthood. During development, ASH-2 knockdown significantly reduces H3K4me3 levels, and this reduction is particularly noticeable in the germline [30]. By contrast, in adult worms, ASH-2 knockdown does not significantly alter H3K4me3, in either the soma or the germline [33,34] (E. L. Greer and

A. Brunet, unpublished). Thus, ASH-2 may interact with SET-2 during development, but form a complex with other methyltransferases in adults and in the soma. Overall, these observations suggest that ASH-2, WDR-5 and SET-2 regulate H3K4me3 in a largely overlapping manner, although each of these proteins may take part in other H3K4me3 regulatory complexes in a particular cell type or at a specific stage of life. As H3K4me3 is generally associated with gene expression [42,43], the time and tissue specificity of H3K4me3 regulation may have crucial consequences on the expression of longevity-related genes.

### Target genes of the H3K4me3-regulating complex

Do deficiencies in members of the H3K4me3 regulatory complex in worms affect all transcriptionally active genes or only a specific subset of genes? Whole-genome microarray analysis identified hundreds of genes regulated by ASH-2 at two stages of life (220 genes at the L3 larval stage and 847 genes at day 8 of life) [30], indicating that ASH-2 does not globally affect transcription, but rather regulates a relatively restricted number of genes. The vast majority of ASH-2-regulated genes are dependent on the presence of an intact germline [30]. Interestingly, a significant number of ASH-2 regulated genes at both ages are involved in lifespan determination [30]. ASH-2 target genes are also enriched for genes exhibiting differential expression with age [30,44]. Together, these results suggest that ASH-2 deficiency and the subsequent decline in H3K4me3 may silence 'pro-aging' genes in the germline, which would serve as a signal to promote the longevity of the soma (Figure 3). Genetic evidence, using worm mutants that cannot produce fertilized eggs but are otherwise phenotypically wild type, suggests that ASH-2 requires the presence of fertilized eggs to regulate the lifespan of the soma [30]. Thus, ASH-2 target genes could regulate the production of hormonal or metabolic signals by fertilized eggs, before the eggshell forms. These signals could diffuse into the soma, thereby preventing aging of somatic cells.

It is possible that deficiencies in H3K4me3 regulators still cause a decrease in global transcription that is not captured by microarray studies because such experiments cannot measure absolute differences in gene expression [45]. Thus, more work is needed to gain quantitative insight into the genes regulated by ASH-2 and other members of the complex. A general decrease in global transcription upon ASH-2 deficiency could lead to a reduction in global protein synthesis. Because reduced protein synthesis has been found to extend lifespan in worms and other species ([46–49], for a review see [50]), a decrease in global transcription/translation could also explain, at least in part, the beneficial effects of ASH-2 deficiency on lifespan. Thus, the mechanisms by which the H3K4me3 regulatory complex affects lifespan are not entirely clear. Comparison across species could give key insight into the role and mode of action of H3K4me3 complexes in longevity.

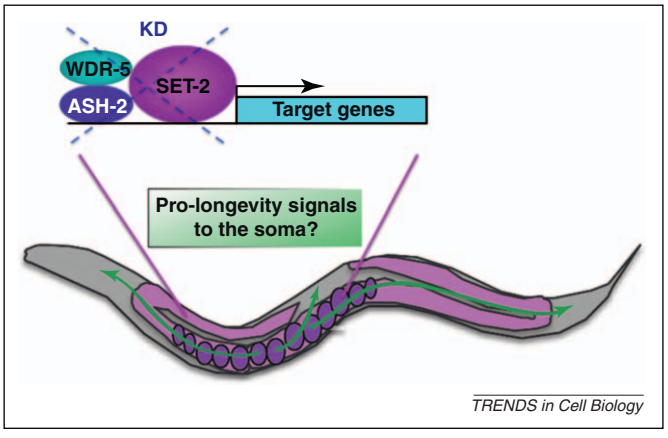


**Figure 3. Interplay between the germline and the soma in the regulation of longevity by ASH-2 complex deficiency.** In adult worms, longevity by deficiencies in the ASH-2 complex requires an intact germline with continuous egg production. It is possible that unknown 'pro-longevity' signals are emitted from the fertilized eggs when ASH-2 complex members are deficient, thereby delaying aging of the soma. KD, knockdown.

### H3K4 methylation regulators in *Drosophila* aging

The role of the H3K4me3 demethylase RBR-2 in promoting longevity appears to be conserved in *Drosophila* (Table 2) [51]. Overexpression of Lid, which is the only fly ortholog of the worm RBR-2 (Figure 4), reduces H3K4me3 levels whereas deficiencies in Lid result in elevated global H3K4me3 [52,53]. Consistent with the worm studies [30], deficiencies in Lid shortened male fly lifespan by 18% [51]. Lid does not affect the lifespan of female flies, suggesting that this H3K4me3 demethylase may impact longevity in a sex-specific manner.

By contrast, Trx, one of the three fly H3K4me3 methyltransferases, does not affect the lifespan of male flies (Table 2) [54]. Whether Trx affects female lifespan and global H3K4me3 levels was not examined in this study. Fly Trx does not have an ortholog in worms [55]. Thus, different H3K4me3 methyltransferase complexes (COMPASS, Trithorax and Trithorax-related) may have opposing roles in longevity, possibly because they act in different tissues. Alternatively, different H3K4me3 methyltransferases may regulate lifespan in a sex-specific manner. Finally, different species may have variable requirements for chromatin regulators during aging, possibly because different tissues are rate limiting for longevity in each species or because of different metabolic requirements.

### H3K4me3 in mammals

Although the importance of H3K4me3 regulators in mammalian longevity has not yet been addressed, intriguing age-dependent changes in H3K4me3 landscape have been observed in the human brain (Table 2) [56]. Genome-wide H3K4me3 landscape in FACS-isolated neurons from the prefrontal cortex of individuals ranging between 0.5 to 69 years of age revealed that neurons from infants ($< 1$ year) exhibit a larger number of loci with H3K4me3 peaks ($\sim$600 loci) than neurons from old adults ($> 60$ years) ($\sim$100 loci) [56]. The subset of infant-specific, H3K4me3-marked genes is enriched in neurogenesis, neuronal growth and differentiation genes, probably reflecting the cellular plasticity of the developing brain. Notably, a subset of loci also displays selective H3K4me3 in old neurons but not in infant neurons, although this smaller set of genes does not show significant enrichment for particular categories of genes [56]. Collectively, these results suggest that the H3K4me3 epigenome landscape is altered as a function of age in

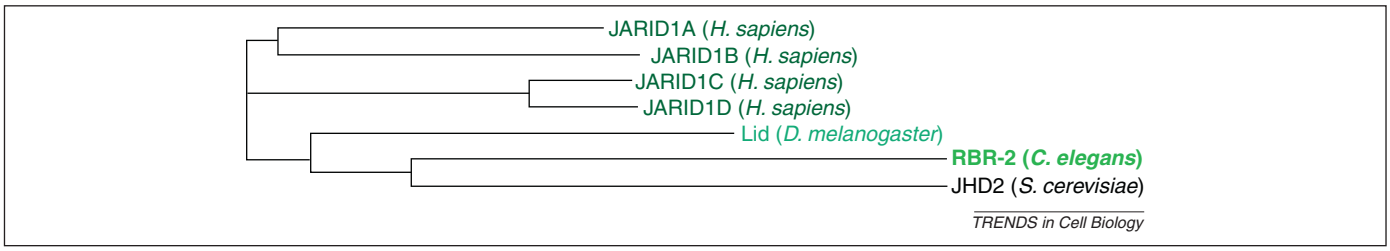


**Figure 4. Phylogenetic analysis of *C. elegans* RBR-2 and its homologs in yeast, flies and humans.** The phylogenetic tree was constructed with ClustalW2 using full-length protein sequences.

neurons. However, in the absence of intermediary ages (young and middle-aged adults), it is difficult to distinguish whether H3K4me3 changes result from aging *per se* or whether they are a consequence of the end of development.

It is also noteworthy that several H3K4me3 regulators have been implicated in the ability of stem cells to self-renew and differentiate, processes that are crucial for proper tissue regeneration and maintenance throughout lifespan [28]. For example, mammalian WDR5, a crucial component of the ASH2L complex, promotes mouse embryonic stem cell (ESC) pluripotency by activating the expression of self-renewal genes [57]. However, it is important to note that WDR5 could regulate ESC pluripotency via proteins other than MLL types of H3K4me3 methyltransferases, as WDR5 has been found to interact with several other proteins [58–60]. MLL1, an H3K4me3 methyltransferase with phylogenetic similarities to *Drosophila* Trx (Figure 1), is essential for the maintenance of fetal and adult hematopoietic stem cells (HSCs) [61,62] and for the production of neurons from postnatal neural stem cells (NSCs) [63]. Furthermore, the H3K4me1/me2 demethylase LSD1 [37] promotes adult NSC proliferation via interaction with the NSC specific transcriptional regulator TLX and by repressing pro-differentiation genes [64]. RBP2 (also known as JARID1A or KDM5A), the H3K4me3 demethylase orthologous to RBR-2 (Figure 4), mediates transcriptional repression during mouse ESC differentiation by associating with polycomb complex PRC2 [65]. Thus, regulators of H3K4me3 appear to play important roles in regulating stem cell properties. Both positive and negative regulators of H3K4me3 appear to be associated with defects in stem cell properties, suggesting that appropriate amounts of this mark are probably essential for stem cells. The ability of H3K4me3 regulators to properly coordinate stem cell renewal and differentiation of stem cells in mammals may be essential to the regenerative potential and longevity of tissues.

In mammalian cells, H3K4me3 has been shown to engage in crosstalk with other marks, in particular the repressive mark H3K27me3 [66], which raises the important question of whether other methylation marks also affect longevity.

## The repressive mark H3K27me3 in *C. elegans* and *Drosophila* longevity and in mammalian stem cell function

In worms, attenuation of the H3K27me3 demethylase UTX-1 by RNAi or heterozygous mutation extends lifespan (Table 2) [38,67]. RNAi knockdown of UTX-1 in worms leads to elevated global H3K27me3 levels [38,67,68]. Contrary to the ASH-2/WDR-5/SET-2 complex, UTX-1 regulates lifespan independently of the germline, suggesting that this demethylase acts principally in the soma to regulate lifespan [38,67]. Furthermore, UTX-1 regulates lifespan in a manner that depends on the insulin–FoxO pathway [38,67]. Interestingly, H3K27me3 levels decrease strikingly with age [38]. Consistent with this observation, *utx-1* mRNA levels increase with age in worms [67]. Together, these studies suggest that preserving high levels of H3K27me3 by inhibiting UTX-1 may be crucial for maintaining youthfulness. These data are also consistent with the loss of epigenetic control of repressed chromatin observed during aging in several species [69,70].

In flies, heterozygous mutations in the complex that methylates H3K27, notably the PRC2 components E(z) and ESC, reduce global H3K27me3 levels and extend the longevity of males (females were not tested) (Table 2) [54]. Mutations in these PRC2 components lead to partial derepression of PRC2 target genes (i.e. Abd-B and Odc1), which may mediate the effect of PRC2 on longevity [54]. Heterozygous mutation of the H3K4me3 methyltransferase Trx reverts the long lifespan of PRC2 mutants, and leads indirectly to a modest restoration of global H3K27me3 levels and PRC2-dependent target gene expression [54], suggesting that the H3K4me3 Trx complex counteracts the H3K27me3 PRC2 complex to regulate lifespan. These results also indicate that, contrary to the model put forward in worms, excessive chromatin repression may in fact shorten lifespan in flies. Why does deficiency in the H3K27 demethylase (in hermaphrodite worms) and also in the H3K27 methyltransferases (in male flies) result in lifespan extension? This discrepancy could be attributed to species-specific differences, possibly because of the amount of proliferative cells versus differentiated cells present in the organism. Alternatively, H3K27me3 levels may not affect lifespan in the same manner, depending on sex, tissue or time of action. Target genes of H3K27me3 complexes may differ depending on the specific regulators that deposit it. It is noteworthy that, in mammalian cells, UTX is part of the Trithorax-related complexes but not a part of the COMPASS or Trithorax complexes [71,72]. Thus, each complex may have a specific role in longevity, even though they target similar marks, possibly because the genomic loci these marks affect are different. Finally, it cannot be excluded that PRC complexes and UTX demethylases have other substrates than H3K27, and that these substrates may differ in worms and flies.

In mammals, H3K27me3 regulators also play important roles in stem cell aging. BMI1, a member of the mammalian H3K27me3 polycomb complex 1 (PRC1), is necessary for

the self-renewal of adult stem cells, such as HSCs [73–76] and NSCs [77–79], in large part through repression of the p16INK4a/p19ARF locus [80–82]. Perturbations in H3K27me3 and H3K4me3 regulators both result in defects in stem cell proliferation, suggesting that a delicate balance between activation and repression of distinct sets of target genes is required during the stem cell aging process. This balance might be particularly important in the context of 'bivalent domains', which are regions of the chromatin containing both H3K4me3 and H3K27me3. Bivalent domains have been suggested to mark regulatory regions of genes that are 'poised' for differentiation in ESCs and potentially other stem cells [66]. Dpy-30, a core component of the mammalian SET1/MLL H3K4me3-regulating complexes, has been shown to be crucial for mouse ESC fate specification by modulating H3K4 methylation in these bivalent domains [83]. It will be interesting to determine whether and how bivalent domains are affected during aging in pools of adult stem cells.

## Other methylation marks in worm, fly and mammalian longevity

Interestingly, additional methyltransferases and demethylases have emerged from *C. elegans* lifespan screens, including the potential methyltransferases SET-9 [30,84], SET-15 [30,84], SET-6 [30], SET-4 [30], BLMP-1 [30] and the demethylase T26A5.5 [38]. Although the exact marks regulated by these enzymes are not yet known in worms, it will be interesting to test the epistatic interactions between H3K4me3 regulators and these other chromatin modifiers.

Several methylation marks have recently been shown to change with age in *Drosophila*. ChIP-chip of several histone marks in 10-day (young) and 40-day (old) female fly heads revealed an age-dependent loss in activating marks (H3K4me3 and H3K36me3) and an increase in repressive marks (H3K9me3) [85], consistent with the notion that, in flies, repressive marks may promote aging. The nuclear distribution of the H3K9me3 mark is also altered in older flies, presumably because this repressive mark aberrantly diffuses into the euchromatin in old individuals. The age-dependent increase in global H3K9me3 does not appear to be accompanied by a change in gene expression at these loci, as measured by microarrays [85]. Thus, the age-dependent increase in H3K9me3 may not be sufficient to alter gene expression. Alternatively, regions of H3K9me3 that might have led to transcriptional silencing may not have been covered by the ChIP-chip genomic arrays (e.g. repetitive regions). Finally, microarrays may underestimate changes in gene expression, particularly when they are global.

In mammals, H4K20me3 – a mark associated with constitutive heterochromatin [86] – increases with age in rat livers [87], as well as in cells derived from human patients with Hutchinson–Gilford progeria, a premature aging syndrome [88]. An elevation of repressive marks with age suggests that excessive transcriptional repression may contribute to the aging process. This conclusion, however, is in contrast to the finding that cells isolated from patients with Hutchinson–Gilford progeria exhibit reduction or complete loss of the heterochromatin mark H3K9me3 [89]. Thus, more work is needed to determine how repressive and activating marks change with age, and to explore whether these changes are a cause or a consequence of aging. An increasing number of histone marks have been found to change during aging, suggesting that different histone marks may act together, in a network, to affect longevity (Figure 5).

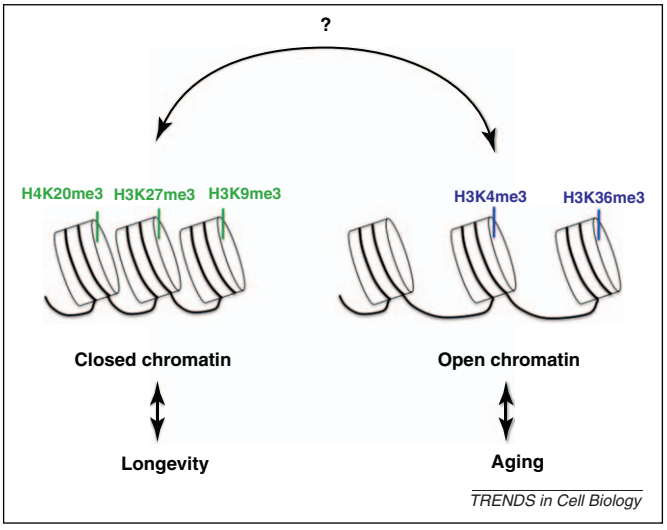


**Figure 5. Potential interactions between histone methylation marks in longevity.** Several different histone methylation marks are regulated during aging in specific tissues (e.g. liver, brain) or the entire organism from worms to mammals. Histone regulators affect aging, at least in worms and flies. Complex interactions between different marks associated with active or repressed chromatin may participate in lifespan regulation.

## Concluding remarks

The emerging evidence for the role of histone methylation regulators in longevity in invertebrates raises a series of questions. First, it will be interesting to test whether environmental interventions such as DR or stress stimuli could mediate longevity by altering histone methylation. It will be particularly informative to test whether regulators of histone methylation, similar to DR, could delay certain aspects of the aging process and slow the occurrence of age-related diseases.

Understanding the mechanisms underlying lifespan extension by regulators of histone methylation is also important. Do these regulators act by specifically altering genes involved in aging or longevity? Or do they induce more global changes, for example in protein translation or in metabolism, which could in turn impact longevity? To address these questions, it will be important to identify the target genes regulated by histone methylation regulatory complexes and examine whether some of these genes are particularly pivotal in mediating the longevity phenotype of histone methylation regulators. It will be intriguing to identify the nature of the signal between fertilized eggs and soma for longevity due to H3K4me3 regulator deficiencies in worms.

Regulators of histone marks are strikingly conserved across species. Thus, dissecting the role of the different complexes in longevity in higher metazoans is of particular importance. It will be interesting to test whether interplay between fertilized embryos and mothers for longevity also occurs in other species. The fact that methyltransferases

that modify the same H3K4me3 histone mark (e.g. SET-2 in worms and Trx in flies) have different effects on lifespan in these species suggests that the answer is likely to be complex. A factor that may contribute to the differences between organisms is the ratio of proliferative cells, such as stem cells and germ cells, to post-mitotic differentiated cells. Another factor that could contribute to differences between species is that changes in chromatin state may affect global metabolism, and that the requirement for metabolic states could vary in different species.

An outstanding question is whether longevity induced by deficiencies in the H3K4me3 complex in the parental generation can be inherited in the subsequent generations, even if the H3K4me3 complex is no longer mutated in descendants. Remarkably, lifespan extension due to deficiencies in members of the H3K4me3 regulatory complex only in parents can be inherited epigenetically for up to three generations [90], providing the first example of transgenerational epigenetic inheritance of longevity. Mutation of one of the H3K4me1/me2 LSD1 demethylases, *spr-5*, in *C. elegans,* leads to progressive epigenetic germline mortality over many generations [91], raising the possibility that changes in this mark may also impact lifespan in a transgenerational manner. Taken together, these data imply that changes in chromatin in one generation could impact the lifespan of subsequent generations.

A greater understanding of the role of chromatin regulators in longevity and the molecular mechanisms by which they act may provide new avenues to extend healthspan and delay the onset of age-related diseases.

**Acknowledgments**

We thank Bérénice A. Benayoun, Eric L. Greer, Jana P. Lim, Travis J. Maures and Elizabeth A. Pollina for critical reading of the manuscript and helpful suggestions. This work was supported by NIH R01-AG31198 grant to A.B. S.H. was supported by an NSF graduate fellowship and a Stanford graduate fellowship.

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