Trimethylation of Lys36 on H3 restricts gene expression change during aging and impacts life span

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Functional data indicate that specific histone modification enzymes can be key to longevity in Caenorhabditis elegans, but the molecular basis of how chromatin structure modulates longevity is not well understood. In this study, we profiled the genome-wide pattern of trimethylation of Lys36 on histone 3 (H3K36me3) in the somatic cells of young and old Caenorhabditis elegans. We revealed a new role of H3K36me3 in maintaining gene expression stability through aging with important consequences on longevity. We found that genes with dramatic expression change during aging are marked with low or even undetectable levels of H3K36me3 in their gene bodies irrespective of their corresponding mRNA abundance. Interestingly, 3′ untranslated region (UTR) length strongly correlates with H3K36me3 levels and age-dependent mRNA expression stability. A similar negative correlation between H3K36me3 marking and mRNA expression change during aging was also observed in Drosophila melanogaster, suggesting a conserved mechanism for H3K36me3 in suppressing age-dependent mRNA expression change. Importantly, inactivation of the methyltransferase met-1 resulted in a decrease in global H3K36me3 marks, an increase in mRNA expression change with age, and a shortened life span, suggesting a causative role of the H3K36me3 marking in modulating age-dependent gene expression stability and longevity.

Keywords: aging; epigenetics; gene expression; H3K36me3; met-1

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Extensive studies in model organisms and human tissues have identified many genes that show age-dependent expression changes (Lee et al. 1999; Zou et al. 2000; Kayo et al. 2001; Fletcher et al. 2002; McCarroll et al. 2004; Zahn et al. 2006; Golden and Melov 2007; Budovskaya et al. 2008; Hong et al. 2008; de Magalhaes et al. 2009; Glass et al. 2013). These age-regulated genes generally participate in a wide variety of biological processes that are associated with age-dependent physiological changes. In mice, most age-regulated gene expression alterations can be either completely or partially delayed by caloric restriction, a well-known intervention that retards aging (Lee et al. 1999, 2000), suggesting that gene expression regulation plays an important and causal role in the aging process.

Epigenetic mechanisms are key to gene expression regulation and other processes of DNA transaction (Kouzarides 2007). Previous studies have clearly demonstrated that changes in DNA methylation, histone modifications, and small RNAs, which are important components of epigenetic regulation, occur during aging in different species (Feser and Tyler 2011; Jung and Suh 2012; Smith-Vikos and Slack 2012; Winnefeld and Lyko 2012; Hannum et al. 2013; Horvath 2013; Wood and Helfand 2013). Emerging evidence in model organisms has bolstered a causative role for epigenetic regulation in longevity determination (Kim et al. 1999; Hamilton et al. 2005; Curran and Ruvkun 2007; McColl et al. 2008; Dang et al. 2009; Greer et al. 2010, 2011; Siebold et al. 2010; Jin et al. 2011; Maures et al. 2011). Caenorhabditis elegans is a leading model to study the molecular basis of aging and longevity determination. Functional studies in C. elegans have identified several chromatin modifiers and histone modification enzymes that promote longevity when...
attenuated [Hamilton et al. 2005; Curran and Ruvkun 2007; Greer et al. 2010, 2011; Jin et al. 2011; Maures et al. 2011]. These studies strongly support the importance of a better understanding of the genome-wide chromatin landscape during aging to gain further insights into possible epigenetic mechanisms of life span modulation.

In this study, we set out to explore the global profile of trimethylation of Lys36 on histone H3 (H3K36me3) in the somatic cells of young and old C. elegans. H3K36me3 is a histone modification mark enriched on the gene body region and associated with active gene transcription (Shilatifard 2006). H3K36me3 can be deposited by elongating RNA polymerase II (Pol II) (Hampsey and Reinberg 2003) and is important for regulating Pol II elongation and preventing cryptic transcription (Carrozza et al. 2005; Venkatesh et al. 2012; Wen et al. 2014). H3K36me3 has also been implicated in RNA splicing (Kolasinska-Zwierz et al. 2009; de Almeida et al. 2011; Kim et al. 2011; Pradeepa et al. 2012) and DNA mismatch repair (Li et al. 2013). Not surprisingly, based on the broad roles of H3K36me3, defects in the enzymes required to maintain H3K36 methylation have been shown to cause developmental defects and disease [Wagner and Carpenter 2012, Fontebasso et al. 2013]. We found that genome-wide distribution of H3K36me3 is largely stably maintained during aging in C. elegans. Interestingly, our data revealed that the H3K36me3 mark is inversely correlated to mRNA expression change during aging. Genes with dramatic expression difference during aging are marked with low or even undetectable levels of H3K36me3 in their gene bodies irrespective of their corresponding mRNA abundance. A similar correlation was also observed in Drosophila melanogaster, suggesting a conserved mechanism for H3K36me3 in suppressing age-dependent mRNA expression alteration. We further identified that the length of the 3′ untranslated region (UTR) strongly correlates with H3K36me3 levels and age-dependent mRNA expression stability, suggesting a novel link between mRNA 3′ end processing and/or microRNA (miRNA) regulation with H3K36me3 modification. Importantly, we demonstrated that the reduction of global H3K36me3 levels through inactivation of the methyltransferase met-1 caused an increase in mRNA expression change with age and a shortened life span. Our findings therefore uncover a new role for H3K36me3 in restraining gene expression alteration during aging, with important consequences on longevity.

Results

H3K36me3 distribution is stably maintained during aging in C. elegans somatic cells

To monitor H3K36me3 patterns in the somatic tissues of C. elegans at different ages, we performed chromat immunoprecipitation (ChIP) coupled with deep sequencing (ChIP-seq) using whole worm extracts from young and old worms lacking germlines. We used germlineless worms to avoid the complication that dramatic changes of histone modifications that occur in germ cells through adulthood would mask changes in aging somatic tissues. The glp-1(e2141) mutant that we used produces very few germ cells (Press et al. 1987) and is slightly longer than the wild type (~10% life span extension) when grown at the restrictive temperature 25°C [data not shown]. Despite the slight life span difference, we believe that relevant age-dependent histone modification patterns can be derived. We examined four independent biological replicates at three time points: day 2 (D2) and D4 adults as the young stage points and D12 adults as the old stage point. Two young time points were chosen because previous studies indicated a dramatic change in proteostasis ability at around D2 [Labbadia and Morimoto 2014], and reproduction generally ends prior to D4 in wild-type worms.

Consistent with previous findings, H3K36me3 was mainly deposited on the gene body region with higher enrichment at the 3′ end [Supplemental Fig. S1A; Pokhokol et al. 2005; Barski et al. 2007; Mikkelsen et al. 2007; Edmonds et al. 2008; Wagner and Carpenter 2012] and was slightly more enriched at exons compared with introns [Supplemental Fig. S1B; Kolasinska-Zwierz et al. 2009]. To evaluate whether aging is associated with genome-wide dynamic changes in the H3K36me3 pattern, we computed the pair-wise Pearson correlation of the histone H3-normalized H3K36me3 data at the three time points for the four biological replicates. The correlation coefficients between all data sets were >0.80, indicating the absence of a dramatic genome-wide H3K36me3 pattern change during aging [Fig. 1A].

Since H3K36me3 largely marks the gene body, we further analyzed the H3K36me3 levels on the gene bodies [transcription start site to transcription termination site according to the ce6 annotation of the C. elegans genome] of all protein-coding genes. Our analysis revealed a bimodal distribution of the H3K36me3 marks on protein-coding genes in C. elegans [Supplemental Fig. S1C, Supplemental Table S2], where gene populations exhibited either high or low levels of normalized H3K36me3 [gene density peaks at normalized H3K36me3 log2 value of 1.6 and −1.5, respectively]. Spearman’s correlation test similarly indicated that H3K36me3 markings on the gene body of protein-coding genes were largely similar during aging, with all pair-wise Spearman’s correlation coefficients >0.87 [Fig. 1B]. In this analysis, we also included the H3K36me3 profiling data from L3 stage larvae [mod-ENCODE ID 3563]. The results showed that the genome-wide H3K36me3 pattern at the L3 stage was very similar to those in adult and aged worms [Spearman’s correlation coefficient >0.84], suggesting that the H3K36me3 pattern is largely established during development. Nevertheless, our analyses revealed that the highly methylated genes had slightly reduced methylation levels with age, whereas the lowly methylated genes had slightly increased levels [Supplemental Fig. S1C].

Despite the generally stable maintenance of the H3K36me3 pattern through aging, we sought to identify genes with consistent changes in H3K36me3 marks with aging. We developed a new ChIP-seq data analysis
pipeline based on the statistical framework of the generalized linear mixed model (GLMM) [see the Materials and Methods for further details] to identify genes and transcripts that show statistically significant changes in H3K36me3 levels between the D2 and D12 adult time points. The GLMM analysis identified 872 transcripts [corresponding to 624 genes] that gained H3K36me3 with age (here termed “K36 up with age”) and 1930 transcripts [corresponding to 1435 genes] that lost H3K36me3 with age (“K36 down with age”), false discovery rate [FDR] <0.05). The dip around 0 in the plots reflects the absence of transcripts with no significant mRNA expression change with age. Gene body H3K36me3 levels and RNA sequencing (RNA-seq) data at D2 and D12 are listed in Supplemental Tables S2 and S3, respectively.

No simple positive correlation between H3K36me3 marks on gene bodies and high mRNA expression abundance for actively expressed genes

To compare H3K36me3 marks with mRNA expression during aging, we performed mRNA sequencing [mRNA-seq] at the identical aging time points using worms prepared in parallel to those for ChIP-seq analysis. Among the 12,447 transcripts that were detectably expressed [FPKM [fragments per kilobase per million mapped fragments] >0.5] at both the D2 and D12 time points, the Cuff-Diff analysis pipeline [Trapnell et al. 2012] identified 3277 transcripts [corresponding to 3218 protein-coding genes] that showed a significant change in mRNA levels through aging [Q-value < 0.01] [Supplemental Table S3]. Comparing H3K36me3 ChIP-seq and mRNA-seq results revealed an absence of H3K36me3 on undetectably and weakly expressed genes \(\log_{10} \text{FPKM} < 0\) [Supplemental Fig. S1E], consistent with previous findings that H3K36me3 marks actively transcribed genes [Shilatifard 2006; Kouzarides 2007]. For genes that are actively expressed at moderate levels \(\log_{10} \text{FPKM} \text{between 0 and 1}\), their mRNA levels positively correlated with their gene body H3K36me3 marking [Spearman’s correlation, \(\rho = 0.17, 0.13, \text{and } 0.14, P\text{-value } < 0.001\)] [Supplemental Fig. S1E]. However, for the highly expressed genes \(\log_{10} \text{FPKM} \text{between 1 and 1.5}\), their mRNA abundance and gene body H3K36me3 levels did not exhibit a significant correlation [Spearman’s correlation, \(\rho = 0.02, P\text{-value } > 0.1\)] [Supplemental Fig. S1E]. Interestingly, for
the most highly abundant genes ($\log_{10} \text{FPKM} > 1.5$), their mRNA levels actually negatively correlated with their H3K36me3 marking (Spearman’s correlation, $\rho = -0.12$, $P$-value < 0.001) [Supplemental Fig. S1E]. Therefore, while H3K36me3 marks are commonly associated with active gene transcription, H3K36me3 levels do not necessarily correlate with mRNA levels [Supplemental Fig. S1F].

We further examined whether the subset of genes that showed age-dependent changes in H3K36me3 markings according to the GLMM analysis would exhibit corresponding changes in mRNA expression levels. In the “K36 up with age” and “K36 down with age” groups, 606 and 1388 transcripts, respectively, were detected in our mRNA-seq analysis. Among them, 210 and 355 transcripts, respectively, exhibited significantly altered mRNA abundance during aging ($Q$-value < 0.05) [Supplemental Table S4]. In general, there appears to be no strict correlation between changes in H3K36me3 levels and corresponding changes in mRNA abundance, as both the “K36 up with age” and “K36 down with age” groups contain a similar proportion of transcripts that exhibited increased, unchanged, or decreased mRNA expression [Fig. 1D]. For the “K36 up with age” group of transcripts, the Spearman’s correlation test identified a positive correlation between a change in H3K36me3 marks and a change in mRNA expression between D2 and D12 adults ($\rho = 0.40$, $P$-value < 0.0001), which are mainly contributed by 76 transcripts in this group with low to median expression levels [FPKM < 10]. Spearman’s correlation test illustrated that changes in H3K36me3 methylation actually negatively correlate with a mRNA abundance change for the “K36 down with age” group ($\rho = -0.16$, $P$-value = 0.002).

H3K36me3 marking on gene bodies negatively correlates with changes in gene expression during aging

We next analyzed whether and how H3K36me3 marks might relate to mRNA expression change with age. We ranked all of the protein-coding transcripts according to their gene body H3K36me3 levels at the young D2 time point and plotted the corresponding $\log_2$ values of age-dependent mRNA abundance changes [i.e., $\log_2$ of D12 FPKM/D2 FPKM]. We observed a striking inverse correlation between H3K36me3 marks and mRNA expression change [Fig. 2A], where transcripts marked by lower levels of H3K36me3 tended to yield greater changes in mRNA levels with age. A similar pattern was also observed when transcripts were ranked according to their gene body H3K36me3 levels at D12 [Supplemental Fig. S2B]. As a different way of representing this trend, genes that showed statistically significant age-dependent mRNA expression changes ($Q$-value < 0.05 or $Q$-value < 0.01) were preferentially marked by lower levels of H3K36me3 [Supplemental Fig. S2C].

To more rigorously examine this inverse correlation, we focused our analysis on the subset of transcripts that were detectably expressed [FPKM > 0.5 at both young and old time points] and that showed statistically significant changes in mRNA abundance with age ($Q$-value < 0.01). Spearman’s correlation test showed that for this group of transcripts (3262 transcripts), the absolute $\log_2$ values of their age-dependent mRNA expression changes and their gene body H3K36me3 levels were strongly negatively correlated (Spearman’s correlation, $\rho = -0.43$, $P$-value < 0.001 when D2 H3K36me3 levels were used, $\rho = -0.41$, $P$-value < 0.001 when D12 H3K36me3 levels were used) [Fig. 2B; Supplemental Fig. S2D]. The absolute $\log_2$ value of the fold change in mRNA abundance was used for the Spearman’s correlation test because the focus was to correlate H3K36me3 marks with the degree of change in mRNA levels and not the direction of the change. Consistent with this strong inverse correlation, transcripts that changed more dramatically during aging were preferentially marked with lower levels of H3K36me3 [Fig. 2D].

In order to further quantify the correlation between gene body H3K36me3 levels and mRNA expression change, we divided this transcript group [FPKM > 0.5 at D2 and D12; $Q$-value < 0.01] into 10 bins with an equal number of transcripts [326 transcripts per bin] and computed the median normalized H3K36me3 level [Fig. 2E] and the variance of age-dependent mRNA abundance change ($\text{variance refers to the statistical term estimating the spread from the mean of age-dependent fold change in mRNA abundance}$) [Fig. 2F] for each bin. Linear regression analysis showed that the variance in mRNA abundance change and the median gene body H3K36me3 levels across the 10 bins is strongly negatively correlated with the coefficient of determination $R^2 = 0.96$ [Fig. 2G]. In addition to protein-coding transcripts, we also observed a similar negative correlation between age-dependent changes in mRNA expression and gene body H3K36me3 levels in long non-coding RNA [lncRNA] genes [Spearman correlation, $\rho = -0.24$, $P$-value = 0.029] [Supplemental Fig. S2F; Supplemental Tables S2, S3].

H3K36me3 marking negatively correlates with gene expression change during aging irrespective of gene length or the abundance and tissue pattern of gene expression

The negative correlation between age-dependent mRNA expression change and gene body H3K36me3 marking did not appear to correlate with mRNA abundance, as genes marked with low to moderate levels of H3K36me3 can have expression levels ranging from very low to very high, although genes marked with high levels of H3K36me3 tend not to exhibit expression abundance at the extreme levels [Fig. 2C; Supplemental Fig. S2A]. To rule out the possibility that lowly expressed genes, which tend to be marked by no or very low levels of H3K36me3, are difficult to quantify using RNA-seq and thus artificially show greater mRNA expression change with age, we further divided the 3262 transcripts [FPKM > 0.5; $Q$-value < 0.01] into three subgroups of equal numbers of transcripts based on their mRNA abundance [low, medium, and high] [Supplemental Fig. S3A]. Spearman’s correlation test demonstrated that for each of the subgroups, age-dependent changes in mRNA expression and H3K36me3 marks still exhibited a statistically significant negative correlation ($\rho = -0.50$, -0.37, and -0.30 for low, medium, high, respectively).
and high mRNA abundance groups, respectively; P-value < 0.001 [Fig. 3A]. For each subgroup, we performed the same binning and computation of gene expression variance and median H3K36me3 levels as discussed above [Fig. 3B]. Linear regression analysis indicated that the negative correlation between H3K36me3 marks and age-dependent mRNA expression variation persisted in all three subgroups ($R^2 = 0.95, 0.84, and 0.96$ for low, medium, high, respectively) [Fig. 3C]. Together, these results indicate that for actively expressed protein-coding genes, their mRNA expression change through aging is inversely correlated with their gene body H3K36me3 marking regardless of their mRNA expression levels.

H3K36me3 levels were previously demonstrated to be affected by both gene length and transcription frequency in yeast [Pokholok et al. 2005; Li et al. 2007]. We further tested whether the negative correlation between H3K36me3 level and mRNA expression change was influenced by gene length. First, we observed a weak positive correlation between absolute values of log$_2$ fold change in mRNA abundance and log$_2$ values of normalized H3K36me3. ($^{***}$) P-value < 0.001. [C] mRNA levels of transcripts with significant age-dependent change [FPKM >0.5; Q-value < 0.01] are plotted according to the rank of H3K36me3 levels at D2. mRNA levels at D2 and D12 are plotted in red and in blue, respectively. [D] Transcripts plotted in B are divided into three subgroups according to the absolute values of their age-dependent log$_2$ fold change (FC) in mRNA abundance: high, $|\log_2 FC| > 3$; medium, $2 < |\log_2 FC| < 3$; and low, $|\log_2 FC| < 2$. The X-axis shows the normalized H3K36me3 levels at D2. The Y-axis shows the proportion of transcripts with the corresponding H3K36me3 level for the indicated subgroup. [E] Transcripts plotted in B were divided into 10 bins of equal numbers of transcripts (except bin 10, which had two extra transcripts) according to their normalized H3K36me3 levels from low to high. Box plot showing normalized H3K36me3 levels of genes in each bin. [F] Variance of the age-dependent mRNA abundance change for each bin is shown. Variance represents the spread from the mean of the log$_2$ values of age-dependent fold change in mRNA abundance for the transcripts in each bin: Variance = $\sum (x - \overline{x})^2 / n - 1$. [G] Regression plot showing variance in mRNA abundance from F as a function of median H3K36me3 levels from E. Gene body H3K36me3 levels and RNA-seq data at D2 and D12 are listed in Supplemental Tables S2 and S3, respectively.
however, this group contained only 80 transcripts, which
significant negative correlation (Supplemental Fig. S3F);
The neuron-specific genes did not yield a statistically
expressed in hypodermis, intestine, and body wall muscle
abundance changes and gene body H3K36me3 markings
negative correlations between age-dependent mRNA
(Spencer et al. 2011). We observed statistically significant
ubiquitously expressed or specifically
H3K36me3 gene body marks and age-dependent changes
this possibility, we investigated the correlation between
tracts, this could lead to a false overall low H3K36me3
and mRNA-seq data were generated from whole worm ex-
differentially expressed among developmental stages and
levels and mRNA expression change during aging
concluded that the inverse correlation between gene
H3K36me3 level and variance in mRNA abundance for each subgroup are shown in
of log2 fold change in mRNA abundance and log2 values of normalized H3K36me3. The linear regression coefficient and
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the longer gene subgroups (R^2 = 0.67, 0.80) [Fig. 3E]. We concluded that the inverse correlation between gene
body H3K36me3 levels and age-dependent mRNA expression change is largely independent of gene length.
Since H3K36me3 is commonly associated with active
gene transcription, genes that are expressed in specific
tissues likely harbor tissue-specific methylation on H3K36,
and ~75% of C. elegans transcripts are estimated to be
differentially expressed among developmental stages and
across cell types (Spencer et al. 2011). As our ChIP-seq
and mRNA-seq data were generated from whole worm extracts, this could lead to a false overall low H3K36me3
marking on tissue-specific genes. In an attempt to exclude
this possibility, we investigated the correlation between
H3K36me3 gene body marks and age-dependent changes
in mRNA abundance using gene sets that are either
ubiquitously expressed or expressed in specific tissues
(Spencer et al. 2011). We observed statistically significant
negative correlations between age-dependent mRNA abundance changes and gene body H3K36me3 markings
for genes that are ubiquitously expressed or specifically expressed in hypodermis, intestine, and body wall muscle
[Spearman’s coefficient −0.52, −0.36, −0.45, and −0.26, respectively; P-value < 0.001] [Supplemental Fig. S3B–E].
The neuron-specific genes did not yield a statistically
significant negative correlation [Supplemental Fig. S3F]; however, this group contained only 80 transcripts, which
might not yield sufficient data for a robust analysis. Overall,
our data suggest that H3K36me3 marks negatively correlate with mRNA expression change with age irrespective of the gene’s tissue pattern of expression.

3’ UTR length is correlated with gene body H3K36me3
levels and mRNA expression change during aging
In addition to gene length discussed above, we examined
whether any other gene structure features would correlate
with H3K36me3 marking in C. elegans protein-coding genes. We surveyed protein-coding region (coding sequence [CDS]) length, 3’ UTR length, and 3’ UTR length. We found that 3’ UTR length strongly correlated with gene body H3K36me3 levels [transcription start site to transcriptional termination site according to c6 annotation] [Fig. 4A] as well as on protein-coding regions (CDS) [ρ = 0.36 and 0.41, respectively; P-value < 2.2 × 10^-16]. It is interesting to note that 3’ UTR length is positively correlated with gene length [Spearman’s correlation coefficient ρ = 0.32; P-value < 0.001], and, as discussed previously, gene length is also positively correlated with H3K36me3 level but with a weaker correlation [ρ = 0.18] compared with that between 3’ UTR length and H3K36me3 level [ρ = 0.36]. We further tested whether mRNA abundance change during aging correlated with 3’ UTR length. As expected based on the correlation of

**Figure 3.** The negative correlation between H3K36me3 levels and gene expression change is independent of mRNA abundance, gene length, or tissue expression pattern. (A) Transcripts plotted in Figure 2B were divided into three subgroups according to mRNA abundance [low, medium, and high]. Transcripts in each subgroup were plotted in the order of normalized H3K36me3 levels at the D2 time point from low [left] to high [right] (shown in red). The corresponding log2 values of age-de- pending fold change in mRNA abundance were plotted in black. (ρ) Spearman’s correlation coefficient between absolute log2 values of fold change in mRNA abundance and log2 values of normalized H3K36me3. (***) P-value < 0.001. (B) Transcripts in each subgroup shown in A [low, medium, and high] were divided into 10 bins of equal numbers of transcripts (except bin 10, which had seven or eight extra transcripts) according to their normalized H3K36me3 levels [data not shown] from low [L] to high [H]. Variance of age-dependent mRNA abundance change for each bin is shown. (C) Regression plot showing var-
ience in mRNA abundance from B as a function of median H3K36me3 levels for each bin. (D,E) The negative correlation between H3K36me3 levels and mRNA expression change is independent of gene length. Trans-
scripts plotted in Figure 2B were divided into five sub-
groups (G1–G5) according to gene length as indicated
e in E. Transcripts in each subgroup were ranked in the or-
der of normalized H3K36me3 levels at the D2 time point from low [left] to high [right] (shown in red in D). The cor-
responding log2 values of age-dependent fold change in mRNA abundance ae plotted in black in D. Each sub-
group was then binned, and subsequent calculation were computed as described for A. (ρ) Spearman’s coefficient between absolute values of log2 fold change in mRNA abundance and log2 values of normalized H3K36me3. The linear regression coefficient and R^2 between H3K36me3 level and variance in mRNA abundance for each subgroup are shown in E. (****) P-value < 0.001.
3′ UTR length with H3K36me3 marking, 3′ UTR length also negatively correlated with age-dependent mRNA expression changes during aging [Spearman’s correlation, \( \rho = -0.24 \), P-value < 2.2 × 10\(^{-16}\)] [Fig. 4B,C]. For the analyses here, the longest 3′ UTR length was used when genes are annotated with more than one 3′ UTR [Mangone et al. 2010]. We detected a similar correlation for the subset of genes with a single 3′ UTR [\( \rho = -0.26 \), P-value < 2.2 × 10\(^{-16}\)], which indicates that 3′ UTR length but not 3′ UTR type determines the correlation.

The negative correlation between H3K36me3 levels and gene expression change is conserved in wild-type C. elegans and in D. melanogaster

Using publicly available data [GSE21784 and modENCODE ID 3565], we examined the correlation between age-dependent mRNA expression change and gene body H3K36me3 marking in wild-type worms with germlines. A comparison of H3K36me3 levels at the L3 stage with the corresponding mRNA expression change from adult D6 to D15 [Youngman et al. 2011] revealed a similar negative correlation between mRNA expression change and gene body H3K36me3 level [\( \rho = -0.42 \), P-value < 0.001] (Supplemental Fig. S2E). Our data suggest that gene body H3K36me3 marking also restricts age-dependent gene expression change in wild-type worms.

To test whether H3K36me3 marks negatively associate with mRNA expression change during aging in different species, we examined H3K36me3 ChIP–chip data and RNA-seq data from young (10 d) and old (40 d) D. melanogaster female heads [Wood et al. 2010]. We analyzed the Drosophila data using the same methods described earlier for the C. elegans data and observed a very similar negative correlation between gene body H3K36me3 levels and age-dependent mRNA expression changes [Spearman’s correlation test, \( \rho = -0.50 \), P-value < 0.001, and linear regression analysis, \( R^2 = 0.92 \)] [Fig. 5A–C]. We further parsed the Drosophila data into three subgroups according to mRNA abundance and again observed that the negative correlation between age-dependent mRNA expression change and H3K36me3 level is independent of gene expression abundance [Spearman’s correlation coefficient, \( \rho = -0.33 \), –0.55, and –0.46, P-value < 0.001] [Fig. 5D]. In summary, we propose that H3K36me3 has a conserved role in restraining dramatic changes in mRNA expression through aging in diverse species.

Reduction of global H3K36me3 levels increases gene expression change during aging and shortens life span

To distinguish between a simple correlation between H3K36me3 marks and changes in mRNA expression and a causative role for H3K36me3 in restricting mRNA expression change with age, we sought to reduce global H3K36me3 levels in adult worms and monitor the consequent mRNA expression through aging. In C. elegans, the met-1 methyltransferase has been suggested to maintain H3K36me3 in somatic cells, and the mes-4 methyltransferase performs that role in germ cells [Andersen and Horvitz 2007]. To investigate the impact of reducing H3K36me3 in somatic cells of C. elegans, we introduced the met-1 (n4337) loss-of-function mutation into the glp-1(e2141) strain background or treated glp-1(e2141) worms...
pendent changes in mRNA expression in to the earlier RNA-seq analysis. We examined the age-de-
spective controls at the D2 and D12 time points, similar (n4337); glp-1(e2141)
aging, we performed mRNA-seq analysis using met-1
met-1; glp-1
genes would influence mRNA expression change through aging in both control worms (Fig. 6B; Supplemental Table S5). A cross-
met-1; glp-1
worms, and transcripts from 4530 genes varied in genes significantly varied during aging in control 1 control worms indicated that transcripts from 3149

met-1; glp-1
and glp-1 worms (Fig. 6A; Supplemental Fig. S4A).

Interestingly, the expression of the ~1800 gene transcripts varied with age significantly only in met-1 mutant worms (~1100 for RNAi worms), but a similar insignificant trend was observed for these transcripts in control worms [Fig. 6C, Supplemental Fig. S4C]. Therefore, the substantial reduction of global H3K36me3 levels (~90% in mutant worms and ~70% in RNAi worms) that occurs in animals with attenuated met-1 likely caused these subsets of genes to become more variable with age. Furthermore, the transcripts that showed significant expression change specifically in the met-1 mutant were enriched with highly methylated genes compared with the transcripts that showed significant expression change in both control and met-1 mutant worms [Supplemental Fig. S4D], suggesting that genes with higher levels of H3K36me3 were more affected by met-1 inactivation.

We next investigated the functional consequence of H3K36me3 reduction on life span. We found that both the met-1 loss-of-function mutation and RNAi knockdown significantly shortened the life span of glp-1 worms (~13%–20% reduction in mean life span) [Fig. 6E, Supplemental Table S7]. Similarly, met-1 mutation in wild-type N2 worms also shortened life span

Figure 5. Gene body H3K36me3 level is negatively correlated with age-dependent mRNA expression change in Drosophila heads. (A) All genes with mappable H3K36me3 ChIP-chip and RNA-seq reads are plotted in the order of normalized H3K36me3 levels at the young time point from low (left) to high (right) [shown in red]. The corresponding log2 values of age-dependent fold change in mRNA abundance are plotted in black. (p) Spearman’s correlation coefficient between absolute log2 values of fold change in mRNA abundance and log2 values of normalized H3K36me3. [***] P-value < 0.001. (B) Genes plotted in A were divided into 10 bins of equal number of genes [except bin 10, which had extra transcripts] according to their normalized H3K36me3 levels from low (left) to high (right). Variance of the age-
dependent mRNA abundance change for each bin is shown. (C) Regression plot showing log2 of the variance in mRNA abundance change from B as a function of median H3K36me3 level. (D) The negative correlation between H3K36me3 level and gene expression change is independent of mRNA abundance. [Middle panel] Genes plotted in A were divided into three groups [low, medium, and high] according to mRNA abundance. Genes in each subgroup were plotted in the order of normalized H3K36me3 levels at the young time point from low (left) to high (right) [shown in red]. [Top panel] The corresponding log2 values of age-dependent fold change in mRNA abundance are plotted in black. Genes in each subgroup [low, medium, and high] were divided into five bins of equal numbers of genes [except bin 5, which had extra genes] according to their normalized H3K36me3 level from low (L) to high (H). [Bottom panel] The variance of age-dependent mRNA abundance change for each bin is shown. (p) Spearman’s correlation coefficient between absolute log2 values of fold change in mRNA abundance and log2 values of normalized H3K36me3. [***] P-value < 0.001.

with met-1 RNAi through development and adulthood. Under our experimental conditions, met-1(n4337); glp-1 (e2141) and met-1 RNAi worms developed at rates similar to control worms [data not shown]. Immunoblotting confirmed that global H3K36me3 levels were reduced ~90% in met-1(n4337); glp-1(e2141) worms and ~70% in met-1 RNAi worms [Fig. 6A, Supplemental Fig. S4A].

To assess whether reduction of H3K36me3 marks on genes would influence mRNA expression change through aging, we performed mRNA-seq analysis using met-1 (n4337); glp-1(e2141) or met-1 RNAi worms and their respective controls at the D2 and D12 time points, similar to the earlier RNA-seq analysis. We examined the age-de-
pendent changes in mRNA expression in met-1 mutant or met-1 knockdown worms compared with controls as described earlier. Our analyses of two biologically independent experiments comparing met-1; glp-1 and glp-1 control worms indicated that transcripts from 3149 genes significantly varied during aging in control glp-1 worms, and transcripts from 4530 genes varied in met-1; glp-1 worms [Fig. 6B, Supplemental Table S5]. A cross-
comparison revealed that 2666 genes exhibited significant mRNA expression changes through aging in both glp-1 and met-1; glp-1 [Fig. 6B], with the degree of change being significantly greater in met-1; glp-1 compared with glp-1 worms [Fig. 6D]. 483 genes showed significant mRNA expression changes only in control glp-1 worms, and 1864 genes showed significant age-dependent changes only in met-1; glp-1 worms [Fig. 6B,C]. Statistical analyses sup-
port that the met-1; glp-1 mutant worms exhibited a significantly greater age-dependent gene expression change compared with control worms [Z-test: 95% confidence interval: 0.26 ± 0.02, NcNemar’s test: P-value < 0.001]. A very similar pattern was observed in met-1 RNAi worms [Supplemental Fig. S4B,C, Supplemental Table S6].

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We propose that global reduction of H3K36me3 in *C. elegans* results in greater gene expression change with age, which has detrimental effects on longevity (Fig. 7).

Low H3K36me3 marking might provide flexibility in gene regulation

Our discovery that H3K36me3 plays an active role in restraining mRNA expression change through aging led us to ask whether H3K36me3 markings on genes might correlate with physiological changes relevant to aging. We performed gene ontology (GO) analysis of genes that showed a large degree of gene expression changes through aging (absolute log2 value of fold change > 2) and were also marked by low levels of normalized H3K36me3 (log2 < 0) with DAVID (Huang et al. 2009). Interestingly, these genes are enriched for functional groups related to innate immunity, redox balance, and heat-shock response (Supplemental Table S3)—all biological processes thought to be critical for modulating aging. An intriguing possibility is that low levels of H3K36me3 marking enable these genes to be more flexibly regulated over time, and their changed expression through aging contributes to longevity determination.

We further speculated that genes that need to be dynamically regulated under specific conditions would be marked by lower levels of H3K36me3. To test this, we performed GO analysis with all protein-coding genes mapped by our H3K36me3 ChIP-seq data. GO terms with at least 20 genes assigned were extracted for further analysis. Median H3K36me3 levels for the genes in each GO term were computed. Interestingly, we observed obvious differences in median H3K36me3 levels among different GO terms.
that lost H3K36me3 marks with age tended to harbor low levels of H3K36me3 in young animals. The genes H3K36me3 marks with age generally started with very low levels of H3K36me3, and accumulated producible changes in H3K36me3 levels with age, albeit usually to a small degree. The genes that accumulated H3K36me3 marking somehow permits flexible gene expression change with age and a shortened life span.

Our study is the first to profile how the genome-wide pattern of H3K36me3 is not correlated with mRNA abundance (Supplemental Fig. S5B). Importantly, this bias distribution of H3K36me3 is associated with active gene transcription, and H3K36me3 is deposited cotranscriptionally. Consistent with this, our data in C. elegans indicate that genes that are silenced [with undetectable RNA-seq reads] lack H3K36me3 marking. However, for genes with detectable mRNA expression, our detailed analysis showed that gene body H3K36me3 marking does not strictly correlate with steady-state mRNA abundance. Specifically, whereas mRNA abundance positively correlates with gene body H3K36me3 levels for the moderately expressed genes, mRNA abundance either does not correlate or even negatively correlates with H3K36me3 levels for the highly expressed genes. This observation with the highly expressed genes differs from that in mammals (Barski et al. 2007). Interestingly, these highly expressed genes tend to be of shorter gene length [average gene length 1.86 kb, median gene length 1.29 kb] and have a shorter 3′ UTR. The gene structure feature of this group of genes might contribute to the anti-correlation between mRNA expression levels and H3K36me3 deposition.

We also observed that gene body H3K36me3 levels correlated with the Pol II 3′ pausing ratio [Spearman’s coefficient Ρ = 0.24; P-value = 2.2 × 10^{-16}] (Kruei et al. 2013), where greater Pol II pausing at the 3′ end of a gene is associated with higher levels of H3K36me3 at the gene even when H3K36me3 markings at the 3′ UTR are excluded. Interestingly, Pol II 3′ pausing as well as H3K36me3 gene body marking correlate with 3′ UTR length. Considering that H3K36me3 deposition is cotranscriptional and the H3K36me3 methyltransferase is associated with elongating Pol II [Hampsey and Reinberg 2003], we propose that Pol II stalling on genes with a longer 3′ UTR results in greater H3K36me3 deposition on the gene.

Our analysis revealed a new function of H3K36me3 in restricting gene expression changes through aging. We found that in whole worms and Drosophila heads, genes marked by low levels of H3K36me3 were more likely to exhibit a greater degree of mRNA abundance alteration between the young and old time points irrespective of how highly or lowly expressed the genes are. The age-dependent mRNA expression variance shows a strong and highly significant correlation with median gene body H3K36me3 levels [correlation coefficient R^2 of ~0.7–0.9]. The correlation that we detected appears specific to age-dependent change in mRNA expression, as the genes that our analyses focused on (exhibiting significant age-dependent mRNA change, Q-value < 0.05) presented highly stable mRNA expression.

**Discussion**

Our study is the first to profile how the genome-wide pattern of H3K36me3 changes with age in C. elegans. We found that global H3K36me3 marks are largely steadily maintained through aging, consistent with previous microarray studies indicating that gene regulation remains largely robust in old worms [Budovskaya et al. 2008]. Nevertheless, we identified ~2000 genes with statistically reproducible changes in H3K36me3 levels with age, albeit usually to a small degree. The genes that accumulated H3K36me3 marks with age generally started with very low levels of H3K36me3 in young animals. The genes that lost H3K36me3 marks with age tended to harbor high levels of H3K36me3 in young animals, suggesting that they are actively expressed in young worms. Consistently, GO analyses indicated that these genes are enriched for functional clusters particularly important for larval development, including growth, development, and sex differentiation [Supplemental Table S4]. Curiously, the age-dependent reduction in H3K36me3 markings on these genes was not accompanied by decreased mRNA expression. Further investigations will be necessary to understand the possible biological significance associated with the age-dependent alterations in H3K36me3 marks on this subset of genes.

In general, H3K36me3 marking is associated with active gene transcription, and H3K36me3 is deposited cotranscriptionally. Consistent with this, our data in C. elegans indicate that genes that are silenced [with undetectable RNA-seq reads] lack H3K36me3 marking. However, for genes with detectable mRNA expression, our detailed analysis showed that gene body H3K36me3 marking does not strictly correlate with steady-state mRNA abundance. Specifically, whereas mRNA abundance positively correlates with gene body H3K36me3 levels for the moderately expressed genes, mRNA abundance either does not correlate or even negatively correlates with H3K36me3 levels for the highly expressed genes. This observation with the highly expressed genes differs from that in mammals (Barski et al. 2007). Interestingly, these highly expressed genes tend to be of shorter gene length [average gene length 1.86 kb, median gene length 1.29 kb] and have a shorter 3′ UTR. The gene structure feature of this group of genes might contribute to the anti-correlation between mRNA expression levels and H3K36me3 deposition.

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Our analysis revealed a new function of H3K36me3 in restricting gene expression changes through aging. We found that in whole worms and Drosophila heads, genes marked by low levels of H3K36me3 were more likely to exhibit a greater degree of mRNA abundance alteration between the young and old time points irrespective of how highly or lowly expressed the genes are. The age-dependent mRNA expression variance shows a strong and highly significant correlation with median gene body H3K36me3 levels [correlation coefficient R^2 of ~0.7–0.9]. The correlation that we detected appears specific to age-dependent change in mRNA expression, as the genes that our analyses focused on (exhibiting significant age-dependent mRNA change, Q-value < 0.05) presented highly stable mRNA expression.
levels between replicates at the same time point. We further demonstrated that H3K36me3 marking actively contributes to the regulation of age-dependent mRNA expression change, as loss-of-function mutation or RNAi depletion of the H3K36me3 methyltransferase met-1 resulted in a reduction in global H3K36me3 in worms and greater mRNA expression change through aging. Although H3K36me3 is well known to be associated with active transcription, its precise role in gene regulation remains elusive. Our findings point to a new function of H3K36me3 that merits further investigation.

How H3K36me3 marks on a gene influence the degree of gene expression change over time remains to be elucidated. One interesting possibility is that H3K36me3 participates in the regulation of local chromatin organization and further restricts gene expression change or deregulation. Indeed, data in Drosophila show that euchromatin with low H3K36me3 is more open, is more enriched for chromatin-binding factors, and replicates earlier during S phase (Filion et al. 2010). Intriguingly, recent data in mammalian cells indicate that reduction of global H3K36me3 levels due to mutations in the SETD2 methyltransferase alters chromatin accessibility, suggesting that H3K36me3 marks can modulate nucleosome occupancy and chromatin structure (Simon et al. 2014). Previous studies have also implicated H3K36me3 marking on suppressing histone exchange on transcribed genes (Smolle et al. 2012, Venkatesh et al. 2012). These findings together with ours suggest that a low level of H3K36me3 marking might define a chromatin environment that provides greater flexibility in gene expression regulation over time. Another intriguing finding from our analyses was that 3’ UTR length correlated with gene body H3K36me3 marks, which in turn correlated with decreased mRNA expression change with aging. Since miRNAs have been proposed to participate in buffering gene expression (Yang et al. 2012), it is possible that longer 3’ UTRs afford greater numbers of miRNA-binding sites and greater buffering potential by miRNAs, which could result in more stable gene expression over time. Further elucidation of a possible link between H3K36me3 marking and miRNA-mediated gene regulation will likely be fruitful. H3K36me3 has also been implicated in preventing cryptic transcription and regulating RNA splicing. Whether those processes play a role in gene expression change through aging remains to be investigated.

Our data indicate that depletion of H3K36me3 leads to significantly shortened life span without compromising development, suggesting that H3K36me3 marking has a key role in longevity assurance. It is likely that the new role that we ascribed to H3K36me3 in maintaining gene expression stability through aging also plays a key role in promoting longevity.

Materials and methods

C. elegans strain growth and harvesting

C. elegans strain glp-1(e2141) and met-1(n4337), glp-1(e2141) stocks were kept at 16°C and grown under standard growth conditions (Brenner 1974). met-1(n4337) was outcrossed with our laboratory N2 three times and further crossed with glp-1(e2141) to generate met-1(n4337); glp-1(e2141). For ChIP-seq and mRNA-seq experiments, embryos prepared from 16°C glp-1(e2141) or met-1(n4337); glp-1(e2141) stocks by bleaching were hatched and cultured at 25°C with ~3000 embryos per 15 cm of nematode growth medium (NGM) plate seeded with 1.5 mL of concentrated E. coli OP50 (30x overnight culture) with 50 μg/mL carbenicillin and 15 μg/mL tetracycline. For D12 samples, worms were refed once on D4. Adult worms at the D2, D4, and D12 stages were washed with ice-cold M9 three times. Worm pellets were stored at −80°C before ChIP and RNA extraction.

D. melanogaster culture and collection

Canton S flies were grown in large 100-mm embryo collection cages (~200 flies per cage) at 25°C with a 12 h light/dark cycle, passing to new food every 2–3 d for 10 d (young samples) or 40 d (old samples). Flies were then collected, sexed, and stored at −80°C until sample preparation.

ChIP and ChIP-seq library preparation

ChIP was performed as described (Ercan et al. 2007, Landt et al. 2012). Worm pellet was ground with a mortar and pestle and cross-linked with 1% formaldehyde in PBS for 10 min at room temperature. Worm fragments were collected by spinning at 3000g for 5 min and resuspended in FA buffer followed by sonication with Bioruptor. Chromatin extract was incubated with H3 antibody [rabbit, Abcam, ab1791], H3K36me3 antibody [rabbit, Abcam, ab9050], and control rabbit IgG overnight at 4°C. Antibodies used were prescreened for specificity using dot blots. The optimal amounts of antibodies used were determined by titration in a preliminary experiment with ChIP-qPCR. Precipitated DNA [10–15 ng] from each sample was used for Illumina sequencing library preparation. DNA from ChIP was first end-repaired to generate a blunt end followed by adding single adenine base for adaptor ligation. The ligation product with adaptor was size-selected and amplified by PCR with primers targeting the adaptor. Up to 12 samples were multiplexed in one lane for single-end 50 nt Illumina HiSeq sequencing. Raw ChIP-seq data have been deposited at Gene Expression Omnibus (GEO; accession no. GSE62720).

For fly samples, chromatin was generated as described, starting with 2000 heads per sample. ChIP was also performed as described [Wood et al. 2010] using 4 μg of H3K36me3 antibody (Abcam, ab9050) with 10 μg of chromatin. Samples were then hybridized and scanned using the GeneChip Drosophila Tiling 2.0R array (Affymetrix) according to the manufacturer’s instructions.

ChIP-seq data analysis

Preprocessings of sequencing reads from the Illumina platform were performed using the FASTX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit). The sequencing reads were aligned to the ce6 version of the C. elegans genome using BWA version 0.6.1 with 6% maximum mismatch [Li and Durbin 2009]. PCR duplicates were removed using SAMtools version 0.1.18 [Li et al. 2009]. Genome-wide estimates of a smoothed tag enrichment profile were performed using SPP version 1.0 with a bandwidth of 200 base pairs (bp) and a step of 100 bp [Kharchenko et al. 2008]. The smoothed enrichment profiles of different replicates at the studied ages were compared at windows of 1000 bp to obtain Pearson correlations [Liu et al. 2011]. To robustly identify enriched and differentially marked units, including genes and
transcripts, at different stages, we used GLMM, which models both fixed time-point effects and random replication effects. For each gene or transcript region of interest, normalized reads (RPM [reads per million mapped reads]) were counted for H3K36me3 and H3 from each biological replicate at different time points and analyzed using GLMM to estimate the time-point effect (i.e., old/young) and the random effect (i.e., variability among replicates). The Benjamini-Hochberg method was used to control for multiple comparisons. The FDR at 0.01 was used as a cutoff for identifying statistically significant units.

The normalized H3K36me3 level of each gene or transcript is presented as the average log2 value of the ratio of H3K36me3 to H3 [H3K36me3 RPM/H3 RPM] from three biological replicates except where otherwise indicated. For some analyses, normalized H3K36me3 levels for gene bodies [transcriptional start site to transcriptional termination site as annotated in ce6], concatenated exons or introns, and 3′ UTR regions were calculated using Homer (Heinz et al. 2010) with ce6 version of the C. elegans genome. For 5′ UTR length calculation, 5′ UTR was defined as the region between transcriptional start site (Chen et al. 2013; Kruesi et al. 2013) and translational start site. Metagene plots of H3K36me3 across gene body regions were generated by using ngs.plot (Shen et al. 2014) with indexed BAM files.

### RNA-seq library preparation and data analysis

Total RNA was extracted from worms harvested at the same stages as ChIP-seq sample preparation for glp-1(e2141), met-1(n4337), glp-1(e2141), or RNAi-treated glp-1(e2141) worms using TRI reagent (Molecular Research Center). mRNA was purified through polyA enrichment, and the mRNA-seq library was prepared with the Illumina TruSeq RNA and DNA sample preparation kit. Three to 12 samples were multiplexed in one lane for single-end 50-nt Illumina HiSeq sequencing. Raw RNA-seq data have been deposited at GEO (accession no. GSE62720).

For fly samples, mRNA was extracted from frozen fly heads using the Dynabeads mRNA Direct kit (Invitrogen). Two-hundred nanograms of mRNA was then used to prepare RNA-seq libraries, which were sequenced on an Illumina HiSeq 2000 at six samples per lane.

For data analysis, tRNA and rRNA reads were first filtered out using Bowtie [Langmead et al. 2009], and the remaining reads were further aligned to ce6 transcript annotation by TopHat or TopHat2 with no novel junctions allowed [Trapnell et al. 2009, 2012]. The uniquely aligned reads with a maximum of two mismatches were kept for differential expression analysis using Cufflinks [Trapnell et al. 2010, 2012]. The relative expression levels at different ages were calculated as the log2 ratio of FPKM using CuffDiff of Cufflinks software version 2.0.0 or 2.2.1 [Trapnell et al. 2012]. The R package CummeRbund version 1.2.0 was used to further analyze the output expression data [Trapnell et al. 2012]. A Q-value cutoff of 0.05 was set for identifying differentially expressed genes.

### Tissue-specific and ubiquitous gene extraction

The gene lists with specific tissue expression profiles were downloaded from the companion Web site for Spencer et al. [2011] [http://www.vanderbilt.edu/wormdoc/wormmap/Selectively_enriched_genes.html]. The selectively enriched gene lists from larval data sets [all neurons, body wall muscle, intestine, and hypodermis] were used for our analyses. For non-tissue-specifically expressed genes, expression profiles of all genes [gene_expr_RNA_avg.X.series.txt.gz] were downloaded from Expression_Matrix [http://www.vanderbilt.edu/wormdoc/wormmap/Expression_Matrix.html]. Genes that have similar expression levels under different developmental stages and cell types were extracted with standard deviations of expression levels of different developmental stages and cell types as criteria. The top 3000 genes with low standard deviation were taken out and mapped with our ChIP-seq and mRNA-seq results. From this gene group, 194 genes were further randomly picked for our correlation analysis to avoid expression-level bias and make a sample size similar to that of tissue-specific genes.

The tissue expression profiles of the genes sets [tissue-specific and ubiquitous] that we picked were further verified by the Web site-embedded tool Tissue Expression Predictions for C. elegans version 1.0 [http://worm-tissue.princeton.edu/search/multi].

### RNAi treatment for mRNA-seq

The met-1 RNAi clone was obtained from the Ahringher bacteria library [Kamath and Ahringer 2003]. Control L4 440 met-1 RNAi bacteria were grown in Luria broth with 50 µg/mL ampicillin at 37°C until OD600 of ~0.75 and concentrated 30 times, and 1.5 mL was seeded on each 15-cm plate. Bacteria were induced with 4 mM IPTG for 4 h before worm embryo seeding. About 3000 worm embryos prepared by bleaching from glp-1(e2141) stock at 16°C were seeded onto each 15-cm RNAi plate. Worms were grown at 25°C and collected at adult D2, D6, and D12. For D12 samples, worms were reared once on D6 with IPTG-induced RNAi bacteria.

### Life span experiment

Embryos from 16°C cultured glp-1(e2141) and met-1(n4337), glp-1(e2141) strains were seeded onto life span plates, hatched, and maintained at 25°C. For N2 and met-1(n4337) strains, embryos laid at 20°C were hatched and maintained at 25°C. L4 stage hermaphrodites were picked for life span measurement. Worms were transferred to freshly induced [overnight with IPTG] RNAi plates every 2 d until approximately D12. Life span was measured by scoring worms every other day or every day. The survival function was estimated using the Kaplan Meier estimator (SPSS software), and statistical analysis was done using the log-rank test. P-value ≤ 0.01 was considered as significantly different from the control population.

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