Loss of SET1/COMPASS methyltransferase activity reduces lifespan and fertility in *Caenorhabditis elegans*

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Abstract

Post-translational modification of histones, particularly lysine methylation, are thought to play a crucial role in the aging process. Histone 3 lysine 4 (H3K4) methylation, a modification associated with active chromatin, is mediated by a family of SET1 methyltransferases acting within conserved multiprotein complexes known as COMPASS. Previous work in model organisms with partial or complete deletion of COMPASS subunits has yielded conflicting results about the requirement for H3K4 methylation during aging. Here, we reassessed the role of SET1/COMPASS-dependent H3K4 methylation in *Caenorhabditis elegans* lifespan regulation and fertility by generating *set-2(syb2085)* mutant animals that express a catalytically inactive form of SET-2, the *C. elegans* homolog of SET1. We show that animals bearing catalytically inactive SET-2 retain the ability to form COMPASS complexes but have a marked global loss of H3K4 dimethylation and trimethylation. Consistent with previous work, reduced H3K4 methylation was accompanied by loss of fertility; however, in striking contrast to earlier studies, *set-2(syb2085)* mutants displayed a significantly shortened, not extended, lifespan and had normal intestinal fat stores. Furthermore, other commonly used *set-2* mutants were also short-lived, as was a *cfp-1* mutant that lacks a non-catalytic SET1/COMPASS component and displays reduced H3K4 methylation. These results challenge previously held views and establish that wild-type H3K4 methylation levels are necessary to achieve a normal lifespan in *C. elegans*.

Introduction

Epigenetic alterations, such as post-translational histone modification and DNA methylation, are well-established hallmarks of cellular senescence and organismal aging (1, 2). Two important consequences of these modifications are transcriptional deregulation and increased genome instability, which are thought to play key roles in aging and age-related diseases (1). Nevertheless, the complexity of the aging epigenome, especially in mammals, has hampered efforts to establish whether and how individual epigenetic alterations contribute to the aging process. Studies in simple model organisms have provided valuable insight into this question. Work in yeast, worms, and flies has shown that nutrient-regulated signaling pathways, such as the insulin-IGF-like and Target Of Rapamycin pathways, play conserved roles in the regulation of mammalian lifespan (3, 4). More recent studies in these model systems have sought to establish a causal link between specific epigenetic modifications and the aging process. For example, overexpression of H3 and H4 histones in wild-type budding yeast is sufficient to extend lifespan, suggesting that the observed loss of histones during replicative aging directly contributes to the aging process (5, 6). Global loss of histones is also observed in aging *Caenorhabditis elegans*, senescent human cells, and human fibroblasts undergoing replicative aging (7-9), suggesting that a decline in core histone proteins may represent a conserved aging mechanism. Loss of heterochromatin is another feature widely regarded as a contributor to the aging process (10). In aging organisms, senescent cells, and models of progeroid syndromes, loss of heterochromatin is associated with a decrease in the abundance of the repressive trimethylated histone 3 lysine 9 (H3K9me3) mark and of heterochromatin protein 1 (HP1) (10, 11). The abundance of another repressive mark, H3K27me3, also declines with time, but the manner in which aging affects this mark differs substantially not only between organisms but also between tissues within the same organism (2, 11). In *C. elegans*, loss of H3K27me3 in aging somatic cells is accompanied by transcriptional upregulation of UTX-1, a demethylase responsible for H3K27me3 removal (12). Notably, reducing UTX-1 levels by RNAi reverses the age-dependent decline in H3K27me3 and prolongs lifespan

(12, 13), suggesting that maintenance of this mark is associated with a delay in chronological aging. In contrast, *Drosophila* carrying mutations that affect Polycomb Repressor Complex 2 components have reduced global levels of H3K27me3 but the males exhibit an extended lifespan (14). While the reason for these opposing effects of H3K27me3 removal on the lifespans of worms and flies is unclear, it most likely involves altered expression of a few key target genes rather than global changes in H3K27me3 distribution (13). Similarly, misregulation of distinct sets of target genes may also account for the extended lifespan of *C. elegans* induced by either inactivation or overexpression of UTX-1 (15).

Histone modifications associated with active chromatin, such as H3K4me3 and H3K36me3, are also subject to age-dependent deregulation and have been implicated in lifespan regulation (16). H3K4me3, a mark associated with active promoters, plays context-dependent functions in gene regulation (17). In yeast, H3K4 methylation is deposited exclusively by the methyltransferase Set1, which acts as part of the multisubunit complex of proteins associated with Set1 (COMPASS) (18, 19). Mammals express at least six proteins capable of methylating H3K4; two Set1-related proteins, Set1A and Set1B, and four more distantly related MLL proteins (MLL-1–4), which are found in distinct SET or MLL COMPASS complexes that share the core regulatory components Swd3/WDR5, Swd1/RbBP5, Bre2/ASH2, and Sdc1/hDPY30 (17, 20–26). In the mouse, all six Set1 and Mll proteins have been shown to be essential for survival (17). While SET1/COMPASS complexes control global H3K4 methylation, MLL/COMPASS-like complexes methylate H3K4 at specific gene targets or regulatory regions (17). In *C. elegans*, the single homologs of SET1 and MLL, SET-2 and SET-16, respectively (27, 28), also display different functions. SET-2 deposits most H3K4me3 marks in both the germline and soma (27, 28), and worms lacking SET-2 are viable but have reduced fertility; in contrast, loss of SET-16 causes embryonic lethality, consistent with studies of mammalian MLL proteins (29, 30).

The role of H3K4me3 in lifespan regulation is controversial and conflicting results have been obtained with several model organisms. Yeast *set1* mutants are short-lived in both replicative and

chronological aging paradigms (31-33). Set1-dependent H3K4me3 promotes chronological longevity in yeast by preventing apoptosis and histone loss, and the mark also contributes to the maintenance of transcriptional patterns required for a normal replicative lifespan (31-33). Conversely, partial loss-of-function mutations or knockdown of genes encoding the SET1/COMPASS components SET-2, WDR-5, and ASH-2 in *C. elegans* have been shown to extend lifespan through changes in lipid metabolism triggered by the germline (34, 35). The lifespan-extending effect observed in *wdr-5* and *set-2* mutants was transmitted transgenerationally, and wild-type descendants of *wdr-5* or *set-2* mutants over several generations were also long-lived (36). More recently, this transgenerational effect was attributed to a generation-dependent accumulation of repressive H3K9me2 (37). However, these studies did not investigate whether loss of H3K4 methylation or acquisition of H3K9 methylation may have contributed to the effects by directly altering the expression of germline genes that affect longevity.

To address the role of H3K4 methylation in *C. elegans* lifespan, we generated a mutant that is unable to mediate H3K4 methylation but retains the ability to form a SET1/COMPASS complex, thereby avoiding interference with potential non-catalytic complex activities. To this end, we generated mutants carrying a catalytically inactive allele of *set-2* using CRISPR/Cas9 editing, and analyzed the effects on SET1/COMPASS complex assembly, H3K4 methylation, fat accumulation, fertility, and, most importantly, lifespan. Interestingly, mutants with catalytically inactive SET-2 displayed some, but not all, of the previously characterized phenotypes of *C. elegans* mutants harboring varying degrees of SET-2 loss of function, with the most striking difference being the shortened, rather than extended, lifespan of our mutants compared with wild-type animals (34). Overall, the results of this study suggest a more complex, and likely context-dependent, requirement for SET1/COMPASS-mediated H3K4 methylation in the maintenance of normal lifespan in *C. elegans*.

Results

A catalytically inactive *set-2* allele reduces global H3K4 methylation in *C. elegans*

Earlier studies suggesting that loss of H3K4 methylation extends *C. elegans* lifespan were performed using *set-2* RNAi knockdown or a *set-2* partial loss-of-function allele, *set-2(ok952)* (34-36). The *set-2(ok952)* mutation is a complex insertion/deletion that encodes a SET-2 protein with an intact catalytic domain but lacks an upstream region; accordingly, *set-2(ok952)* animals show only a small reduction in global H3K4me3 levels in the soma and germline and no obvious phenotype (27) (Fig. 1A). To more directly investigate how SET1/COMPASS-dependent methylation contributes to lifespan in *C. elegans*, we engineered a catalytically inactive *set-2* allele, *set-2(syb2085)*, by replacing the conserved histidine residue at position 1447 of the SET domain with lysine using CRISPR-Cas9 technology (Fig. 1A) (38). Immunofluorescence staining of *set-2(syb2085)* animals revealed a marked decrease in H3K4me3 (~70%) in the germline compared with wild-type (N2) animals (Fig. 1A–C). Moreover, the H3K4me3 reduction in *set-2(syb2085)* animals was comparable to that observed in *set-2(bn129)* null mutants (Fig. 1A–C) and in *cfp-1(tm6369)* mutants lacking the SET1/COMPASS component CFP-1 (29) (Fig. S1). Consistent with the earlier work, H3K4me3 levels in the germline of *set-2(ok952)* animals were relatively modestly reduced compared with wild-type worms (27) (Fig. 1C). Similar results were obtained when the germlines were immunostained for H3K4me2, which is also mediated by SET1/COMPASS in *C. elegans* (Fig. 1C) (29). The immunostaining results were confirmed by western blot analysis of synchronized populations of early embryos followed by semi-quantitation of H3K4me2 and H3K4me3 (Fig 2A, B). No significant differences in H3K4me2/3 levels were detected between wild-type and *set-2(ok952)* embryos, whereas both histone marks were reduced by ~60% in *set-2(bn129)*, *set-2(syb2085)*, and *cfp-1(tm6369)* mutants. Total H3 expression was comparable among the strains. Taken together, our results demonstrate that H3K4 methylation in *set-2(syb2085)* animals is reduced to the level seen in *set-2* null animals, consistent with complete loss of *set-2* catalytic activity.

To verify that loss of SET-2 catalytic activity in *set-2(syb2085)* mutants does not interfere with SET-2 protein stability or SET1/COMPASS integrity, we performed co-immunoprecipitation

and mass spectrometry-based proteomic analyses using a newly generated *C. elegans* strain expressing a *gfp::cfp-1* transgene. These experiments showed that immunoprecipitates of GFP::CFP-1 from lysates of *set-2(syb2085)* mutant embryos contained SET-2, the core SET1/COMPASS components ASH2, RbBP5, WDR5, and DPY30, and the unique subunit WDR-82 (Fig. 2C, Table S1). Similar results were obtained in CFP-1 immunoprecipitates from *set-2(ok952)* mutant embryos (Fig. 2C, Table S1), while none of the SET1/COMPASS subunits were detected in immunoprecipitates from *set-2(bn129)* null mutants, as expected (Table S2). Taken together, these results indicate that SET1/COMPASS formation is maintained in *set-2(syb2085)* mutant animals, suggesting that disruption of non-catalytic SET-2 or complex activities are unlikely to contribute to the loss of H3K4 methylation in these mutants.

Partial or complete *set-2* inactivation or loss of SET-2 catalytic activity shortens lifespan

As noted above, previous work suggested that loss of H3K4 methylation in *set-2(ok952)* mutants results in lifespan extension, potentially *via* modulation of fat metabolism (35). To determine whether loss of H3K4me2/3 in the catalytically inactive SET-2 mutants was associated with similar effects on lifespan, we compared *set-2(syb2085)* animals with N2 wild-type worms and *set-2(ok952)*, *set-2(bn129)*, and *cfp-1(tm6369)* mutants, all of which display varying H3K4me2/3 levels. Unexpectedly, we observed a significant and reproducible reduction in the mean lifespan not only of *set-2(syb2085)*, *set-2(bn129)*, and *cfp-1(tm6369)* mutants but also of *set-2(ok952)* mutants, which had previously been described as exhibiting increased longevity compared with wild-type animals (Fig. 3A, Table S3). Our finding that each of the SET1/COMPASS mutants displayed decreased lifespans compared with wild-type animals are consistent with recent work showing similar effects on longevity in *set-2(bn129)* mutants, *ash-2* mutants (39), and early generations of *wdr-5* mutants (37).

Because the previous study examining *set-2(ok952)* animals attributed the extended lifespan to altered lipid metabolism (37), we used Oil Red O staining to quantify levels of neutral lipids, the main form of lipid storage in *C. elegans*. However, we observed no significant differences in neutral

lipid content between wild-type animals and either *set-2(ok952)* or *set-2(syb2085)* animals, whereas lipid stores were increased in *daf-2(e1370)* mutants, which have a defect in insulin signaling that results in increased lipid storage and extended lifespan (Fig. 3B, Table S3) (40, 41). Consistent with our results, in an independent study lipid content was found to be unaltered in *wdr-5* loss-of-function mutants (42). These results demonstrate that loss of catalytic activity in SET1/COMPASS and a corresponding reduction in global H3K4me2/3 not only shortens lifespan in *C. elegans* but does so without modifying neutral lipid content.

Loss of SET-2-dependent H3K4 methylation is responsible for loss of fertility

We considered that the reduced lifespan exhibited by *set-2* mutants could result from developmental defects (43). However, we found that the development time of *set-2(syb2085)* and *set-2(ok952)* animals was only modestly lengthened compared with that of wild-type animals (~67 h, ~64 h, and ~62 h, respectively; Fig. S2). This finding suggests that the shortened lifespan of *set-2(syb2085)* animals is unlikely to be due to major developmental defects, although we cannot exclude a contribution from more subtle defects in neuronal development (44).

Inactivation of SET1/COMPASS components has been shown to disrupt (29) or have no effect (34) on *C. elegans* fertility. Therefore, we analyzed fertility phenotypes in *set-2(syb2085)*, *set-2(bn129)*, and *set-2(ok952)* animals. We found that loss of SET-2 catalytic activity resulted in fertility defects similar to those of *set-2* null animals, including decreased brood size under normal growth conditions (20°C) and progressive sterility at a higher temperature (25°C), although the defects were less severe than in *set-2(bn129)* mutants (Fig. 4A, B) (45). As previously reported, the fertility phenotypes of *set-2(ok952)* animals were not significantly different from those of wild-type animals (Fig. 4A, B) (46). Collectively, these results indicate that SET1/COMPASS-dependent H3K4 methylation plays a crucial role in maintaining germline homeostasis in *set-2(syb2085)* and *set-2(bn129)* animals.

Discussion

Previous studies using partial knockdown of SET1/COMPASS subunits have led to the commonly held view that loss of H3K4 methylation increases lifespan (1, 2). More recent studies suggested that this effect is observed only after multiple generations of knockdown of SET1/COMPASS components and depends on the accumulation of repressive H3K9me2 (37). In the present work, we sought to clarify whether SET1/COMPASS-dependent H3K4 methylation affects longevity by examining *C. elegans* mutants in which the catalytic activity of SET-2 was selectively inactivated, thereby leaving other functions of the protein and/or COMPASS unaltered. We found not only that SET-2-mediated H3K4 methylation is essential for a normal lifespan, but also that, in contrast to earlier studies, even a mild reduction in SET-2 activity is detrimental to lifespan, as demonstrated by *set-2(ok952)* animals.

We observed decreased longevity in *set-2(bn129)* mutants, which fail to form SET1/COMPASS complexes, as well as in *set-2(syb2085)* animals. Both mutants also exhibited similar global loss of H3K4me2/3 methylation in the germline and soma, decreased fertility at 20°C, and progressive sterility at the stressful temperature of 25°C. Recent reports for several histone-modifying complexes suggest that they play important functions independent of catalytic activity (47). Our results strongly suggest that the shortened lifespan and loss of fertility phenotypes of *set-2* mutants result from loss of H3K4 methylation rather than catalytic-independent functions of SET-2. Of note, we found that *cfp-1* mutants, which lack the CFP-1 chromatin-targeting subunit of the COMPASS complex, also exhibited reduced lifespan. Thus, although we cannot exclude the possibility that loss of methylation of a non-histone substrate may contribute to the observed phenotypes of *set-2(syb2085)* animals, our results are most consistent with a crucial role for SET1/COMPASS-dependent H3K4 methylation in maintaining a normal lifespan, in addition to its previously documented role in promoting germ cell survival (29, 46, 48).

An earlier study reported that the lifespan of *set-2(ok952)* mutant animals was approximately 20% longer than that of wild-type animals (34), which contrasts with the observations in the present

study. We were also unable to recapitulate the increase in lipid storage previously implicated in the lifespan extension of *set-2(ok952)* animals (35). Indeed, neither *set-2(ok952)* nor *set-2(syb2085)* mutants showed a detectable change in intestinal lipid content compared with wild-type worms in our study. We found that the mutant SET-2 protein in *set-2(ok952)* animals retains its ability to associate with other SET1/COMPASS components and exhibits only a small decrease in H3K4 methylation compared with wild-type animals, consistent with the presence of an intact SET-2 catalytic domain (27, 48). The reasons for the apparent discrepancies in lifespan of *set-2(ok952)* animals in the present and earlier study are unclear but could be due to subtle experimental differences. The genetic background of *C. elegans* mutants have been shown to influence lifespan (49), and additional mutations could have contributed to the observed differences in the lifespan of *set-2(ok952)* mutants. (34). More recent studies suggest that changes in lifespan may not be observed for ≥ 20 generations in animals with reduced H3K4 methylation, as shown for *C. elegans* mutants lacking WDR-5 (37). We note, however, that WDR-5 is a common component of both SET1 and MLL COMPASS complexes; it is present in other multiprotein complexes with histone-modifying activity in *C. elegans* and other species (50-54) and it has known COMPASS/H3K4 methylation-independent functions (55). Therefore, the context in which WDR-5 influences lifespan remains to be established.

Our results show a direct correlation between loss of H3K4me_{2/3} modification in the germline and fertility defects in *C. elegans*. Although *set-2(syb2085)* and *set-2(bn129)* mutants were sterile, *set-2(ok952)* mutant animals were fertile, consistent with only a modest loss of H3K4me_{2/3} in these animals. Sterility in mutants lacking SET-2 catalytic activity may be related to altered chromatin organization or global deregulation of the germline transcriptional program, as we previously observed in the *set-2(bn129)* mutants (56, 57). Interestingly, a similar correlation was not observed between H3K4 methylation and the longevity phenotype, in that all three *set-2* mutants showed a reduction in lifespan despite having markedly different global H3K4 methylation levels. These results suggest that selective alteration in methylation patterns on selected genes, rather than a global decrease, may be responsible for the observed phenotypes. In this regard, changes in H3K27me₃ levels

that affect the expression of relatively few genes have been shown to influence *C. elegans* lifespan (12, 13). Likewise, SET1/COMPASS-dependent H3K4me3 maintains normal chronological lifespan in yeast by specifically promoting histone gene expression (32). Our results could also be explained by the existence of a critical threshold of global H3K4 methylation below which longevity is decreased.

The results of our study provide novel insights into the controversy surrounding the impact of H3K4 methylation on longevity. In yeast, loss of SET1/COMPASS significantly shortens replicative lifespan, most likely *via* reduced expression of a set of genes that are normally induced during aging (33). In *C. elegans*, overexpression of the RBR-2 H3K3me3 demethylase extends lifespan (34), while mutations or RNAi-mediated knockdown of the same enzyme have been shown to either increase (7, 58, 59) or decrease (12, 34) lifespan, depending on the allele used. Decreased longevity in *rbr-2* mutants has been attributed to the presence of an additional mutation in the original genetic background (58). In *Drosophila melanogaster*, RNAi-mediated depletion or mutation of Lid, the RBR-2 ortholog, resulted in increased levels of H3K4me3 and reduced lifespan in males (60). Interestingly, depletion of LSD-1, another H3K4 demethylase that targets H3K4me1 and H3K4me2, has been shown to extend lifespan in *C. elegans* (12, 61). Taken together, these data and the results of the present study argue against a simple model in which H3K4 methylation and demethylation play opposing roles in lifespan regulation (34). Additional studies using well characterized mutants and tissue-specific RNAi/deletion approaches will be required to clarify this complex relationship and to establish the contexts in which selective loss of SET1/COMPASS-dependent H3K4 methylation alters the chromatin landscape during aging.

Materials and Methods

Genetics and strains

Strains were cultured under standard laboratory conditions (62). The wild-type N2 (Bristol) was used as reference strain. The strains used are as follows: PFR401, *set-2(ok952)* III; PFR253, *set-2(bn129)* III, (29); PFR588, *cfp-1(tm6369)* IV; PHX2171, *set-2(syb2085)/qC1[dpy-19(e1259) glp-1(q339) qIs26]* III (this work); PHX1012, *cfp-1(syb1012)* IV (this work); PFR727, *cfp-1(syb1012)* IV; *set-2(ok952)* III (this work); PFR728, *cfp-1(syb1012)* IV; *set-2(syb2085)* III (this work); PFR558, *qaIs22[HA::wdr-5.1;Cbunc-119(+)]*; *set-2(bn129)*III; *wdr-5(ok1417)* III (54); CB1370, *daf-2(e1370)* III; XA6226, *mrg-1(qa6200)/qC1 [dpy-19(e1259) glp-1(q339) qIs26]* III (CGC).

All mutants were outcrossed to wild-type N2 at least four times. *cfp-1(syb1012)* and *set-2(syb2085)* were generated by CRISPR/Cas9 technology at SunyBiotech (Fuzhough City, Fujian, China). *cfp-1(syb1012)* is a knock-in allele carrying a degron-GFP tag inserted at the 5' end of the endogenous *cfp-1* coding sequence. For *set-2(syb2085)*, the conserved histidine residue at amino acid position 1447 within the catalytic SET domain was replaced by lysine (38). Two synonymous point mutations were introduced in order to avoid cutting of the repair template by Cas9. Wild-type and *syb2085* sequences are shown below, with the H1447K substitution underlined and synonymous mutations capitalized:

wt: aagcgtggaaattttgctcgatttatt AAT CAC tcgtgc CAA cctaattgctacgcgaaggta

syb2085: aagcgtggaaattttgctcgatttatt AAC AAG tcgtgc CAG cctaattgctacgcgaaggta

To avoid the potential accumulation of epigenetic modifications (37), *set-2(syb2085)* mutants were maintained in a heterozygous state by crossing them with XA6226 carrying the *qC1* balancer chromosome (45).

Lifespan analysis

Lifespan assays were conducted at 20°C according to standard protocols (63). Worms were synchronized by bleaching and transferred at the L1 stage. Worms were maintained on solid Nematode Growth Medium (NGM) seeded with *Escherichia coli* strain OP50-1. 5-Fluoro-2'-deoxyuridine was not added to the plates. The L4 stage was considered day 0. Hermaphrodites were transferred onto fresh seeded plates every day for the first week of the lifespan assay. Animals that failed to display heat-provoked movement were scored as dead, and animals that crawled off the plates or “bagged” worms were censored. P values were calculated using the log-rank (Mantel–Cox) method. Statistical analysis of individual lifespan studies was performed using the Oasis online software (64). Statistical analysis of experiments shown in the main text and replicate experiments are provided in Table S3.

For experiments using the balanced strain PHX2171, non-roller *set-2(syb2085)* homozygotes were isolated and grown at 20°C for at least two generations before initiating the analysis to avoid the maternal effect.

Brood size assay

Ten L4 larvae were isolated on individual plates seeded with OP50-1 bacteria at 20°C and allowed to develop into egg-laying adults overnight. Adult animals were then transferred to fresh plates every 12 h until reproduction ceased. Plates were scored for the number of viable progeny 24 h after removal of the mother.

Developmental time measurement

Twenty gravid adults were allowed to lay eggs on NGM plates spotted with OP50-1 for 1 h and then removed. The time to egg-laying adulthood was measured from this point. Eggs were allowed

to hatch and 10 animals were randomly picked and transferred to individual plates. Plates bearing single young adults were checked every hour for the presence of eggs.

Transgenerational fertility assays

Six independent lines were established from freshly thawed wild-type animals, *set-2(ok952)* mutants, *set-2(bn129)* mutants maintained as homozygotes, and homozygous *set-2(syb2085)* mutants obtained from balanced strain PHX2171. For each line, six homozygous L4 stage animals were transferred to single plates seeded with OP50-1 and grown at 25°C. From each generation, six worms were again picked and transferred to single plates until the animals became sterile (fewer than 10 larvae/plate), as previously described (29). When at least 200 larvae were counted on a plate, the line was considered fully fertile.

Oil Red O staining

Worms were synchronized by bleaching and grown to the young adult stage at 20°C on NGM plates seeded with OP50-1. Worms were washed off the plates with 1X phosphate-buffered saline containing X% Tween (PBST), placed in Eppendorf tubes, pelleted at low speed for 30 s, and washed three times with PBST. Worms were incubated with rocking for 3 min in 600 µL of 40% isopropanol, the isopropanol was then removed, and the worms were mixed with 600 µL of Oil Red O (3:2 ratio of 5 mg/mL Oil Red O/100% isopropanol and sterile water). Animals were incubated on a rotator at room temperature for 2 h, pelleted at low speed, and washed for 30 min in 600 µL PBST. Aliquots of 10 µL of stained animals were deposited onto freshly prepared 3% agarose pads, covered with coverslips, and imaged with the same exposure settings at 10X magnification with a Zeiss AxioImager Z1 equipped with a CoolSNAP color camera. Quantification of staining was performed using FIJI image processing software (35). Briefly, the original TIFF images were background subtracted, converted to grayscale, inverted, and thresholded. The intestinal cells of whole worms were selected

using the oval brush tool, saved to the Region of Interest manager, and mean Oil Red O intensity was quantified.

Western blot analysis

Embryos or L4 stage worms were pelleted in M9 buffer and flash frozen in liquid nitrogen. Pellets were recovered in TNET buffer (50 mM Tris-HCl pH 8.0, 300 mM NaCl, 1 mM EDTA, and 0.5% Triton X-100 supplemented with cOmplete Easypack protease inhibitor cocktail [Sigma]). Samples were mixed 1:1 by volume with Lysing Matrix Y (MP Biomedicals) and lysed using a Precellys 24 homogenizer (Bertin) at 2 bursts of 20 s ON at 6000 rpm with 10 s OFF between bursts. Samples were then centrifuged at 4°C for 5 min at 20,000 g and protein concentrations in the supernatants were measured using the Bradford assay (Bio-Rad Protein Assay Dye). Aliquots equivalent to 1 µg and 3 µg protein were separated on NuAGE 12% Bis-Tris Gels using MOPS 1X (Invitrogen) running buffer, and then transferred to 0.45 µM nitrocellulose membranes (Bio-Rad). Membranes were blocked with PBST containing 5% non-fat dry milk, and incubated overnight at 4°C with anti-H3 (Cell Signaling Technology #14269 at 1:2500 dilution in PBST containing 1% BSA), anti-H3K4me2 (Active Motif #39913 at 1:2000), or anti-H3K4me3 (AB Clonal #A2357 at 1:2000) primary antibodies. The membranes were washed then 4 times in PBST, incubated with secondary antibodies: IRDye 800CW goat anti-Rabbit (Licor # 926-32211 at 1:10 000) and IRDye 680 RD goat anti-Mouse (Licor # 925-68070 at 1:10 000). Images were obtained with the Chemidoc MP Imaging System (Bio-Rad) and fluorescent signals were quantified using Image Lab software (Bio-Rad).

Immunofluorescence microscopy

Gonads were fixed essentially as previously described (65) and incubated overnight at 4°C with mouse anti-H4K3me3 (Wako #305-34819 at 1:500 dilution in PBST) or rabbit anti-H3K4me2 (Invitrogen #710796 at 1:20,000) primary antibodies. The slides were washed with PBST, and incubated at room temperature for 50 min with goat anti-mouse Alexa Fluor 680 (Invitrogen/Molecular

Probes #A21057 at 1:1000) or goat anti-rabbit Alexa Fluor Plus 641 (Invitrogen/Molecular Probes #A32733 at 1:1000) secondary antibodies. The slides were washed again in P and images were acquired with a YOKOGAWA CQ1 spinning disk confocal microscope. Maximum intensity projections of whole germlines acquired with the same settings were opened in ImageJ, and 5 germlines per strain were processed identically. Intensities of nuclei from the distal germline (mitotic and transition zones) or the mid-germline (early and mid-pachytene regions) were measured and averaged.

Immunoprecipitation and mass spectrometry-based proteomic analyses

Details of the immunoprecipitation and mass spectrometry-based proteomic analyses are provided in Supplementary Information. Immunoprecipitations were performed on frozen embryos prepared by hypochlorite treatment from strains grown at 20°C on enriched NGM.

Acknowledgments

Strains were obtained from the Caenorhabditis Genetics Center (CGC), supported by the NIH Office of Infrastructure Programs (P40 OD010440), and the National BioResource Project. We acknowledge the Discovery Platform and Informatics Group at EDyP (U1292 INSERM/CEA/UGA, Institut de Recherche Interdisciplinaire de Grenoble, IRIG), and the SFR Biosciences (UAR3444/CNRS, US8/Inserm, ENS de Lyon, UCBL) : PLATIM. We would like to thank Dr. Cécile Bedet, Laura Faccin and Lisa-May Alvarez for technical support.

This project is supported by the Agence Nationale de la Recherche (ANR) [15-CE12-0018-01] and [ANR-19-CE12-0025] and the Centre National de la Recherche Scientifique (CNRS). The proteomic experiments were partially supported by ANR under projects ProFI (Proteomics French Infrastructure, ANR-10-INBS-08) and GRAL, a program from the Chemistry Biology Health (CBH) Graduate School of University Grenoble Alpes (ANR-17-EURE-0003).

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Figure 1

Figure 1. Loss of SET-2 enzymatic activity in *C. elegans* markedly reduces H3K4me2 and H3K4me3 in the germline. (A) Schematic showing the *set-2* alleles used in this study. Black and shaded boxes represent conserved domains (RRM, SET, and post-SET). The point mutation introduced in *set-2*(*syb2085*) is shown in the SET domain. (B) Schematic showing the regions of the germline used for quantification of immunofluorescence signals: mitotic zone (MZ), transition zone (TZ), and pachytene (PA). (C) Confocal images of dissected gonads from wild-type animals and *set-2* mutants stained with Hoechst and anti-H3K4me3 antibody (left panels) or anti-H3K4me2 (right panels). Scale bar, 10 μ m. Lower bar graphs show quantification of H3K4me2/3 signals in the MZ+TZ and PA regions. Data show the mean pixel intensities $\pm$ SEM, n=5. ns, not significant, *P<0.05, **P<0.001, ****P<0.0001 for mutants vs wild-type worms by one-way ANOVA followed by Dunnett's test.

Figure 2

Figure 2. Loss of SET-2 catalytic activity causes a global reduction in H3K4me2 and H3K4me3 in the early *C. elegans* embryo. (A) Western blot analysis of proteins from embryos of wild-type *C. elegans* and the indicated *set-2* or *cfp-1* mutants. Blots were probed with anti-H3K4me3, anti-H3K4me2, and anti-H3 antibodies. Left and right lanes for each strain represent samples with 1 µg and 3 µg total protein, respectively. (B) Quantification of H3K4me3 (left) and H3K4me2 (right) band intensities from blots represented in (A). Mean ± SEM, n=3. ns, not significant, *P<0.05, **P<0.001, ****P<0.0001 for mutants vs wild-type worms by one-way ANOVA followed by Dunnett's test. (C) List of selected proteins (and their mammalian homologs) identified by mass spectrometry of proteins immunoprecipitated with GFP::CFP-1 from embryos carrying the indicated *set-2* alleles. SET-2/SET1 and SET16/MLL core complex components are highlighted in blue and gray, respectively. SC; spectral counts.

Figure 3

Figure 3. Loss of SET-2-dependent methylation reduces lifespan. (A) Lifespan assays of wild-type *C. elegans* and *set-2* and *cfp-1* mutants. Mean reductions relative to wild-type animals are 17.6% for *set-2(ok952)* ($P < 0.001$), 26% for *set-2(syb2085)* ($P < 0.0001$), 23.3% for *set-2(bn129)* ($P < 0.0001$), and 18.3% for *cfp-1(tm6369)* ($P < 0.001$). P values were calculated using the Mantel–Cox log-rank method. Replicate experiments are shown in Table S3. (B) Quantification of Oil Red O signal intensity (upper panel) and images of Oil Red O-stained wild-type, *set-2(ok952)*, *set-2(syb2085)*, and *daf-2(e1370)* animals (lower panels). Magnification = 10X. Data represent the mean intensity $\pm$ SEM of $n=30$ worms. ns, not significant; **** $P < 0.0001$ by one-way ANOVA followed by Dunnett's test.

Figure 4

Figure 4. SET-2 catalytic activity is required for germline immortality. (A) Total number of progeny of wild-type, *set-2(ok952)*, *set-2(syb2085)*, and *set-2(bn129)* animals grown at 20°C. Mean $\pm$ SEM, n=10. ns, not significant; *P<0.05, ***P<0.001 for *set-2(syb2085)* and *set-2(bn129)* vs wild-type, respectively, by one-way ANOVA followed by Dunnett's test. (C) Transgenerational fertility assay of wild-type animals and the indicated *set-2* mutants performed at 25°C. Scoring was based on five biological replicates, with 6 independent lines each, error bars show SEM. Wild-type animals can be maintained for more than 40 generations without loss of fertility (54).