Current Biology, Vol. 14, 1639–1643, September 21, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.08.062

The MES-2/MES-3/MES-6 Complex and Regulation of Histone H3 Methylation in *C. elegans*

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Summary

The *C. elegans* proteins MES-2 and MES-6, orthologs of the Polycomb group (PcG) chromatin repressors E(Z) and ESC [1, 2], exist in a complex with their novel partner MES-3 [3]. The MES system participates in silencing the X chromosomes in the hermaphrodite germline [4, 5]. Loss of maternal MES function leads to germline degeneration and sterility [6]. We report here that the MES complex is responsible for di- and trimethylation of histone H3 Lys27 (H3-K27) in the adult germline and in early embryos and that MES-dependent H3-K27 marks are concentrated on the X's. Another H3-K27 HMT functions in adult somatic cells, oocytes, and the PGCs of embryos. In PGCs, the MES complex may specifically convert dimethyl to trimethyl H3-K27. The HMT activity of the MES complex appears to be dependent on the SET domain of MES-2. MES-2 thus joins its orthologs *Drosophila* E(Z) and human EZH2 among SET domain proteins known to function as HMTs (reviewed in [7]). Methylation of histones is important for long-term epigenetic regulation of chromatin and plays a key role in diverse processes such as X inactivation and oncogenesis [8]. Our results contribute to understanding the composition and roles of E(Z)/MES-2 complexes across species.

Results and Discussion

We demonstrate here that the MES complex has nucleosomal histone H3-specific methyltransferase activity. Nuclear extracts were prepared from *C. elegans* embryos and immunoprecipitated by antibodies against MES-6. Western blot analysis revealed that anti-MES-6 antibodies specifically precipitated MES-6 and coprecipitated MES-2 and MES-3 (Figure 1). The resulting supernatants and immunoprecipitates were analyzed for HMT activity with nucleosomal substrates purified from chicken erythrocytes or equivalent amounts of free histone H3, and the labeled products were separated by SDS-PAGE. The immunoprecipitated MES complex contained robust HMT activity on nucleosomal histone H3, whereas the control preimmune precipitate did not (Figure 1). The MES complex had no detectable activity on free histone H3 (Figure 1B). The human EZH2/EED complex had similarly strong activity for nucleosomal H3 and barely detectable activity for free H3 (Figure 1B), as demonstrated previously [9]. We conclude that the HMT activity and substrate specificity of MES-2 and EZH2 complexes are conserved from worms to humans.

Immunofluorescence microscopy revealed that the MES complex is required for H3-K27 methylation. The fly E(Z)/ESC complex and the orthologous human EZH2/EED complex methylate H3-K27 [9–12] and, according to some reports, also H3-K9 [11, 12]. To investigate the residue specificity of MES-mediated H3 methylation, we stained wild-type and *mes* mutant worms with purified antibodies specific for H3 dimethyl-Lys27 (H3-di-mK27), H3-tri-mK27, H3-di-mK9, or H3-tri-mK9. *mes* mutant germlines did not show alterations in H3-di-mK9 or H3-tri-mK9 staining ([4] and data not shown) but displayed dramatic alterations in H3-di-mK27 and H3-tri-mK27. In the wild-type, H3-di-mK27 and H3-tri-mK27 are abundant in virtually all nuclei (Figures 2A, 2C, 2E–2G, 3A, 3C, 4A, 4C, 4E, and 4H). In contrast, in *mes-2, mes-3*, and *mes-6* worms, both modifications are absent from most of the germline (Figures 2B, 2D, and 2H; data not shown) and from early embryo nuclei (Figures 3B and 3D; data not shown). *mes-2* alleles with point mutations within the SET domain {*mes-2(bn48)*, S628P and K629E, and *mes-2(bn72)*, Y673H [1]} had H3-di-mK27 and H3-tri-mK27 patterns similar to those of protein null alleles (data not shown). Thus, assuming that the *bn48* and *bn72* proteins are correctly assembled into MES complexes, as suggested by molecular epistasis results [1, 2], the SET domain of MES-2 appears to be required for H3-K27 methylation. In conclusion, the MES-2/MES-3/MES-6 complex has HMT activity for histone H3 and is solely responsible for H3-K27 methylation in most regions of the germline and in early embryos.

The H3-di-mK27 and H3-tri-mK27 staining patterns displayed some significant differences both in wild-type and in *mes* mutant tissues. We describe the distribution of H3-di-mK27 and then tri-mK27, first in the wild-type and then in mutants, and then discuss the implications of the differences. In the wild-type adult germline, H3-di-mK27 marks appear to be equivalently distributed over all chromosomes, with a banded or nonuniform appearance (Figures 2A and 2E); they are present in all germline nuclei except sperm, which lack all assayed histone modifications [13]. In wild-type embryos, H3-di-mK27 persists at high levels on chromatin in all nuclei (Figures 3A, 4A, 4C, and 4E), but surprisingly, the level of H3-di-mK27 in the P_4 cell begins to decline just before the cell's division to produce the PGCs, Z2 and Z3, at the ~100 cell stage (Figures 4A and 4C). By the end of embryogenesis, H3-di-mK27 is barely detectable in Z2 and Z3 (Figure 4E).

Examining *mes* worms, we found that at least one additional *C. elegans* HMT catalyzes H3-di-mK27 methylation; robust staining was visible in nuclei of adult somatic tissues (e.g., gonad distal-tip and sheath cells, Figure 2B and data not shown) and in oocytes (data not shown). In the adult germline, this non-MES HMT activity

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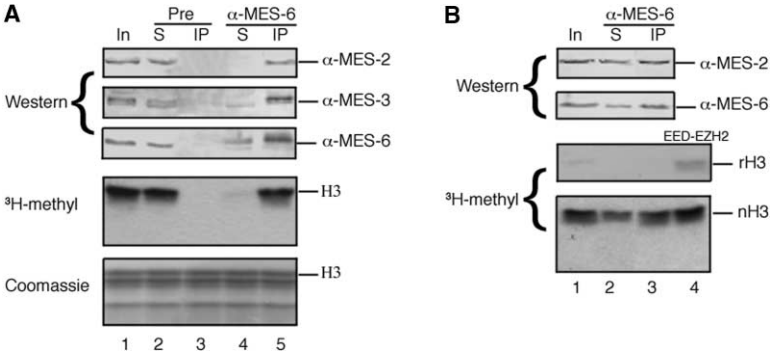

Figure 1. The *C. elegans* MES Complex Contains Nucleosomal Histone H3-Specific Methyltransferase Activity

(A) Western blot analysis (top three panels) indicates that all three MES proteins were immunoprecipitated by antibody to MES-6 (lane 5). The immunoprecipitated complex as well as controls was subjected to HMT assays with equal amounts of nucleosomal substrates, and the labeled products were separated by SDS-PAGE (bottom two panels). Abbreviations are as follows: Pre, preimmune serum; In, input (10% of the amount of extract used for immunoprecipitation); S, supernatant (10% of the total); and IP, immunoprecipitate (split three ways for MES-2 and MES-6 Western blot, MES-3 Western blot, and HMT assay).

(B) The MES protein complex and the human EED-EZH2 complex were assayed for HMT activity with free recombinant histone H3 (rH3) or nucleosomes (nH3) as substrate, and the labeled products were separated by SDS-PAGE. In, S, and IP are as in (A) except that the IP was split five ways among the Western blot and several HMT assays.

appears in late pachytene/early diplotene (data not shown). Notably, after fertilization, sperm chromatin gains H3-di-mK27 marks during decondensation (as observed for other histone modifications [13]), then the marks decline dramatically in both the sperm- and oocyte-derived pronuclei during the 1 cell stage (Figures 3E and 3F; data not shown). The rapid loss of a histone methylation mark is not unprecedented in *C. elegans* embryogenesis [14] and may be due to histone exchange or cleavage of histone tails [15]. H3-di-mK27 persists in the other meiotic products, the polar bodies (Figure 3B). Surprisingly, given the relatively normal somatic development of *mes* mutants, *mes-2, mes-3*, and *mes-6* mutant embryos lack detectable H3-di-mK27

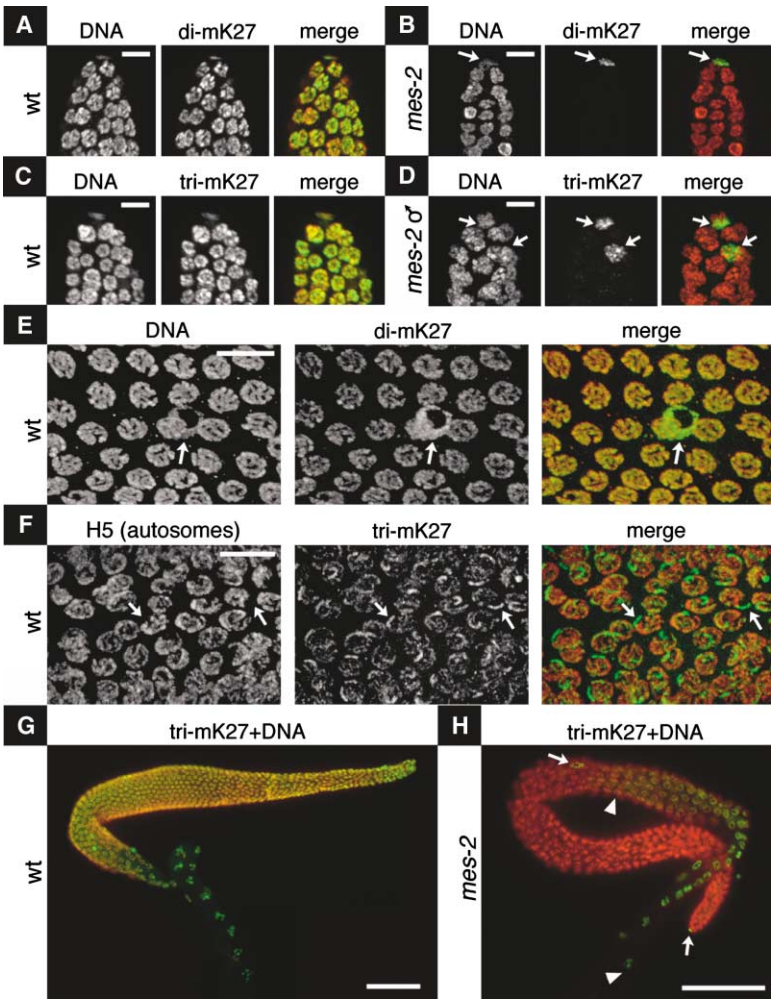

Figure 2. Methylation of H3-K27 in the Distal-Medial Germline Requires MES Function

Chromatin (labeled DNA) is stained with PA3 (A–E) or DAPI (G and H) and is shown in red. H3-mK27 stain is shown in green.

(A and C) Distal germline chromatin in wild-type gonads contains H3-di-mK27 (A) and H3-tri-mK27 (C).

(B and D) H3-di-mK27 (B) and H3-tri-K27 (D) are absent from chromosomes in distal germline nuclei in *mes-2(bn11)* M^+ hermaphrodites (B) and *mes-2(bn27)* M^- males (D), but present in the somatic distal-tip cells (arrows). (M^+ is a *mes* mutant that inherited a maternal load of *mes* gene product from a *mes*/+ parent. M^- is a *mes* mutant derived from a *mes*/*mes* parent.)

(E) H3-di-mK27 marks all chromosomes in wild-type pachytene nuclei and in a somatic gonad sheath nucleus (arrow).

(F) H3-tri-mK27 is enhanced on X chromosomes (arrows) in wild-type hermaphrodite pachytene nuclei. Autosomes (red) are marked with mouse monoclonal antibody H5, which recognizes an active, phosphorylated form of RNA polymerase II associated preferentially with the autosomes in the *C. elegans* germline [4, 5].

(G) H3-tri-mK27 is present in all nuclei of a wild-type hermaphrodite gonad.

(H) In a *mes-2(bn11)* M^+ hermaphrodite gonad, H3-tri-mK27 is detectable only in somatic nuclei (arrows) and in germline nuclei from late pachytene through oogenesis (region between arrowheads). The scale bar represents 5 μm (A–D), 10 μm (E and F), and 50 μm (G and H).

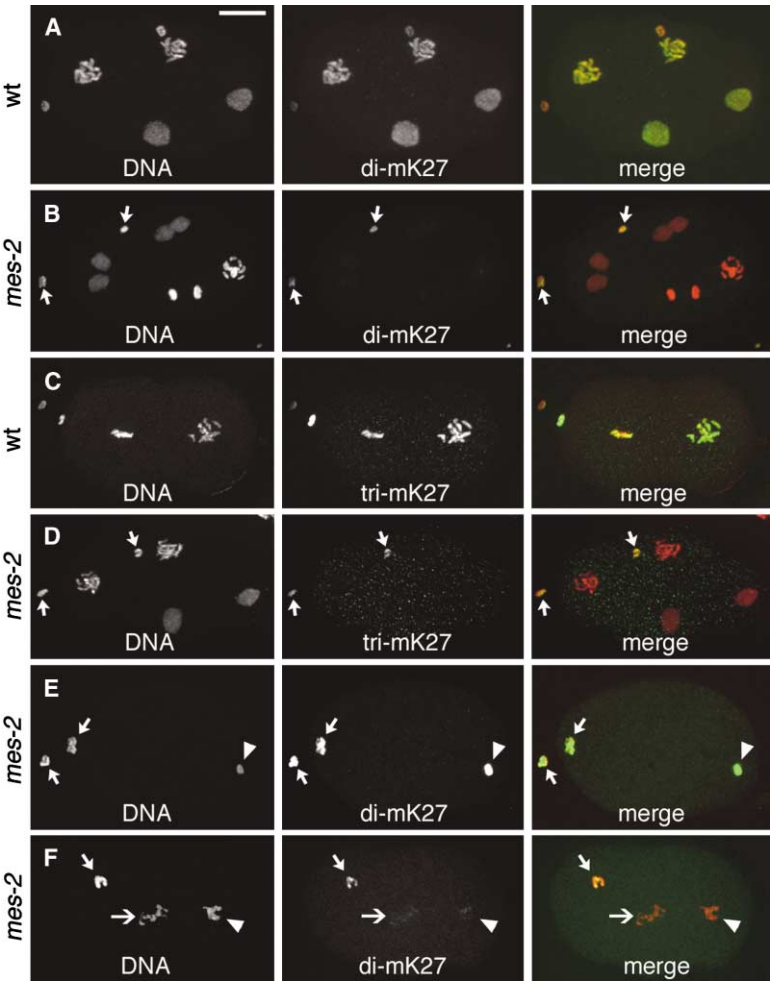

Figure 3. Methylation of H3-K27 in Early Embryos Requires MES Function

PA3 (DNA, red) marks chromatin. H3-mK27 is in green.

(A and C) Wild-type embryos are shown. H3-di-mK27 (A) and H3-tri-mK27 (C) are on all chromosomes. (B and D–F) *mes-2* M$^-$ embryos are shown. ([B, E, and F] *bn11*; [D] *bn76*). H3-di-mK27 (B) and H3-tri-mK27 (D) are undetectable on chromosomes in embryonic nuclei but are present in the two polar bodies (arrows in [B] and [D]). In meiosis II embryos (E), both products of meiosis I (arrows) are heavily marked with H3-di-mK27, as is the sperm DNA (arrowhead). After completion of meiosis (F), both female (open arrow) and male (arrowhead) pronuclei show marked reduction in H3-di-mK27. Polar bodies (one of two shown by a closed arrow) remain well stained. The scale bar represents 10 μm in all panels.

methylation in all somatic nuclei prior to the ~100 cell stage (Figures 3B, 4B, and 4D; data not shown). At later stages, low to moderate H3-di-mK27 staining appears in a few somatic cells (Figures 4F and 4G; data not shown). This reduced level of H3-K27 methylation (compared to wild-type, Figure 4E) may underlie the subtle homeotic transformations observed in some *mes-2*, *mes-3*, and *mes-6* mutant males [16]. Unexpectedly, a H3-K27 dimethylation activity commences at the ~100 cell stage in *mes* embryos, exclusively in the PGCs (Figures 4B and 4D; data not shown). Thus, the pattern of H3-di-mK27 is regulated by another HMT activity as well as by the MES complex within the germ cells of embryos.

The H3-tri-mK27 pattern in wild-type adult germlines (Figures 2C, 2F, and 2G) is similar to that of H3-di-mK27 except for one notable difference: H3-tri-mK27 marks are concentrated and uniformly distributed on the X chromosomes but have a banded appearance (similar to that of H3-di-mK27) on the autosomes (Figure 2F). Because H3-K27 methylation is generally a repressive chromatin modification [8], the enhanced H3-tri-mK27 on the X chromosomes is in agreement with silencing of the X chromosomes in the *C. elegans* germline [4, 5]. H3-tri-mK27 (but not di-mK27) is also concentrated on the inactive X chromosome in mouse embryos [17]. In wild-type embryos, H3-tri-mK27 closely resembles di-mK27 in distribution up until Z2 and Z3 are born. Later, in contrast to the disappearance of the di-mK27 mark in Z2 and Z3, tri-mK27 levels in Z2 and Z3 are similar to or slightly above those in somatic nuclei (Figure 4H).

In *mes* mutants, H3-tri-mK27 is absent from distal and medial germline (Figures 2D and 2H; data not shown) and embryonic nuclei (Figures 3D and 4I; data not shown), the same tissues that lack H3-di-mK27. *mes* adult somatic cells have trimethylated H3-K27 (e.g., gonad distal-tip and sheath cells; Figures 2D and 2H; data not shown). In *mes* proximal gonads, H3-tri-mK27, like H3-di-mK27, appears at late pachytene/early diplotene and persists in oocytes (Figure 2H and data not shown) and polar bodies of embryos (Figure 3D and data not shown).

H3-tri-mK27 marks in *mes* embryos resemble di-mK27 at early stages of embryogenesis (Figure 3D and data not shown); however, tri-mK27 levels remain very low or undetectable in Z2 and Z3, as well as in all somatic nuclei (Figure 4I). Therefore, it appears that a non-MES activity is efficient at producing di-mK27 marks (Figures 4F and 4G), but not tri-mK27 marks (Figure 4I), in Z2 and Z3 of *mes* embryos; in other words, MES function is not required for H3-K27 dimethylation but is required for trimethylation in the PGCs. In wild-type embryos, the MES complex appears to prevent the accumulation of

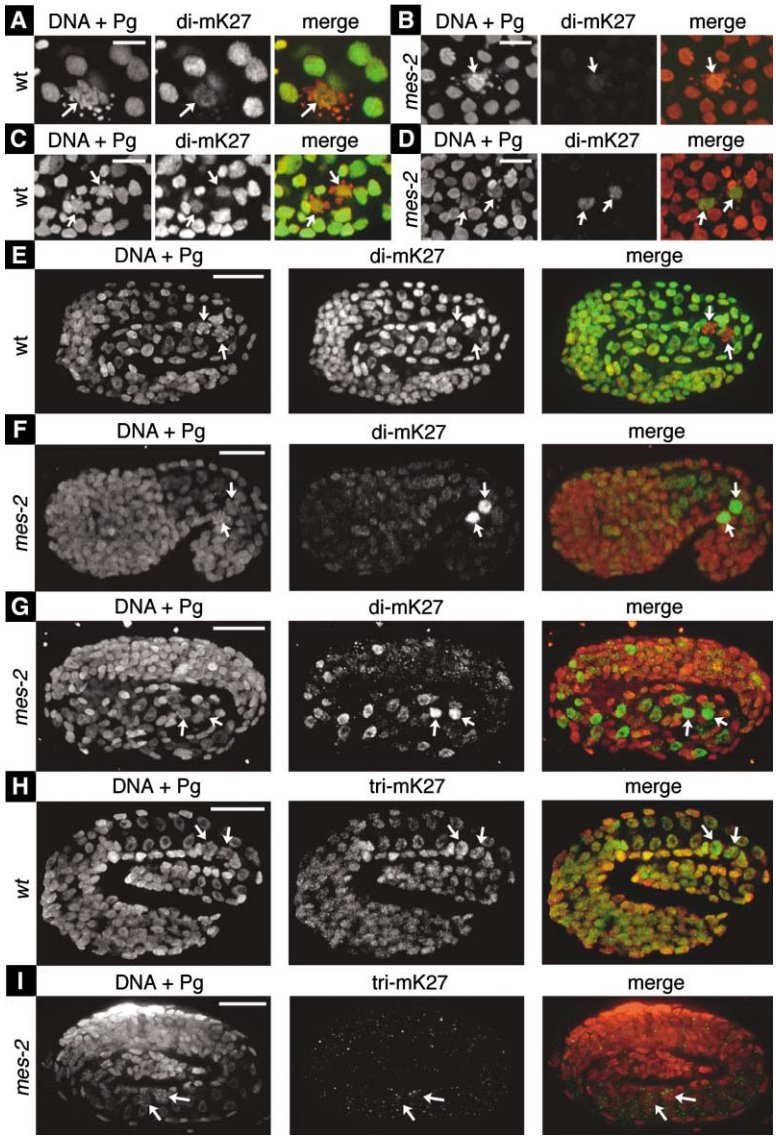

Figure 4. The Roles of the MES Complex and a Non-MES HMT in H3-K27 Methylation in Somatic Cells and the PGCs of Embryos

Germ cells (arrows) are marked with an antibody (OIC1D4, red) to P granules (Pg). DNA (stained with PA3) is also red. H3-mK27 is in green.

(A, C, E, and H) Wild-type embryos are shown. Compared to surrounding somatic nuclei, the nuclei in the germline blastomere P_4 (A) and its PGC daughters Z2 and Z3 (C and E) show a reduction in H3-di-mK27. Z2 and Z3 do not show a reduction, and often show a slight elevation, in H3-tri-mK27, in comparison to that of somatic nuclei (H).

(B, D, F, G, and I) *mes-2* M^- embryos are shown ([B, D, and F] *bn11*; [G] *bn76*; [I] *bn48*). H3-di-mK27 first appears faintly in P_4 (B) and becomes stronger in Z2 and Z3 ([D] ~100 cell stage; [F] comma stage; [G] 3-fold stage). H3-di-mK27 appears in some somatic nuclei as well (F and G). H3-tri-mK27 levels remain low or undetectable in the somatic cells and PGCs of late embryos ([I] 3-fold stage). The scale bar represents 5 μm (A–D) and 10 μm (E–I).

di-mK27 and to promote the accumulation of tri-mK27. The MES complex may downregulate H3-di-mK27 by blocking the expression or activity of the non-MES HMT or by rapidly converting di-mK27 to tri-mK27. If the second model were true, we might expect tri-mK27 in wild-type embryos to be high in the PGCs, to complement the lower levels of di-mK27 in those cells. In some embryos, the level of tri-mK27 staining in PGCs was indeed slightly elevated in comparison to that of neighboring well-stained somatic nuclei, but absolute levels of methyl marks are difficult to quantify (e.g., Figure 4H).

It has been a mystery why male (XO) progeny of *mes* mothers are generally not sterile [18]. One possibility is that males possess an alternative H3-K27 HMT and thus do not depend on the MES system. The observation that *mes-2*, *mes-3*, and *mes-6* mutant males, like mutant hermaphrodites, lack H3-di-mK27 and H3-tri-mK27 in germ nuclei (Figure 2D and data not shown) argues against that theory.

Our results reveal that temporal and spatial regulation of H3-K27 methylation patterns in *C. elegans* is complex and under the control of at least two HMTs. Within the adult germline, the MES complex is responsible for H3-K27 di- and trimethylation during all stages except terminal oogenesis. The MES-mediated concentration of methyl marks on the X chromosomes is consistent with the proposed role of the MES complex in silencing the X's. MES-4, another SET domain protein [4] that is not part of the MES-2/MES-3/MES-6 complex [3], is not required for H3-K27 methylation; *mes-4* mutants showed wild-type H3-K27 di- and trimethylation patterns (data not shown). MES-4 is localized to autosomes and absent from the X's, and it might act in opposition to the MES-2/MES-3/MES-6 complex in establishing X repression in the germline [4]. Surprisingly, in the PGCs in embryos, the MES complex is not required for H3-K27 dimethylation and in fact prevents accumulation of H3-di-mK27. This could reflect dimethylation of H3-K27 by a non-MES HMT and subsequent efficient conversion of H3-di-mK27 to H3-tri-mK27 by the MES complex. This theory is

consistent with the emerging view that different HMTs are responsible for different degrees of methylation (i.e., mono-, di-, and trimethylation), which in turn are correlated with different chromatin states [19–21]. Trimethylation appears to be a critical epigenetic mark. For example, H3-tri-mK4 catalyzed by yeast Set1 is a mark of actively expressed genes, and H3-tri-mK9 catalyzed by mammalian SUV39 is a mark of constitutive heterochromatin [21–23]. MES-2-mediated H3-tri-mK27 may mark silenced chromatin in germ cells, in a manner similar to the role of EZH2-mediated H3-tri-mK27 in silencing of the inactive X chromosome [17]. Our understanding of the consequences of H3-K27 methylation in *C. elegans* will be advanced by identification of the other H3-K27 HMT(s) and the gene targets of methylation.

Our finding that *mes* mutant embryos lack the robust H3-K27 methylation seen in somatic cells of wild-type embryos and yet develop into healthy (but sterile) adults raises questions about the roles of H3-K27 methylation in the soma. *mes* mutants do show low-penetrance homeotic transformations in the soma, and in certain genetic backgrounds they display sexual transformations [16, 18]. This suggests that the MES-2 complex has redundant or relatively insignificant functions in maintaining repression in somatic cells. Thus, although the E(Z) and EZH2 complexes in flies and vertebrates serve major roles in maintaining repression of numerous genes in somatic cells (reviewed in [8, 24]), similar control by the MES-2 complex may have been largely usurped by other regulators in the course of evolution.

Supplemental Data

Additional data and detailed Experimental Procedures are available at www.current-biology.com/cgi/content/full/14/18/1639/DC1/.

Acknowledgments

We thank Leslie Phillips for technical assistance and Eric Witze and Joel Rothman for sharing their nuclear extract procedure before publication. This work was supported by National Institutes of Health grants GM34059 (S.S.) and GM068804 (Y.Z.).

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