

HIS-24 Linker Histone and SIR-2.1 Deacetylase Induce H3K27me3 in the *Caenorhabditis elegans* Germ Line[∇]

Martina Wirth,¹ Franziska Paap,¹ Wolfgang Fischle,¹ Dirk Wenzel,² Dmitry E. Agafonov,^{3,4} Timur R. Samatov,^{3,4} Jacek R. Wisniewski,⁵ and Monika Jedrusik-Bode^{1*}

Laboratory of Chromatin Biochemistry,¹ Electron Microscopy Group,² and Department of Cellular Biochemistry,³ Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany; Institute of Protein Research, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia⁴; and Department of Proteomics and Signal Transduction, Max Planck Institute for Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany⁵

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HIS-24 linker histone and SIR-2.1 deacetylase are involved in chromatin silencing in *Caenorhabditis elegans*. Depletion of SIR-2.1 results in cytoplasmic retention of HIS-24 in oocytes. However, the molecular working mechanisms of HIS-24 and SIR-2.1 are unclear. We show here a synergistic function of SIR-2.1 and HIS-24 that are together essential for maintenance of the H3K27me3 mark in the germ line of *C. elegans*. We demonstrate the synthetic effects of the two factors on brood size, embryogenesis, and fertility. SIR-2.1 and HIS-24 associate with the subtelomeric regions but apparently do not interact directly. We report that SIR-2.1 deacetylates H3K9 at subtelomeric regions and suggest that deacetylation of H3K9 is a prerequisite for H3K27 methylation. In turn, we found that HIS-24 specifically interacts with the histone H3 K27 region, when unmodified or in the trimethylated state. Overall, our data indicate that SIR-2.1 and HIS-24 contribute to the propagation of a specialized chromatin state at the subtelomeric regions and elsewhere in the genome.

The chromosome ends of the nematode, *Caenorhabditis elegans*, are protected from degradation and fusion by DNA-protein complexes called telomeres (17). The telomeres consist of tandem DNA repeats, (TTAGGC)_n, which are bound by specific proteins promoting the function and integrity of the telomeres (27). The lack of telomerase, an enzyme adding telomeric DNA repeats to chromosome ends, leads to genome instability and end-to-end chromosome fusion (18).

Adjacent to the terminal telomeric repeats lie subtelomeric regions, which are defined by block repeats with sequence homology between chromosomes. The subtelomeres are described as a transition zone between telomeres and chromosome sequences (7, 27). In contrast to telomeres, the biological role of subtelomeres is still poorly characterized, although in budding yeast and *Tetrahymena* the subtelomeres may influence the lengths of the adjacent telomeres. It is suggested that this effect is mediated by the compact heterochromatin structure of the subtelomeric regions, rather than by specific sequence elements and/or tandem repeats (19). Chromatin modifications on histone H3 lysine residues seem to play a direct role in establishing transcriptionally repressive subtelomeric heterochromatin and therefore have an effect on the expression level of nearby vital genes in yeast (19).

In most organisms, telomeres and subtelomeres are packaged in a heterochromatin structure, whereas in *C. elegans* heterochromatin has not been yet defined cytologically (8). In vertebrates, enzymes known to be involved in heterochromatin formation at telomeres are proteins of the sirtuin *sir2* gene

family, SIRT1 and SIRT6, as well as the histone methyltransferases Suv4-20h1 and Suv4-20h2 (3, 20). Their deficiency results in telomere dysfunction and derepression of telomere recombination (3, 20). SIRT1 and SIRT6 are NAD⁺-dependent histone deacetylases that modify histone H3 at lysine position K9 (H3K9). SIRT1 promotes heterochromatin formation by additionally modifying H4K16, causing the loss of the H3K79me2 mark that is associated with transcriptionally active chromatin, and recruiting linker histone variant H1.b (15, 25). Interestingly, a decrease in human linker histone H1 variants to 50% of normal levels leads to an increase in telomere length caused by telomeric recombination or by enhanced telomerase activity (22). However, our understanding of the role of linker histone in telomeric chromatin is still incomplete.

C. elegans contains eight linker histone variants but only one, HIS-24, promotes germ line development and influences histone H3 methylation in germ line. Its partially cytoplasmic retention in germ cells is dependent on the chromatin-modifying Polycomb group genes (*mes-2*, *mes-3*, *mes-4*, and *mes-6*), as well as on the *C. elegans* sirtuin gene *sir-2.1* (12, 14).

In the present study we show that SIR-2.1 and HIS-24 are associated with the subtelomeric regions in *C. elegans*. We report that HIS-24 specifically interacts with H3K27me3 in the germ line of *C. elegans* and propose that SIR-2.1 and HIS-24 influence germ line development by promoting the modification of H3K27 and H3K9 at the subtelomeric regions. Interestingly, the loss of H3K27me3 in an *mes-2* background causes decreased binding of HIS-24 to the subtelomeres. Our results indicate that it is not specific sequence elements-tandem repeats, but chromatin structure and their modifications at the subtelomeres and elsewhere in the genome that contribute to fertility and normal embryogenesis in *C. elegans*. Furthermore, we show that similar molecular mechanisms that regulate chromosome modification at the subtelomeres exist in both invertebrates and vertebrates.

* Corresponding author. Mailing address: Max Planck Institute for Biophysical Chemistry, Laboratory of Chromatin Biochemistry, Am Fassberg 11, D-37077 Göttingen, Germany. Phone: (49) 551-201-1446. Fax: (49) 551-201-1337. E-mail: mjedrus@gwdg.de.

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MATERIALS AND METHODS

Strains. Maintenance, culture, and genetic manipulation of *C. elegans* were carried out according to standard procedures (4). Bristol strain (N2) was used as the wild-type strain. Strains with the following genotypes were obtained from the *Caenorhabditis* Genetics Center: *sir-2.1(ok434)III*, *his-24(ok1024)X*, and *mes-2(bn11) unc-4(e120)lmnC1 dpy-10(e128) unc-52(e444)II*. The double transgenic strain *his-24(ok1024)X sir-2.1(ok434)III* was generated by crossing strains. A PCR-based analysis was used for the *ok1024* and *ok434* alleles.

Two independent lines were established and tested for the mortal (*mrt*) phenotype. All assays were initiated with sibling F₂ homozygotes from the *his-24; sir-2.1* mutant strain. Six L1 larvae (F₂) were grown for 2 weeks on a small agar plate seeded with *Escherichia coli* OP50 as food at 25°C and could grow for two generations per dish before starvation. Then, six L1 larvae (F₄) were transferred to a fresh plate, and every 2 weeks the *his-24; sir-2.1* double-mutant strain was passaged to F₂₂ (1). Hatching rates were assessed by individual plating of 15 L4 larvae of *his-24(ok1024)X sir-2.1(ok434)III* at the F₂ to F₂₂ generations and screening after 24 h for sterility or male production after 4 days at 25°C.

Brood size and embryonic lethality. The numbers of progeny and dead embryos at 25°C were scored daily. The embryos were stained with 500 ng of DAPI (4',6'-diamidino-2-phenylindole)/ml for 10 min as previously described (14).

Immunoprecipitation and mass spectrometry. Mixed populations of L4 and adult worms were homogenized according to the method of Cheeseman and Desai (6). Approximately 3.5 mg of total precleared protein was incubated with the specific rabbit polyclonal anti-HIS-24 antibody. Finally, the protein complexes were incubated with protein G-Sepharose (Pierce). For mass spectrometry, the immunoprecipitated proteins were resolved on gradient Bis/Tris gels (4 to 12%; Bio-Rad), visualized by silver staining, and analyzed by mass spectrometry. The results from wild-type and *his-24* mutant strains were compared, and the differences were analyzed by Western blotting with rabbit polyclonal anti-SIR-2.1 antibody (1:1,000; kindly provided by A. Gartner), polyclonal rabbit anti-HIS-24 antibody (1:10,000), and anti-H3K27me3 antibody (1:1,000; kindly provided by T. Jenuwein).

Immunofluorescence analysis. Wild-type worms and EC602 [*unc-119(ed3)III; eels602[unc-119(+ his-24::gfp)]*], *his-24(ok1024)X*, *sir-2.1(ok434)III*, and *his-24(ok1024)sir-2.1(ok434)III* double mutants were fixed with 1.85% paraformaldehyde and stained as previously described (12). One percent of *his-24::gfp* (EC602) transgenic worms showed slight expression of HIS-24::GFP in the germ line. The dissected gonads of these transgenic worms were stained with an antibody raised against green fluorescent protein (GFP) and SIR-2.1. The following antibodies were used: anti-HIS-24 (1:1,000), anti-GFP (1:500; Roche Applied Science), anti-H3K27me3 (kindly provided by T. Jenuwein), anti-H3K9me2 (Upstate catalog no. 07-212), anti-H3K9me3 (Upstate catalog no. 07-442), anti-H3K36me3 (Abcam catalog no. ab9050), anti-H3K56ac (Epitomics catalog no. 2134-1), anti-H3K79me-1,2,3 (Abcam catalog no. ab28940), and anti-RNA polymerase II CTD (Abcam catalog no. ab817). Alexa 555-goat anti-rabbit, Alexa 488-goat anti-rabbit, and/or Alexa 488-goat anti-mouse secondary antibodies at a dilution of 1:500 (Molecular Probes) were used. The samples were then mounted with Vectashield and analyzed by using a Leica SP5 laser scanning microscope. Images were acquired as a series of 0.5- μ m-thick optical sections, processed, and then merged.

Immunoelectron microscopy of ultrathin cryosections. Samples were cut, fixed with 2% paraformaldehyde in 0.1 M sodium phosphate (pH 7.4) for 24 h at 4°C, and postfixed with 4% paraformaldehyde–0.1% glutaraldehyde for 2 h on ice. Cryosections were prepared as previously described (16, 28), labeled for the indicated antigens, and examined with a Philips CM120 electron microscope and a TVIPS charge-coupled device camera system.

Western blot analysis. *C. elegans* lysates were prepared and analyzed by Western blot as previously described (12). The membranes were incubated with anti-H3K27me3 (kindly provided by T. Jenuwein) at 1:1,000, anti-acetyl-H3 (Upstate catalog no. 06-599) at 1:10,000, anti-H3K9ac (Upstate) at 1:1,000, and anti-H3K9K14ac and anti-H4K16ac (Upstate) and then stripped before incubation with anti-H3 (Abcam catalog no. ab1791) at 1:20,000, using Restore Plus Western blot stripping buffer (Pierce catalog no. 46430).

RNA isolation and reverse transcription-PCR. RNA was isolated as previously described (14). cDNAs encoding full-length *sir-2.1* and truncated *sir-2.1* were cloned into pEU3-NII-StrepII using XhoI and NotI restriction sites after reverse transcription-PCR amplification from total RNA of wild-type and *sir-2.1(ok434)III* mutant strains using reverse transcriptase SuperScript III (Invitrogen) according to the manufacturer's instruction. Primer sequences are available on request.

Histone deacetylase assay. The deacetylase assays were essentially performed as described by Verdin et al. (26) using a chemically acetylated H4 peptide as a

substrate. For the histone deacetylase assay, the cDNAs of full-length *sir-2.1* and the *sir-2.1* deletion allele were cloned into pEU3-NII-StrepII. pEU3-NII-StrepII containing the cDNA of HP1 β was kindly provided by Szobosz Sörös. Recombinant SIR-2.1, truncated SIR-2.1, and HP1 β were translated in vitro using wheat germ extract (24). Since wheat germ extract does not exhibit intrinsic deacetylase activity, in vitro-translated proteins were directly used for the enzymatic assay without further purification. The total reaction volume was 100 μ l containing 5 μ l of in vitro-translated protein. Reactions were incubated at 25°C for 2 h or overnight. The protein concentrations of the in vitro-translated proteins were determined by quantifying the incorporation of [¹⁴C]leucine into full-length protein bands using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography.

ChIP. Chromatin immunoprecipitation (ChIP) assay was performed as previously described (21) with some modifications. L4s and adults were homogenized in ice-cold lysis buffer with protease inhibitors and 0.1% Triton using an equal volume of cubic zirconium beads (BioSpec Products, catalog no. 11079105z). The mixture was vortexed for 10 min at 4°C and then sonicated by using a Branson 250 sonifier. To ca. 2.8 mg of total protein/ml, ~5- μ g portions of the following polyclonal anti-rabbit antibodies were added: anti-HIS-24, anti-HIL-4 (cross-reactive with all H1 variants except H1.X, with strong reactivity for HIL-4) (14), anti-SIR-2.1 (kindly provided by A. Gartner), anti-H3, anti-H3K9ac, anti-H3ac, and H3K27me3. ChIP assays were quantified by real-time PCR using SYBR green PCR Master Mix (Applied Biosystems) and an MJ Research sequence detection system (catalog no. R107a). All reactions were analyzed in triplicate. Primer sequences are available on request.

Peptide pulldowns from total worm lysates. Peptide pulldowns were performed as previously described (29). For H3 and H4 peptide binding experiments, 10 μ g of the biotinylated peptide was first coupled to streptavidin-agarose beads (Pierce). The H3 peptides were either methylated on K27 (amino acids [aa] 19 to 38), methylated on K9 (aa 1 to 20), unmethylated (aa 1 to 20), or methylated H4 peptides (aa 12 to 27). Worm lysates were incubated for 2 h with the beads and washed seven times with PD-150 buffer (20 mM HEPES [pH 7.9], 150 mM KCl, 0.2% Triton, 1 \times protease inhibitor [Roche], 20% glycerol), and bound proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in Laemmli buffer.

RESULTS

***His-24; sir-2.1* double mutants phenocopy aspects of mortal germ line (*mrt*) mutants and display pleiotropic defects distinct from *his-24* and *sir-2.1* phenotypes.** The gene-specific effects of linker histone H1 might result from its interaction with sequence-specific DNA-binding proteins or specific regulatory factors such as BAF, Msx1, and SIRT1 (25). In worms, the interaction between H1 and specific regulators is still not clearly understood. Recently, we have reported that SIR-2.1 in *C. elegans*, a homolog of human SIRT1, is involved in the mislocalization of the linker histone variant HIS-24 in the germ line (12). Both HIS-24 and SIR-2.1 influence chromatin silencing in the germ line of *C. elegans* (13, 14). To investigate the function of *sir-2.1* and *his-24* within the germ line, we generated the double-mutant *his-24; sir-2.1* strain. We observed that sterility of the double-mutant strain increased from one generation to next. The maximum sterility of 21% of 1,258 screened worms was observed at generations F₁₂ and F₁₄ at 20°C and 25°C in contrast to *his-24* and *sir-2.1* single-mutant strains (Fig. 1A). Several developmental defects such as short germ line arms (12% of 123 screened gonad arms; Fig. 1B) and masculinization of the germ line (germ line without oocytes [5% of 123 screened gonad arms]; Fig. 1B) were found. In contrast, the *sir-2.1(ok434)III* mutant strain is superficially wild type, and only 9% of *his-24(ok1024)X* mutant worms are sterile. These findings suggest a synergistic effect of SIR-2.1 on the observed phenotype in the double-mutant *his-24; sir-2.1* strain (14). The sterility of the *his-24; sir-2.1* double-mutant strain was slightly reduced at 15°C and observed in 14.2% of 1,451

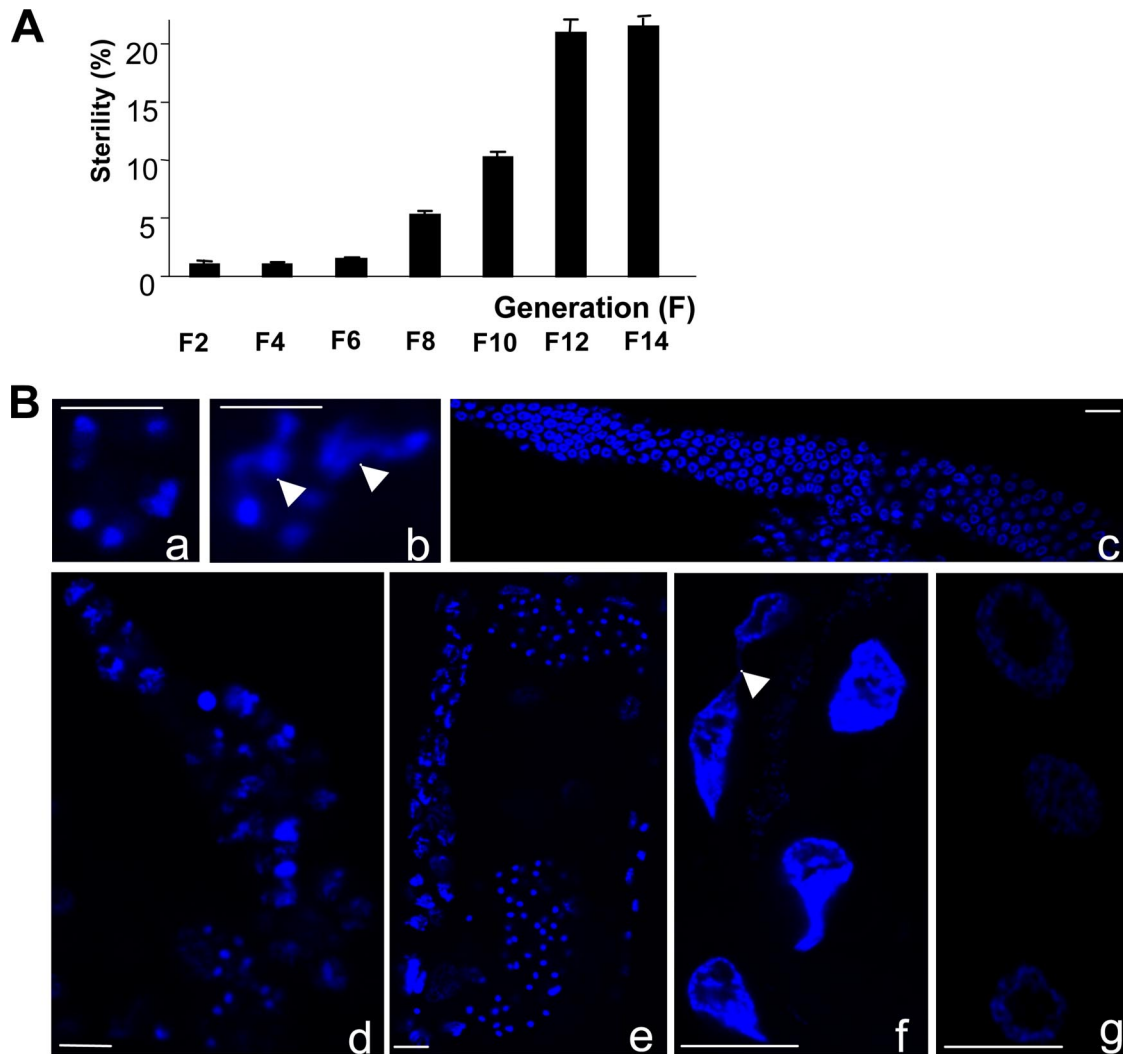


FIG. 1. (A) The sterility of double *his-24*; *sir-2.1* mutants is dependent on the generation number. The maximum of ca. 21% sterile worms was observed in the F_{14} generation. The worms were scored for sterility using two independent *his-24*; *sir-2.1* double-mutant lines. Error bars indicate the standard deviation (SD). (B) *his-24(ok1024)X*; *sir-2.1(ok434)III* double-mutant strains display many defects in chromosome morphology (b, d, e, and f) compared to wild-type worms (a, c, and g). An unequal distribution of chromatin between daughter nuclei cells in the polyploid cells of intestine (f) and fusions between chromosomes in oocytes were observed by DAPI staining (b). Short germ line arms (d) and masculinization of the gonad (e) were found. (c) Gonad arm of wild-type hermaphrodite. Arrows point to the chromosome fusion in the oocyte (b) and nuclei of polyploid cells of intestine (f). Scale bars: 5 μm (a and b) and 10 μm (c to g).

worms. Interestingly, the double-mutant strain was not 100% sterile if passaged for multiple generations, which is typical for the mortal germ line mutants (*mrt*) or telomerase reverse transcriptase (*trt-1*) mutants. Nevertheless, the double-mutant strains displayed end-to-end chromosome fusion in the soma and germ line nuclei, suggesting defects in genome stability (Fig. 1B; Tab.1) (1, 18). Moreover, decreased brood size at all generations and increased numbers of dead embryos were observed (Table 1). The brood size of the *his-24*; *sir-2.1* double-mutant strain was strongly decreased, to 50% of wild-type worms or the single-mutant *his-24(ok1024)* or *sir-2.1(ok434)* strains. In addition, 5 of 24 gonads of the double mutant (21%) contained oocytes with an abnormal number of chromosomes at diakinesis (Table 1).

To further support the observed end-to-end chromosome fusion as a result of telomere-telomere fusions, we performed

TABLE 1. Phenotypic characterization of mutant strains at 25°C^a

Strain	No. of gonads with DAPI-stained chromosomes at diakinesis (<i>n</i>) ^b	% Dead embryos (<i>n</i> = 4) ^c	Mean brood size \pm SD (<i>n</i> = 4)
Wild type strain	24 (24)	1.2 (942)	284 \pm 34
<i>sir-2.1(ok434)III</i> strain	23 (24) [1]	1.0 (840)	241 \pm 21
<i>his-24(ok1024)X</i> strain	10 (10)	4.3 (767)	236 \pm 14
<i>sir-2.1(ok434)III/his-24(ok1024)X</i> strain	19 (24) [5]	10.2 (542)	134 \pm 45

^a HIS-24 and SIR-2.1 act synergistically to control the brood size, embryonic lethality, and number of chromosomes at diakinesis. The wild-type worms and mutant strains of the F_{12} generation were screened for the number of gonads with oocytes containing an abnormal number of chromosomes at diakinesis. For each strain, the progeny of four hermaphrodites (*n* = 4) were phenotypically characterized, and the numbers of dead embryos and brood sizes were counted.

^b *n*, number of screened gonad arms with DAPI. The numbers of gonads with oocytes containing an abnormal number of chromosomes are indicated in brackets.

^c The numbers of screened worms are indicated in parentheses.

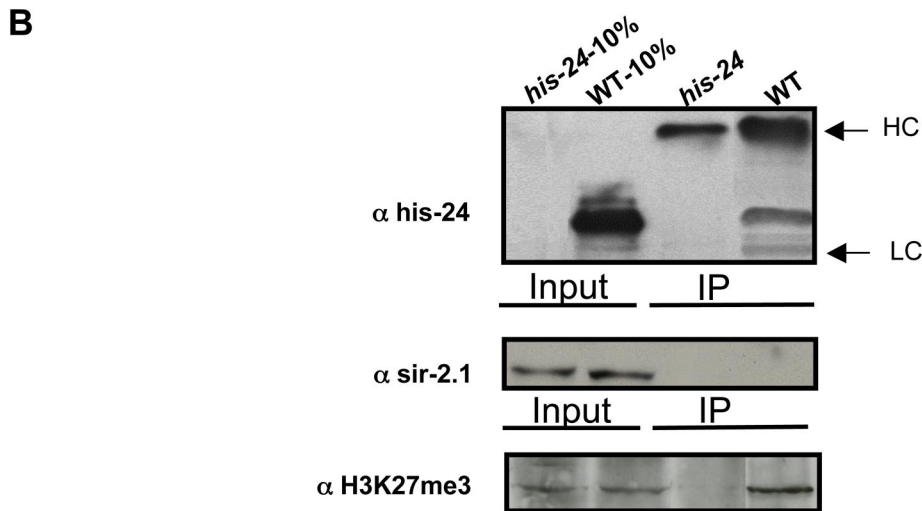
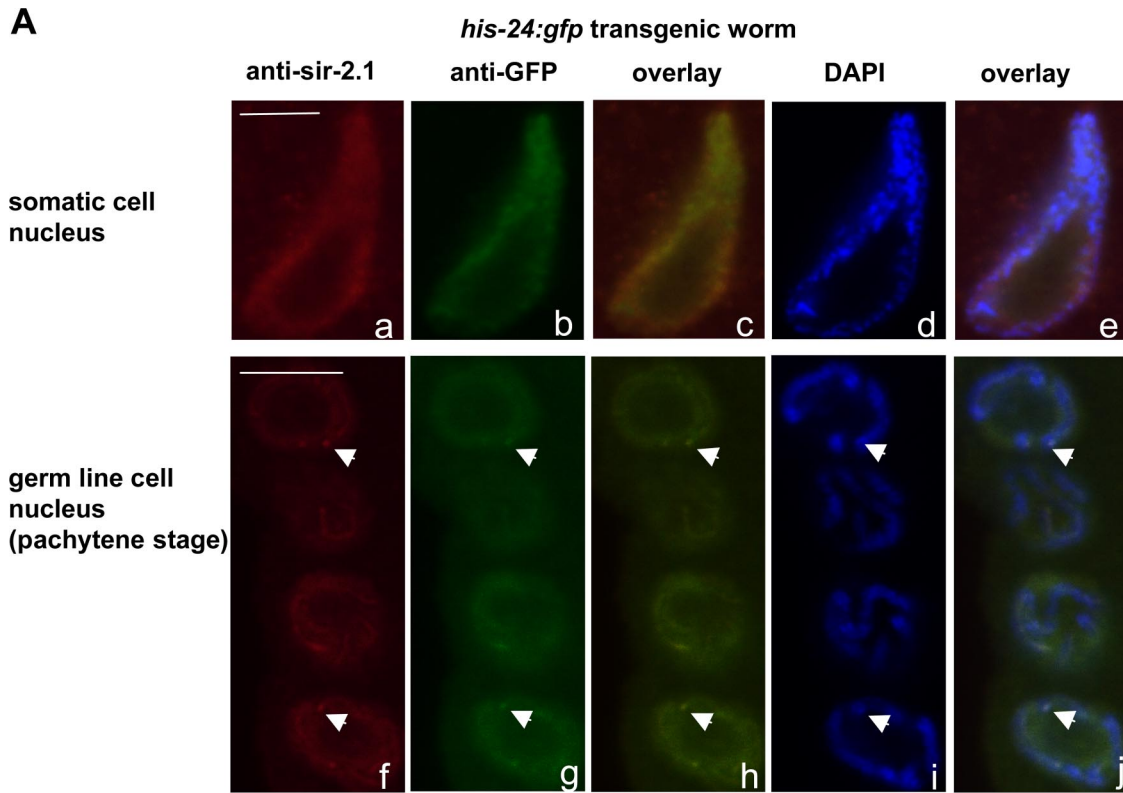
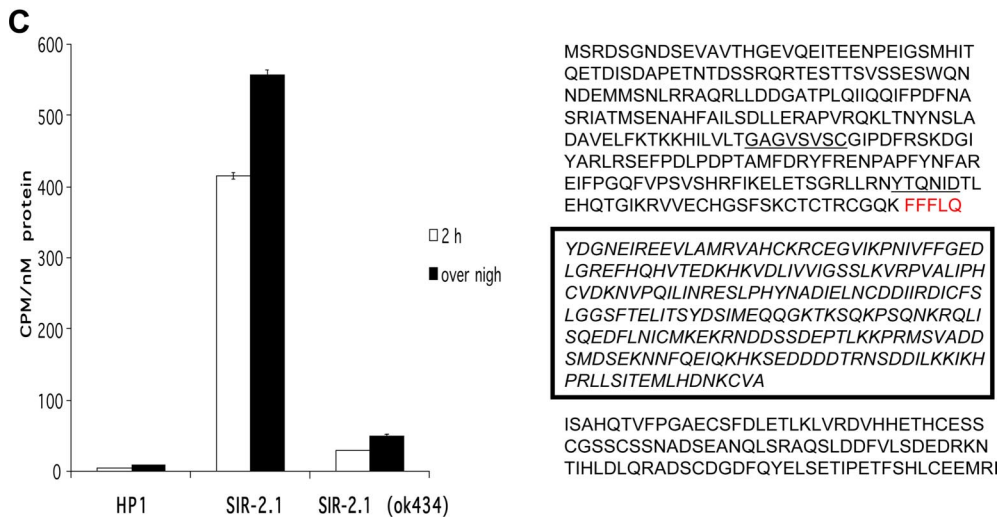
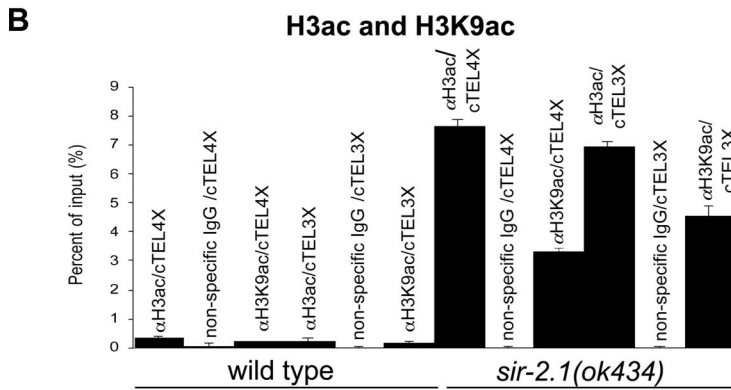
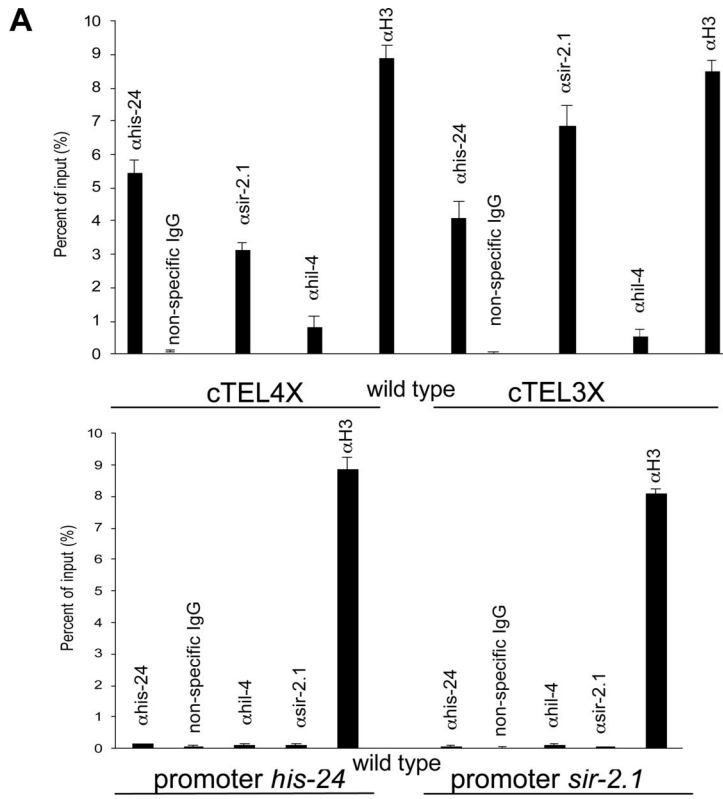


FIG. 2. (A) HIS-24::GFP colocalizes with SIR-2.1 in the polyploid cell nucleus of intestinal cells and at the ends of chromosomes of germ cells in the pachytene stage. Both proteins were detected using specific antibodies against GFP and SIR-2.1 (see arrow points). Corresponding overlay pictures are shown (c, e, h, and j). Scale bar, 5 μ m. (B) Coprecipitation of wild-type worms and *his-24(ok1024)*X-null mutant strain with antibodies raised against HIS-24 as a control. Protein extracts were prepared from lysates of the wild-type and *his-24(ok1024)*X mutant strains. The precipitates were analyzed by Western blotting with anti-SIR-2.1 and anti-H3K27me3 antibodies. SIR-2.1 does not coprecipitate with HIS-24, in contrast to H3K27me3. HC, heavy chain; LC, light chain.

Southern blot analysis. No significantly different rate of telomere shortening or lengthening in the *his-24*; *sir-2.1* double mutant compared to the *sir-2.1* and *his-24* single mutants and the wild type were detected (data not shown). Therefore, we conclude that HIS-24 and SIR-2.1 act differently from other *mortal* mutants and do not regulate telomere lengths. Moreover, SIR-2.1 and HIS-24 play an essential role in germ line

development, as well as significantly reduce the brood size and subsequent embryonic survival.

SIR-2.1 colocalizes with HIS-24, but the proteins do not interact directly. To test whether HIS-24 interacts with SIR-2.1, we performed immunofluorescence studies, as well as immunoprecipitation experiments, followed by mass spectrometry analysis. Although we observed costaining of SIR-2.1 and HIS-24 in the



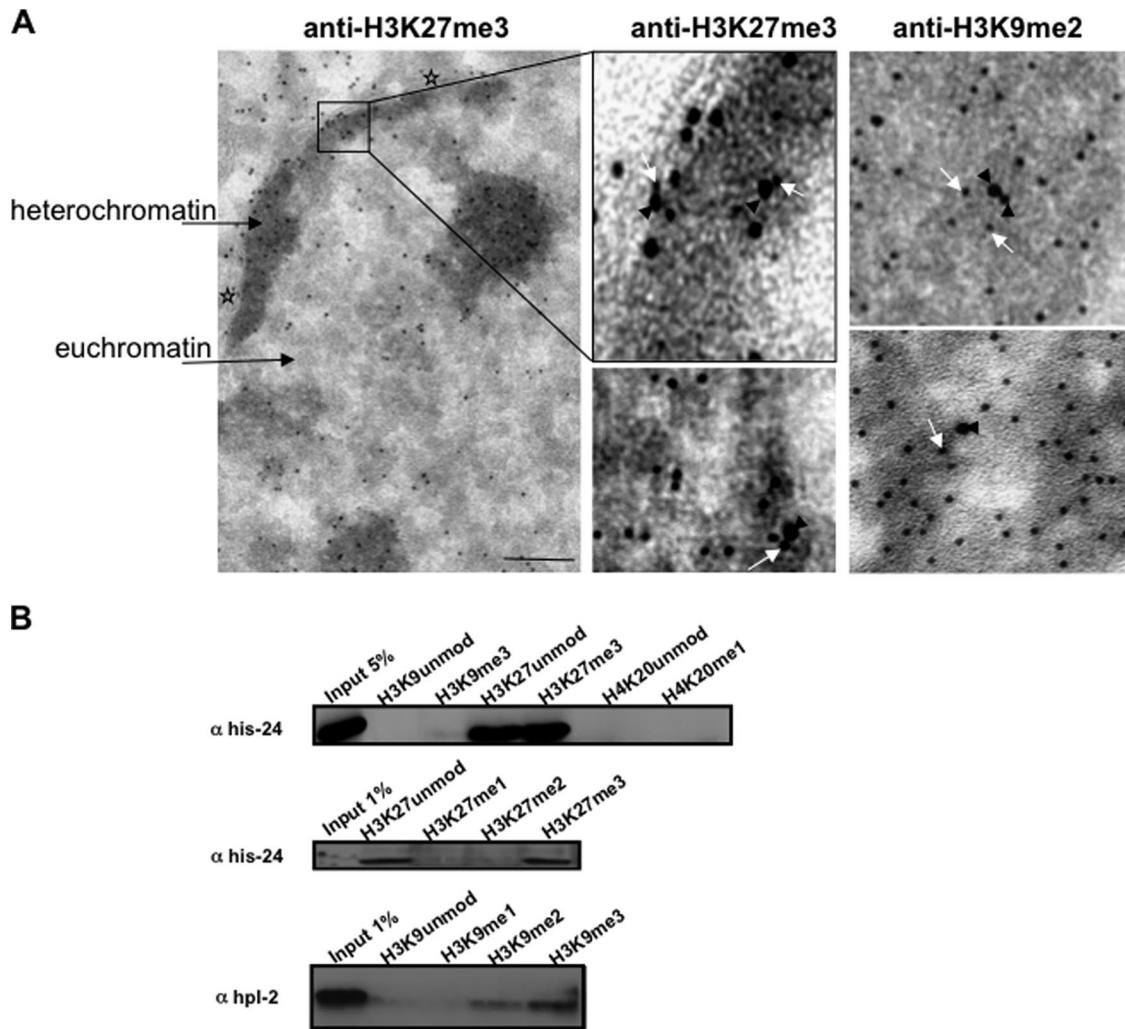


FIG. 4. (A) Electron microscopy of HIS-24 and H3K27me3 in the germ line of an adult hermaphrodite at the pachytene stage (left and middle panels). *C. elegans* germ line nuclei display extended segments of heterochromatin (electron-dense and dispersed), as well as less-dense regions, i.e., the euchromatin. HIS-24 is partially colocalized with H3K27me3 in the nuclei of germ line (the middle panel shows two different images of two nuclei in the germ line). In contrast, HIS-24 is not colocalized with H3K9me2; however, both proteins are localized in the heterochromatin regions (right panel, two different images of two nuclei at the pachytene stage). An arrowhead points (black) to HIS-24 labeling (10-nm gold), an arrow points (white) to H3K27me3 labeling (5-nm gold) or to H3K9me2 (5-nm gold), and stars indicate the positions of the nuclear membrane. Scale bar, 200 nm. (B) HIS-24 specifically recognizes the H3 tail around the H3K27 region in peptide pull-down assays using total worm lysates. In the top panel, interaction of HIS-24 with peptides representing different histone tail regions and in distinct modification stages was assayed by experiments with total worm extracts. The middle panel shows the binding of HIS-24 to histone H3 K27 peptides of different methylation stages. In the bottom panel, HPL-2 (a homolog of HP1) was used as a positive control for determining the binding specificity to the H3K9me2 and H3K9me3 peptides.

nuclei of soma and at the ends of chromosomes at the pachytene stage (Fig. 2A), we did not detect SIR-2.1 immunoreactivity in HIS-24 precipitates isolated from wild-type worms (Fig. 2B), nor did we find SIR-2.1 in mass spectrometric analyses (data not

shown). Therefore, it is possible that the interaction of HIS-24 with SIR-2.1 is indirect via other factors or that both proteins are involved in parallel pathways of the same process: germ line development and/or chromatin modification.

FIG. 3. (A) Quantitative ChIP using total protein isolated from wild-type worms and antibodies to HIS-24, SIR-2.1, H3, and HIL-4. The primer sets used for quantitative ChIP analysis were directed to the subtelomeric region of chromosome III (cTEL3X), IV(cTEL4X), and promoters of *sir-2.1* and *his-24* genes as a control. A control experiment using specific antibody against the core histone H3 demonstrating the HIS-24 specificity to the subtelomeric regions compared to the promoters of *sir-2.1* and *his-24* was performed. Error bars indicate the SD. (B) SIR-2.1 deletion causes specific H3K9 acetylation and global H3 acetylation on subtelomeres compared to full-length expressed SIR-2.1. Error bars indicate the SD. (C) Histone deacetylase assay for full-length SIR-2.1 and truncated SIR-2.1. The loss of enzymatic activity of truncated SIR-2.1 compared to full-length SIR-2.1 was evaluated. HP1 was used as a negative control. The truncated SIR-2.1 protein lacks 229 aa encoded by a part of exon 3 (613 bp from 1,821 bp) (black box). The second exon is spliced in frame to the rest of the third exon, generating five additional amino acids in between (red). Highly conserved amino acid motifs of SIR-2 proteins (from yeast to humans) are underlined and are present in the truncated SIR-2.1 protein.

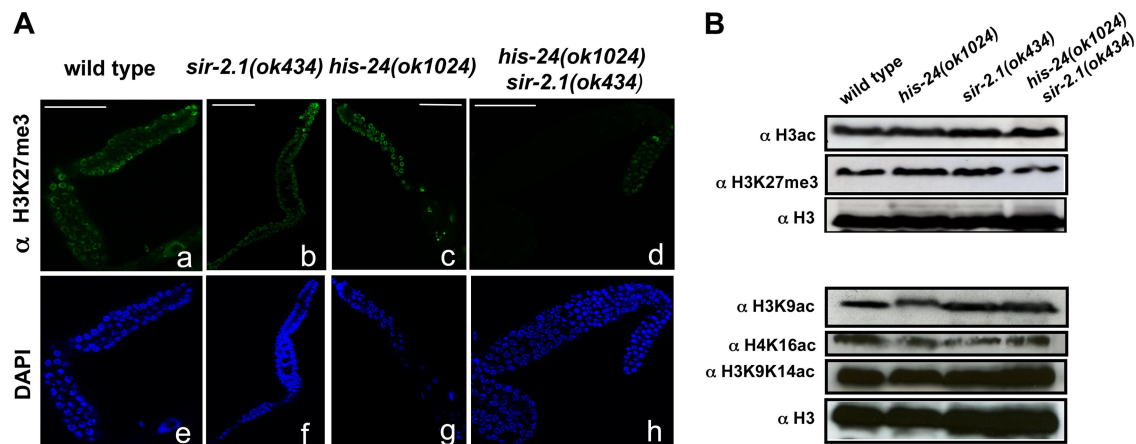


FIG. 5. (A) Loss of H3K27me3 staining in the pachytene stage of the germ line of *his-24*; *sir-2.1* double mutant (d). The H3K27me3 chromatin mark is detected only in the somatic nuclei, distal tip cells, and mitotic zone of the germ line. In contrast, H3K27me3 staining is present in all nuclei of the germ line of the wild type, *sir-2.1(ok434)III*, and *his-24(ok1024)X* mutant strains (a, b, and c). The corresponding DAPI staining pictures are also shown (e, f, g, and h). Scale bar, 50 μ m. (B) The reduction of H3K27me3 level seen in immunoblotting from whole-cell extracts of *his-24*; *sir-2.1* double mutant. No changes in *his-24(ok1024)X* and *sir-2.1(ok434)III* control worms were detected. There were no changes in the *his-24*; *sir-2.1* double-mutant, *sir-2.1* mutant, and *his-24* mutant levels of H3 acetylation.

HIS-24 and SIR-2.1 associate with telomeres in vivo. The observed *his-24*; *sir-2.1* phenotype suggested an influence of these proteins on telomere function. Recently, it has been shown that SIRT6 has enzymatic activity at telomeric chromatin (20). Therefore, our initial interest was to determine whether SIR-2.1 is involved in histone acetylation at the telomeres and whether SIR-2.1 and HIS-24 can access the telomere ends in vivo in *C. elegans*. Notably, SIR-2.1 and HIS-24 are abundantly expressed proteins and are associated with chromatin, but their localization at the telomeres is difficult to detect by immunofluorescence (9, 12).

As shown by quantitative ChIP analysis, HIS-24 specifically binds to the subtelomeric regions cTEL3X and cTEL4X with a 25-fold increase compared to the *his-24* or *sir-2.1* promoter regions (Fig. 3A and B). In contrast, we did not see any changes of H3 binding to the subtelomeric regions compared to the *his-24* or *sir-2.1* promoter regions, indicating that HIS-24 is specifically enriched at the subtelomeric regions (Fig. 3A).

cTEL3X and cTEL4X are single-copy loci and thus specific for their respective telomeres (27). The subtelomeric region of cTEL4X contains tandem (satellitelike) repeats 20 bp in length in contrast to cTEL3X.

Importantly, the HIS-24 antibody precipitates significantly more subtelomeric material than the HIL-4 antibody, which is cross-reactive to *C. elegans* linker histone variants (Fig. 3A) (14). Therefore, we conclude that only the linker histone HIS-24 and not any of the seven other linker histone variants is associated with subtelomeric regions (Fig. 3A). This interpretation is also supported by our previous findings showing that only linker histone HIS-24 is able to enhance the telomeric position effect in budding yeast (13).

In similar experiments, we found SIR-2.1 binds with higher affinity to cTEL3X than to cTEL4X. Further, SIR-2.1 deficiency and the lack of its enzymatic activity causes hyperacetylation of both subtelomeric regions cTEL3X and cTEL4X (Fig. 3B). Since the truncated SIR-2.1 protein from the deletion strain *sir-2.1(ok434)III* has no enzymatic activity, we sought to

determine whether SIR-2.1 can deacetylate specific lysine residues of H3 at the subtelomeric regions (Fig. 3C). Quantitative ChIP analysis using an antibody against H3K9ac indicates that in the absence of SIR-2.1 H3K9ac is increased, implying that *C. elegans* SIR-2.1 can specifically deacetylate H3K9 at the subtelomeric regions. Furthermore, lack of SIR-2.1 influences HIS-24 binding to the subtelomeric regions. In the *sir-2.1* background HIS-24 binds fourfold less compared to the wild type, implicating the involvement of SIR-2.1 on HIS-24 recruitment to subtelomeres (see Fig. 6B). These results suggest that SIR-2.1 homologs in invertebrates and vertebrates have the same enzymatic substrates and that SIR-2.1 is important for HIS-24 recruitment to the subtelomeric regions.

HIS-24 has a stimulatory effect on H3K27me3 in the germ line. Previously, we have reported that HIS-24 in the *mes-3* (named for *maternal effect sterility*) background has an influence on the presence of H3K27me3 in the pachytene stage of germ line cells. In addition, MES proteins cause mislocalization of HIS-24, similar to SIR-2.1 (12). Notably, MES are Polycomb group proteins, and MES-2 is a histone methyltransferase for H3K27me3 in the germ line (2). Therefore, we investigated whether HIS-24 colocalizes with H3K27me3 in the nuclei of germ line cells and whether MES-2 has an influence on HIS-24 binding to subtelomeres. We found that HIS-24 colocalizes with H3K27me3 in the pachytene stage of germ line nuclei and that the lack of MES-2 and H3K27me3 in the germ line affects HIS-24 association with subtelomeres (Fig. 4A and 6B).

The majority of the HIS-24 and H3K27me3 colocalized proteins are found close to the membrane of the nucleus in the electron-dense region of the meiotic stem-cell population. In addition, part of HIS-24 could be detected in the cytoplasm of germ line cells, as reported previously (Fig. 4A) (12). To further investigate the relationship of HIS-24 and H3K27me3, we performed pull-down studies with modified histone peptides. We found that HIS-24 specifically interacts with H3K27me3 and H3K27 unmodified peptides, but not with the correspond-

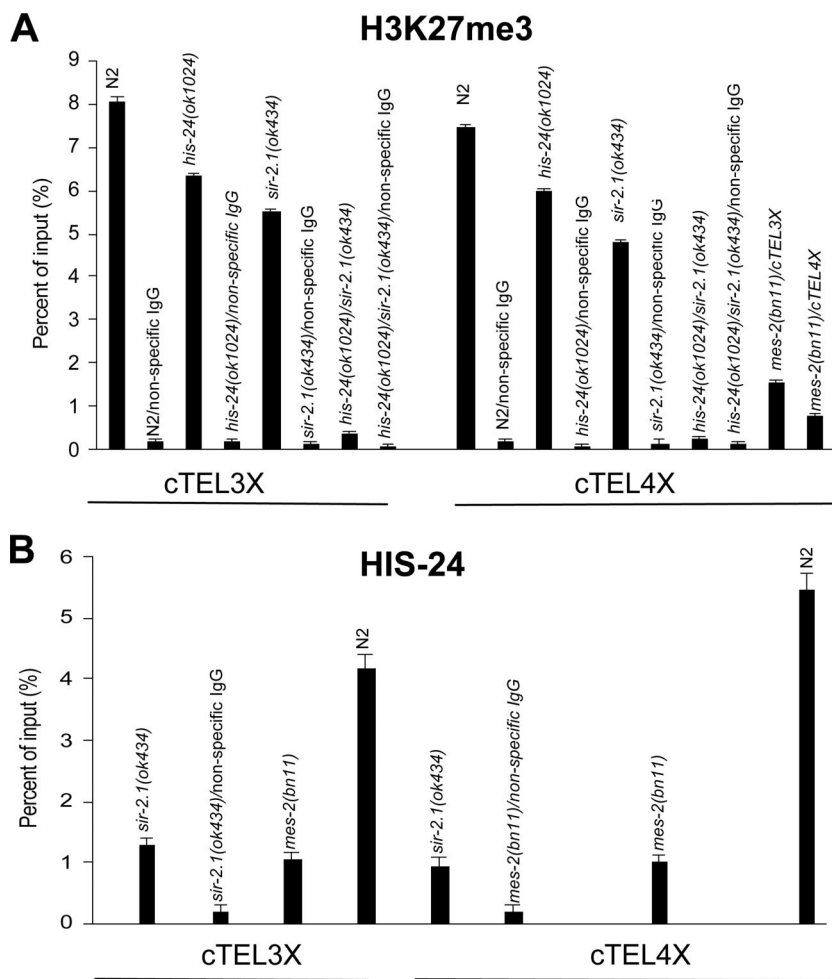


FIG. 6. (A) The H3K27me3 mark is present at the subtelomeres cTEL3X and cTEL4X. A deficiency in HIS-24 and SIR-2.1, as well as MES-2, causes the loss of H3K27me3 at the subtelomeres compared to the wild type. (B) The lack of SIR-2.1 and MES-2 has an influence on HIS-24 binding to subtelomeres compared to the wild type. Error bars indicate the SD.

ing H3K9 or H4K20 peptides (Fig. 4B). We hypothesize that the unusual HIS-24 binding to both peptides is mediated by different factors (MES complex) or that HIS-24 could have more than one binding pocket. Interestingly, the lack of HIS-24 does not directly influence H3K27me3 levels in the worm (Fig. 5). In contrast, only the depletion of both SIR-2.1 and HIS-24 causes a deficiency of H3K27me3 in germ line cells, suggesting a role for HIS-24 and SIR-2.1 in mediating chromatin modifications, specifically H3K27me3 (Fig. 5). In addition, a slightly reduced level of H3K27me3 at subtelomeres was observed in the *his-24* and *sir-2.1* mutant backgrounds compared to the complete lack of H3K27me3 in the *his-24*; *sir-2.1* double-mutant background (Fig. 6A). Next, we sought to determine whether the loss of MES-2, and therefore of H3K27me3, has an influence on the recruitment of HIS-24 to the subtelomeres. As shown, the lack of MES-2 influences the binding of HIS-24 to the subtelomeres, implicating an essential role of the H3K27me3 mark mediated by MES-2 for the presence of HIS-24 at the subtelomeric regions (Fig. 6B). Finally, we sought to determine whether SIR-2.1 and HIS-24 have an influence on other chromatin histone modifications.

No changes were observed on dissected double-mutant worms in analyses with antibodies to H3K9me2, H3K9me3, H3K36me3, H3K56ac, H3K79me1,2,3, RNA polymerase II CTD, H3K9ac, H3K9K14ac, H4K16ac, and hyperacetylated H3 (data not shown). There were also no changes in the acetylation levels in Western blots (Fig. 5B). These data demonstrate that SIR-2.1 preferentially catalyzes the deacetylation of H3K9 at the subtelomeric regions and does not influence global acetylation levels of H3K9, H3K9K14, and H4K16 in *C. elegans*.

DISCUSSION

A linker histone binding to H3K27. In the present study, we report for the first time that HIS-24 specifically binds to H3K27 unmodified and H3K27me3 peptides. Although we currently cannot explain why HIS-24 has this unusual dual binding behavior in vitro, we think that in the context of binding to subtelomeric regions, the H3K27me3 mark is important. First, the lack of MES-2, which is required for H3K27me3 in germ line cells (2), causes the loss of HIS-24 at the subtelomeric

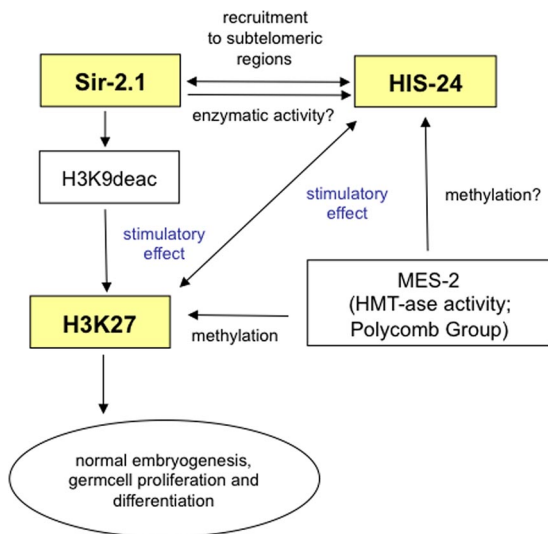


FIG. 7. Model explaining how HIS-24 and SIR-2.1 are essential for the maintenance of the H3K27me3 mark in the germ line of *C. elegans*.

regions (Fig. 6B). Second, HIS-24 is essential for H3K27me3 at subtelomeres in a *sir-2.1* and *mes-2* background (12; the present study). Third, HIS-24 and H3K27me3 are found together close to the nuclear membrane (Fig. 4A). We hypothesize that H3K27me3 and its interaction with HIS-24 might produce a stronger effect on chromatin compaction than HIS-24 interaction with linker DNA or either component alone.

Establishment and maintenance of H3K27me3 in the *C. elegans* germ line. We propose that SIR-2.1 and HIS-24 establish repressive chromatin by their very specific stimulatory effect on the H3K27me3 modification in germ line cells, which may assure the proper regulation of chromatin structure and gene expression. We found that HIS-24 and SIR-2.1 both associated with the subtelomeres but that they did not interact directly. Although do not yet know how these two factors cooperate, we believe enzymatic modification of HIS-24 by SIR-2.1 is an intriguing possibility. Notably, SIRT1 deacetylates linker histone variant H1.b at lysine position 26 in vitro (25).

SIR-2.1 plays an important role for the recruitment of HIS-24 as well as H3K27me3 to the subtelomeres, as we have shown by using quantitative ChIP. Obviously, H3K9ac and H3K27me3 exclude each other at the subtelomeric regions (Fig. 3B and 6A). Interestingly, the MES complex (Polycomb group proteins) promotes accumulation of H3K27me3 in the *C. elegans* germ line as previously reported (2). The histone methyltransferase enzyme for H3K27me3 is MES-2. Loss of MES-2, as well as SIR-2.1, causes the lack of HIS-24 and H3K27me3 at the subtelomeres. We suggest that SIR-2.1 and HIS-24 are part of the Polycomb group gene (PcG) silencing complex in the *C. elegans* germ line and that both factors functionally interact (Fig. 7). It has been reported that methylation of histone H3K27 by the PRC complex (i.e., the Polycomb repressive complex) is dependent on the presence of histone H1 (5, 15, 25) and that a triple H1 knockout of embryonic stem cells leads to a twofold reduction in H3K27 meth-

ylation (10). Therefore, we propose that HIS-24 recognizes the H3K27me3 mark and at the same time is also essential for H3K27me3 maintenance. This interpretation is consistent with our previous data, where SIR-2.1 and HIS-24 influence chromatin silencing in the germ line and HIS-24 promotes H3 methylation (12, 13, 14). Further, the loss of SIR-2.1, MES-2, and/or HIS-24 has an influence on the occurrence of the H3K27me3 mark at the subtelomeric regions (Fig. 7).

Interestingly, the lack of both proteins HIS-24 and SIR-2.1 causes the loss of H3K27me3 in *C. elegans* germ line similar to the lack of HIS-24 and MES-3 as previously reported (12). Furthermore, SIR-2.1 and HIS-24, as well as MES-3 and HIS-24, show functional interaction with respect to fertility and brood size, suggesting that they function in the same developmental pathways in *C. elegans* (12) (Fig. 7). In *Drosophila*, SIR-2 interacts with PcG genes, and the *sir-2* mutation enhances the phenotype of Polycomb group mutants. However, *sir-2* mutants are viable under standard laboratory condition (11).

We hypothesize that maintenance of the H3K27me3 mark during germ cell differentiation and proliferation may be essential for the sterility and brood size of *C. elegans*. However, we cannot exclude that the observed phenotype is due to independent effects of HIS-24 and SIR-2.1 on transcription and is not solely dependent on H3K27me3 and the binding of HIS-24. Notably, *C. elegans* homologs of several chromatin modification and remodeling complexes have been found to play crucial roles in germ line development, germ line-soma distinction, or genome instability (2, 23).

In the present study we identified a novel link between SIR-2.1, PcGs, H3K27me3, and linker histone HIS-24. Future studies are required to understand the molecular relations between SIR-2.1 and PcGs. Modulation of the enzymatic activity of E(Z) methyltransferase by SIR-2.1 remains an intriguing possibility.

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