Caenorhabditis elegans Dosage Compensation Regulates Histone H4 Chromatin State on X Chromosomes

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Dosage compensation equalizes X-linked gene expression between the sexes. This process is achieved in *Caenorhabditis elegans* by hermaphrodite-specific, dosage compensation complex (DCC)-mediated, 2-fold X chromosome downregulation. How the DCC downregulates gene expression is not known. By analyzing the distribution of histone modifications in nuclei using quantitative fluorescence microscopy, we found that H4K16 acetylation (H4K16ac) is underrepresented and H4K20 monomethylation (H4K20me1) is enriched on hermaphrodite X chromosomes in a DCC-dependent manner. Depletion of H4K16ac also requires the conserved histone deacetylase SIR-2.1, while enrichment of H4K20me1 requires the activities of the histone methyltransferases SET-1 and SET-4. Our data suggest that the mechanism of dosage compensation in *C. elegans* involves redistribution of chromatin-modifying activities, leading to a depletion of H4K16ac and an enrichment of H4K20me1 on the X chromosomes. These results support conserved roles for histone H4 chromatin modification in worm dosage compensation analogous to those seen in flies, using similar elements and opposing strategies to achieve differential 2-fold changes in X-linked gene expression.

Higher eukaryotes require a balanced karyotype. Most aneuploidies are not tolerated, and those that are have detrimental phenotypic consequences. However, differences in the sex chromosome dose are well tolerated in many species due to dosage compensation (DC). Dosage compensation equalizes both sex chromosome-linked gene expression to autosomes and sex-linked gene expression between the sexes (56). In the fly *Drosophila melanogaster*, XY males upregulate the single X chromosome 2-fold, a mechanism which results in each sex having the functional equivalent of two X chromosomes and two sets of autosomes and therefore a balance of gene expression (19, 72). X chromosome upregulation is thought to occur in both sexes in mammals and worms (14, 24, 53), resulting in the need to prevent X-linked hyperexpression in females and hermaphrodites. To achieve that, in mammals, XX females inactivate one of the two X chromosomes (27, 41, 58), while in the worm *Caenorhabditis elegans*, XX hermaphrodites downregulate both X chromosomes 2-fold (11, 50).

Dosage compensation mechanisms in mammals and flies involve changes in chromatin. Mammalian X chromosome inactivation is initiated by the *Xist* noncoding RNA and is accomplished by spreading facultative heterochromatin over the entire inactive X (Xi) chromosome (27, 41, 58). The active X (Xa) and Xi chromosomes can be distinguished by unique sets of activating and repressive epigenetic marks, respectively. The Xi chromosome is depleted of H3K4me2/3 and acetylation of H3K9, H4K5, K8, K12 and K16, H3R17me2, and H3R26me but is enriched for H3K9me2/3, H3K27me3, H4R3me2, H4K20me1, H2AK119ub1, and macro-H2A relative to the active X chromosome and autosomes (27, 41, 58). To achieve upregulation of the X chromosome in flies, the male-specific lethal (MSL) complex loads across the single X chromosome in males, dependent on *msl-2* expression (36). The histone acetyltransferase activity of MOF (a subunit of the MSL complex) leads to hyperacetylation of histone H4 lysine 16, a chromatin mark widely associated with gene activation (1, 6, 69). Another histone mark, phosphorylation of histone H3 serine 10 by JIL-1 kinase, also contributes to fly dosage compensation (33, 42, 62). In worms, the dosage compensation complex (DCC) binds to both X chromosomes in hermaphrodites to downregulate

late gene expression. The DCC consists of condensin I^{DC}, which contains two SMC (structural maintenance of chromosomes) proteins (DPY-27 and MIX-1) and three CAP (chromosome-associated polypeptide) proteins (DPY-26, DPY-28, and CAPG-1) and a recruitment complex composed of SDC-1, SDC-2, SDC-3, and the associated proteins DPY-30 and DPY-21 (8, 9, 11, 29, 43, 50, 76, 78). The DCC is thought to load across X chromosomes in a two-step manner: binding to a group of high-affinity recruitment sites (rex) and spreading in a transcription-dependent, DNA sequence-independent manner to sites unable to recruit on their own (dox) (15, 32). Some rex sites are able to recruit only as extrachromosomal arrays and not as part of a duplication of a small region of X chromosomes (waystations) (5). Condensin I^{DC} is homologous to condensin, the highly conserved mitotic chromosome organization and segregation machinery, suggesting that dosage compensation in the worm is achieved by partial condensation of the X chromosomes. Whether this is accompanied by DCC-mediated changes in chromatin structure at the level of the nucleosome, analogous to those documented in mammals and flies, is not known.

Previous studies reported a decrease in HTZ-1 (histone H2A variant) occupancy (60, 77) and decreased levels of H3K4me3 on dosage-compensated X chromosomes (59). Other work has shown an increase in nucleosome occupancy at X-linked gene promoters that is sequence, and not DCC, dependent (16). Genome-wide mapping of chromatin marks by the modEncode project revealed a small decrease in activating marks and a small increase in repressive marks on the X, as well as a large increase in the repressive mark H4K20me1 (21, 45). Whether these chroma-

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tin changes are a result of DCC action in worms remains unknown.

In this report, we present evidence that the mechanism of dosage compensation in *C. elegans* involves genome-wide redistribution of chromatin marks, including depletion of H4K16ac and enrichment of H4K20me1, on the X chromosome compared to autosomes. These results suggest that regulation of H4K16ac is a conserved feature of the *Drosophila* and *C. elegans* dosage compensation mechanisms, both of which involve a 2-fold regulation of transcript levels from the dosage-compensated chromosome. In addition, H4K20me1 enrichment, indicative of transcriptional repression, is conserved between mammalian and *C. elegans* dosage compensation despite the differences in the degree of transcriptional repression during these processes.

MATERIALS AND METHODS

Strains. Animals were maintained on NG agar plates using standard methods. The following strains were used in this study: MT14911 *set-4(n4600)* II, SS1075 *set-1(tm1821)* III/ hT2g, TY0420 *dpy-27(y57)* III, TY1140 *sdsc-2(y46)* X, TY1724 *sdsc-3(y129)* V, TY1936 *dpy-30(y228)* V/nT1 [*unc-? (n754) let-?*] (IV; V), TY2386 wild type (WT) (N2), TY4381 *dpy-28(s939)* III/qC1 III, TY3936 *dpy-21(e428)*, TY4161 *sdsc-1(y415)* X, TY4341 *dpy-26(n199) unc-30(e191)* IV/nT1 [qls51] (III;IV), TY4403 *him-8(e1489)* IV; *xol-1(y9) sex-1(y263)* X, and VC1009 *sir-2.1(ok434)* IV.

Strains defective in dosage compensation. We analyzed worms homozygous for strong loss-of-function alleles descended from heterozygous mothers [*m+z-*, *dpy-26(n199)*, *dpy-28(s939)*, *dpy-30(y228)*]; worms homozygous for weaker loss-of-function alleles, in which the function of the gene was further depleted by RNA interference (RNAi) [*sdsc-2(y46)*, RNAi], *dpy-27(y57)*, RNAi]; worms homozygous for a weak loss-of-function allele in *sdsc-3(y129)*; and worms homozygous for strong loss-of-function alleles in the two exceptional dosage compensation genes which do not lead to hermaphrodite lethality [*dpy-21(e428)*, *sdsc-1(y415)*].

RNAi. RNA interference by feeding was performed with the Ahringer laboratory RNAi feeding library (34). Concentrated RNAi [*dpy-27(y57)* on *dpy-27* RNAi, WT on *set-1* RNAi] was performed as follows: 100 ml of LB was inoculated with the RNAi construct-containing bacteria from the Ahringer library in the presence of ampicillin and tetracycline and grown 16 h at 37°C. IPTG (isopropyl- β -D-thiogalactopyranoside) (20% [vol/wt]) was added at a dilution of 1:1,000, and incubation continued for 2 h at 37°C. The culture was spun down at 4,000 rpm for 10 min and resuspended in 700 μ l of LB. A 100- μ l volume was plated onto nematode growth medium (NGM) plates containing ampicillin and IPTG and used for RNAi beginning the following day. First-generation RNAi feeding [*sdsc-2(y46)* grown on *sdsc-2* RNAi] was performed as follows: L1-stage larvae were placed on plates seeded with RNAi bacteria and grown to adulthood. Third-generation RNAi feeding (WT on concentrated *set-1* RNAi) was performed as follows: P₀ adults from the process described above were transferred to fresh RNAi plates and allowed to lay eggs; L4-stage larvae from this F₁ generation were transferred to a third set of new RNAi plates and allowed to lay embryos, and these worms (the “F₂” generation) were grown to adulthood and examined. Second-generation RNAi feeding (all other analyses) was performed as follows: P₀ adults from the first-generation RNAi feeding were transferred to fresh RNAi plates and allowed to lay eggs, and these progeny (“F₁” generation) were grown to adulthood and examined. Multiple RNAi knockouts (see Fig. 6) were performed sequentially (using a method similar to that described in reference 65). P₀ adults grown from L1-stage larvae on plates spotted with RNAi against the first factor were moved to plates seeded with RNAi against the second factor and allowed to lay eggs. The progeny (“F₁” generation) were grown to adulthood and examined. RNAi procedures were conducted in the order shown by the data row labels.

Antibodies. Antibodies specific to DPY-27 and CAPG-1 were described previously (9). Commercial antibodies used for immunofluores-

cence (IF) analysis were as follows: H3K9ac (rabbit; Abcam ab4441) (1:100); H4K16ac (rabbit; Millipore 07-329) (1:500); H4K20me1 (rabbit; Abcam ab9051) (1:100); H4K20me2 (rabbit; Millipore 07-367) (1:100); H4K20me3 (rabbit; Abcam ab9053) (1:100); and H3K27me3 (rabbit; Millipore 07-449) (1:100). Secondary antibodies were purchased from Jackson ImmunoResearch. Antibody specificity was tested using the following peptides: H4K20me1 (Abcam ab17043) and H4K16ac (Millipore 12-346). The dimodified H4K16acK20me1 peptide with sequence KGGAK(ac)RH RK(me1)VLRDNIQ was synthesized by Biomatik.

Microscopy. Antibody staining of dissected adults, immunofluorescence *in situ* hybridization (immuno-FISH), and detergent extraction were performed as described previously (10, 60). Images were captured with a Hamamatsu Orca-Erga close-coupled-device (CCD) camera mounted on an Olympus BX61 motorized Z-drive microscope using a 60 $\times$ APO oil immersion objective. These images are projections of optical sections with a Z spacing of 0.2 μ m. Scale bars were added using ImageJ (available at <http://rsb.info.nih.gov/ij/>; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD) and a template image created in Slidebook.

Image analysis. Three-dimensional (3D) image stacks were collected for each nucleus analyzed at 0.2- μ m Z-spacing. Fluorescence intensity quantification for staining of histone modifications was completed in Slidebook by a method similar to those used previously by other groups in a variety of experimental systems (25, 52, 73). Images were collected by setting exposure times such that the fluorescence intensity for each channel fell within the dynamic range of detection (approximately 2/3 of the maximal intensity for the sample).

For each image, masks were set using the “mask $\rightarrow$ segment” function. The mask is established by a user-defined intensity threshold value applied over an image in order to distinguish real signal from background signal and autofluorescence. The same standard of background signal exclusion was applied to all nuclei from the same worm, based on the levels of background signal and autofluorescence observed. The average signal intensity within a mask, calculated in three dimensions and for each channel individually, was measured by the use of Slidebook, calculating the intensity value of each pixel within a masked volume and averaging all values within this mask. This was done for each channel of an image, and average histone modification staining intensity values were recorded for both the “X chromosome(s)” mask and the “histone modification (whole-nucleus)” mask. The whole-nucleus mask value was calculated from the histone modification signal rather than from the DAPI (4',6'-diamidino-2-phenylindole) signal. For each histone modification, the ratio of “average X chromosome(s) histone modification intensity” to “whole nucleus histone modification intensity” was then calculated for each nucleus within an experimental set. This ratio was then averaged over all nuclei within an experimental set to calculate the final “X chromosome: nucleus” mean histone modification intensity values shown on each graph. Descriptive statistics (standard deviation and sample size) were also calculated. Sample sizes are listed in each figure. Values shown represent means $\pm$ 1 standard deviation of the mean. Fluorescence intensity differences were evaluated by Student's *t* test using the MINITAB 12 Student release.

High-resolution ChIP-chip metagene analysis. Chromatin and DCC chromatin immunoprecipitation with microarray technology (ChIP-chip) data sets (H4K16ac EE, DCC identification no. 3182; H4K20me1 EE, DCC identification no. 2765; H4K20me1 L3, DCC identification no. 2784; DPY-27 EE, DCC identification no. 3435; DPY-27 L4, DCC identification no. 630) were downloaded from modMine 25 (modEncode; <http://intermine.modencode.org/>). EE refers to early-biased embryo samples composed of roughly 50% dosage-compensated and 50% non-dosage-compensated tissue (61). The unzipped raw data and annotation files were then uploaded to the Cistrome/Galaxy server (44), referenced to the ce4 (WS170) genome, for analysis. First, we ran each experiment using MA2C (71) and default settings to call peak regions of signal. Then, the MA2C output files were used in CEAS (66) analysis. We ran CEAS using default

settings but with the addition of four gene lists to the analysis (DC genes, non-DC genes, autosomes, and X chromosome). The “All” gene list includes both the expressed and nonexpressed genes included in WS170. The DC and non-DC gene lists were constructed from the lists of 373 dosage-compensated and 290 non-dosage-compensated genes defined previously (32). The autosomal and X chromosome gene lists were compiled from a WS226 genome download from WormMart (<http://caprica.caltech.edu:9002/biomart/martview/>). Cistrome recognizes all 17,143 genes on autosomes and all 2,778 genes on X chromosomes in the WS170 genome build from these autosome and X chromosome gene lists. The “average gene profiles” were extracted from each CEAS output report. The *x* axis (shown in base pairs) marks the 3-kb-scaled metagene body and 1 kb upstream and downstream of the transcript start and stop sites. The *y* axis represents the average normalized signal from two replicate ChIP-chip experiments (4 replicates for DPY-27 L4 data).

Western blot analysis. Embryos were collected from mutant or RNAi-treated gravid adults. Lysates were prepared by sonicating embryos for 10 15-s bursts in homogenization buffer (50 mM HEPES [pH 7.6], 1 mM EDTA, 140 mM KCl, 0.5% NP-40, 10% glycerol, and protease inhibitor cocktail [Calbiochem]). Cellular debris was pelleted by centrifugation at $5,000 \times g$ at 4°C for 15 min. Equal amounts (7.5 μ g) of each sample were loaded into 15% acrylamide gels for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to nitrocellulose and blotted with the following antibodies: H4K20me1 (Abcam ab9051) at 1:10,000 or H4K20me3 (Abcam ab9053) at 1:1,000 and α -tubulin (Sigma T6199) at 1:1,000.

RESULTS

Dosage compensation-dependent changes in chromatin. To look for evidence for the involvement of histone modifications in rebalancing chromosome dosage effects in *C. elegans*, we used IF to assay levels of various histone modifications. To mark the X chromosome territory, we used either IF with antibodies to DCC components (CAPG-1 or DPY-27) or fluorescence *in situ* hybridization with X chromosome probes (Xpaint). We chose this method because IF offers a rapid method to test many antibodies or modifications with relative ease at single-cell resolution (so that we could focus on the dosage-compensated soma). Microscopy can also be used on the limited amount of starting material obtained from mutants where ChIP analysis would not be possible. We analyzed intestinal nuclei, which are 32-ploid, facilitating easier visualization and quantification of antibody staining. We quantified the ratio of average histone modification staining on X chromosomes versus the entire nucleus using intensity-based masks (see Materials and Methods), which is similar to methods used previously in other systems (25, 52, 73). Because dosage compensation in *C. elegans* involves a downregulation of gene expression on both hermaphrodite X chromosomes, we expected some activating histone modifications to be underrepresented or some repressive modifications to be enriched or a combination of the two.

We observed significant depletion of H4K16ac and confirmed enrichment of H4K20me1 on hermaphrodite X chromosomes (Fig. 1) (21, 45). H4K5ac, H4K8ac, H4K12ac, H3K9ac, and H3K27me3 staining on the hermaphrodite X chromosomes showed no statistically significant differences (Fig. 1 and data not shown). We observed similar levels of depletion of H4K16ac and enrichment of H4K20me1 whether we used DCC antibodies or Xpaint FISH to mark the X chromosome (Fig. 1A and B), indicating that the harsh fixation conditions involved in FISH did not affect our quantification. Since the H4K16ac and H4K20me1 modifications lie very close to each other on the histone tail, we

FIG 1 Histone modification differences on hermaphrodite X chromosomes. (Left) Representative IF images of intestinal nuclei from WT hermaphrodites or males stained with antibodies against H3K9ac, H4K16ac, or H4K20me1 (green) and DCC subunit CAPG-1 (red) (A) or probed with Xpaint fluorescent *in situ* hybridization probes (FISH; red) (B and C) to mark the X chromosomes. DNA (DAPI) is shown in blue. Scale bars = 5 μ m. (Right) Fluorescence intensity quantification for panels A to C. Average green intensity values were determined for both the green and red channels. The values for the ratio of histone modification staining in the X chromosome channel to histone modification staining in the histone modification channel were determined and averaged over many nuclei. Mean values $\pm$ 1 standard deviation and sample size (n) are indicated. Asterisks indicate results statistically significant at $P < 0.001$ compared to the results for males. H4K16ac was reduced by about 25% and H4K20me1 was enriched by 40% on the WT hermaphrodite X chromosomes compared to whole-nucleus average.

performed peptide blocking IF experiments to ensure that binding to one modification was not inhibited by the presence of the other (data not shown). Signal from the H4K16ac antibody was blocked by both a K16ac peptide and a peptide modified on both residues, but not by a K20me1 peptide, indicating that the antibody is able to bind a K16-acetylated histone tail whether or not K20 is monomethylated. Similarly, signal from the H4K20me1 antibody was blocked by both a K20me1 peptide and a dimodified peptide but not a K16ac peptide (data not shown).

We next asked whether the observed depletion of H4K16ac on hermaphrodite X chromosomes is dependent on the hermaphrodite-specific activity of the DCC by analyzing wild-type (WT) male worms and hermaphrodites carrying mutations in DCC subunits (see Materials and Methods). In both males (Fig. 1C) and DCC mutant hermaphrodites (Fig. 2), H4K16ac was no longer depleted on the X chromosome. In fact, it was enriched on the X chromosomes of DCC mutant hermaphrodites. Similar results were obtained when we used IF to mark the X chromosomes in *sdc-1* or *dpy-21* animals (DCC function is compromised in these strains, but the DCC still loads onto the X chromosomes) (Fig. 2A), as well as when we used Xpaint FISH for all mutants analyzed

FIG 2 H4K16ac depletion is lost in dosage compensation mutants. (A and B) (Left) Representative images of intestinal nuclei from WT hermaphrodite and dosage compensation mutant worms stained with antibodies against H4K16ac (green). The X chromosomes (red) are marked with antibodies against either CAPG-1 (A) or Xpaint FISH (B). DNA (DAPI) is shown in blue. Scale bar = 5 μ m. (Right) Fluorescence intensity quantification shows enrichment of H4K16ac on the X chromosome in all DCC mutants tested. (C) Fluorescence intensity quantification for additional DC mutant strains analyzed by H4K16ac IF and Xpaint FISH; images not shown. Asterisks in all panels indicate results statistically significant at $P < 0.001$ compared to the results for control WT hermaphrodites. *dpy-21(e428)* shows the greatest degree of enrichment.

(Fig. 2B and C). H4K16ac levels are enriched on the X chromosomes in dosage compensation mutant hermaphrodites, but not on the X chromosomes in wild-type males (Fig. 1C), suggesting the existence of additional sex-specific differences in X chromatin regulatory mechanisms beyond DCC function.

Mutations in DCC subunits either reduced or eliminated H4K20me1 enrichment (Fig. 3). Interestingly, in dosage compensation mutants, H4K20me1 signal intensity appeared to increase on autosomes. This is consistent with the idea that in these mutants, X chromosome expression increases while autosomal expression decreases (32). These data indicate that DCC function is necessary for both reduction of H4K16ac and enrichment of H4K20me1 on hermaphrodite X chromosomes.

To gain further insight into how DCC activity may relate to these chromatin changes, we also made use of publicly available ChIP-chip data sets produced by the modEncode consortium (<http://www.modencode.org>) (21, 45) for high-resolution analysis of H4K16ac, H4K20me1, and DPY-27 occupancy across the genome. Using Cistrome (44), a web-based platform for high-throughput data analysis, we constructed metagene profiles (Fig. 4) for each feature at two time points, early-biased mixed embryonic stages and larval stage L3 or L4. No larval data set is currently available for H4K16ac. We compared average ChIP signal profiles of all 2,778 X-linked genes and 17,143 autosomal genes identified

FIG 3 H4K20me1 enrichment is lost in dosage compensation mutants. (Left) Representative images of intestinal nuclei from WT hermaphrodite and dosage compensation mutant worms stained with antibodies against H4K20me1 (green); the X chromosomes (red) are marked by anti-CAPG-1 (A) or Xpaint FISH (B). DNA (DAPI) is shown in blue. Scale bar = 5 μ m. Asterisks indicate results statistically significant at $P < 0.001$ compared to the results for control WT hermaphrodites (one asterisk) or for both WT and *sdca-1(y415)* hermaphrodites (two asterisks). (Right) Fluorescence intensity quantification shows that the enrichment of H4K20me1 on the X chromosome is reduced in DCC mutants, except in *dpy-21(e428)*, where no enrichment is seen.

FIG 4 High-resolution analysis of chromatin regulation during development. Metagenome profiles illustrate the results of H4K16ac, H4K20me1, and DPY-27 ChIP-chip analysis performed using data sets from <http://modencode.org/> or the NCBI GEO database and either WT early-biased (EE) embryos (left column) or stage L3 or L4 WT larval tissue (right column). The x axis represents a 3-kb-scaled gene body with 1 kb upstream and downstream of the transcribed region. The y axis shows the average ChIP-chip profile values. Data corresponding to “All,” “X chromosomes,” and “Autosomes” include results from both expressed and nonexpressed genes from genome build ce4 (WS170), while data corresponding to “DC genes” and “non-DC genes” include only results from expressed X-linked genes. A comparison of the X chromosome data to the autosome data confirms our IF results concerning H4K16ac and H4K20me1. Further, these results suggest that H4K16ac occupancy, but not H4K20me1 or DPY-27 occupancy, may be a predictor of dosage compensation status on X chromosomes.

in genome build ce4 (WS170). We also compared profiles of X-linked genes subjected to dosage compensation (DC genes) and X-linked genes whose expression is not influenced by the DCC (non-DC genes). DC genes are defined as the 365 genes whose expression is elevated at least 1.5-fold in dosage compensation mutant embryos, and non-DC genes are defined as the 287 genes whose expression does not change significantly in these samples (32). The ChIP-chip data confirm that H4K16ac is underrepresented and H4K20me1 and DPY-27 are enriched on X-linked genes compared to autosomes (Fig. 4). Unexpectedly, there was not a substantial difference between the distributions of DPY-27 and H4K20me1 on DC versus non-DC genes (Fig. 4). This is consistent with previous observations that DPY-27 occupancy is not predictive of dosage compensation status (32). However, H4K16ac levels are considerably higher on non-DC genes than DC genes (Fig. 4, row 1), both at the promoter and throughout the gene body, suggesting that lower levels of this mark may be a distinguishing feature of dosage compensation.

SIR-2.1, SET-1, and SET-4 mediate changes on the dosage-compensated X chromosomes. We next set out to determine

which histone-modifying activity is responsible for the reduction of H4K16ac levels on the X chromosomes. Budding yeast Sir2 and its homologs in other organisms are conserved H4K16 deacetylases (30). Depletion or mutation of *sir-2.1*, a *C. elegans* Sir2 homolog (75), led to the loss of the H4K16ac depletion seen in control vector RNA interference (RNAi)-treated worms (Fig. 5). Depletion of any of the other histone deacetylases (HDACs) (*sir-2.2*, *sir-2.3*, *sir-2.4*, *hda-1* [RPD3 homolog], *hda-2*, *hda-3*, *hda-4*, *hda-5*, *hda-6*, *hda-10*, or *hda-11*) did not result in a similar increase in H4K16ac levels on the X chromosomes. We conclude that SIR-2.1 is responsible, at least in part, for the reduction in H4K16ac observed on dosage-compensated X chromosomes. However, we cannot rule out the possibility that additional factors, including decreased histone acetyltransferase activity on X chromosomes, also contribute to the depletion of H4K16ac.

Next, we wanted to determine which histone methyltransferase is responsible for the enrichment of H4K20me1 on the X chromosomes. We tested two candidates: SET-1, the *C. elegans* gene most closely related to PR-SET-7, the enzyme which monomethylates H4K20 (17); and SET-4, the gene annotated as

FIG 5 The activity of SIR-2, SET-1, and SET-4 is needed for the depletion of H4K16ac on the X chromosomes. (Left) Representative immunofluorescence images of intestinal nuclei from worms treated with control vector, *sir-2.1*, or *set-1* RNAi as well as from WT, *sir-2.1(ok434)*, and *set-4(n4600)* worms stained with antibodies against CAPG-1 (red) and H4K16ac (green). DNA (DAPI) is shown in blue. Scale bars = 5 μ m. (Right) Fluorescence intensity quantification. Asterisks indicate results statistically significant at $P < 0.001$ compared to the results for control WT or vector RNAi-treated hermaphrodites (one asterisk) or both WT and *sir-2.1(ok434)* hermaphrodites (two asterisks). *sir-2.1* and *set-1* RNAi and/or mutation abolishes the partial H4K16ac depletion, while *set-4* mutation may lead to enrichment of H4K16ac on the X chromosomes similar to that seen with most DCC mutants.

the *C. elegans* homolog of SUV4-20, the methyltransferase which mediates di- and trimethylation of H4K20 (Wormbase [http://www.wormbase.org], release WS222). By IF and Western blot analysis, H4K20me1 levels were eliminated in *set-1* mutants or after *set-1* RNAi (Fig. 6), consistent with SET-1 depositing this mark. In *set-4(n4600)* mutants (2), or after *set-4* RNAi, overall H4K20me1 levels increased, and the signal was evenly distributed across the nucleus (Fig. 6), suggesting that SET-4 may antagonize H4K20 monomethylation on autosomes. This finding is consistent with the observed increase in H4K20me1 levels in flies carrying a mutation in the *set-4* homolog *Suv4-20* (64). Western blot analysis also revealed that SET-4 activity is needed for wild-type levels of H4K20me3 (Fig. 6B). IF experiments using antibodies specific to H4K20me2 and H4K20me3 produced only low-level nuclear staining that was not dependent on the presence of SET-4 (data not shown), possibly due to cross-reactivity with another protein. This finding prevented us from assessing the spatial distribution of H4K20me3 in the nucleus. The X chromosome enrichment of H4K20me1 was reduced or eliminated in both *set-1* or *set-4* mutant and depleted worms, indicating that X chromosome enrichment of this mark requires the activities of both SET-1 and SET-4.

Previous studies suggested that H4K20me1 antagonizes H4K16ac (54). Consistent with this model, knockdown of *set-1* or mutation of *set-4* abrogated both the enrichment of H4K20me1 and the H4K16ac reduction on the X chromosomes (Fig. 5 and 6A). Conversely, a mutation in *sir-2.1* affected only depletion of H4K16ac and did not alter H4K20me1 enrichment on the X chromosomes (Fig. 5 and 6), suggesting that SIR-2.1 may act downstream of SET-1 and SET-4 (see Discussion).

Genetic requirements for dosage compensation. We further asked whether *set-1*, *set-4*, and *sir-2.1* are genetically required for dosage compensation. In the *him-8(e1489); xol-1(y9) sex-1(y263)* strain, males die due to inappropriate activation of the DCC but

FIG 6 Enrichment of H4K20me1 on dosage-compensated X chromosomes requires the function of SET-1 and SET-4 but not SIR-2.1. (A) (Left) Representative immunofluorescence images of intestinal nuclei from worms treated with control vector, *sir-2.1*, or *set-1* RNAi, as well as from WT, *sir-2.1(ok434)*, and *set-4(n4600)* worms stained with antibodies against CAPG-1 (red) and H4K20me1 (green). DNA (DAPI) is shown in blue. Scale bars = 5 μ m. (Right) Fluorescence intensity quantification. Asterisks indicate results statistically significant at $P < 0.001$ compared to the results from control WT or vector RNAi controls. *set-1* and *set-4* RNAi and/or mutation abolishes the enrichment of H4K20me1 on the X chromosomes, but a mutation in *sir-2.1* does not. (B) Western blot analysis of H4K20me1 (left) and H4K20me3 (right) levels. Embryonic extracts derived from vector, *set-1*, *set-4*, or *set-8* (a methyltransferase not involved in H4K20 methylation) RNAi-treated WT worms or *set-4(n4600)* or *sir-2.1(ok434)* strains probed with anti-H4K20me1, anti-H4K20me3, and antitubulin antibodies are shown. Whereas *set-4* RNAi and mutation increase H4K20me1 levels compared to vector controls, *set-1* RNAi abrogated H4K20me1 and *sir-2.1* mutation did not significantly affect H4K20me1. Tubulin is shown as a loading control. H4K20me3 levels diminish after *set-1* RNAi and are undetectable after *set-4* RNAi or in *set-4(n4600)* mutants. Note that the H4K20me3-specific antibody cross-reacts with a protein of higher molecular mass (marked by an asterisk).

can be rescued by RNAi depletion of factors necessary for dosage compensation (60). Significant male rescue was observed after knockdown of the DCC subunit *dpy-27* or consecutive depletion of *set-4* and *set-1* (Fig. 7A). Depletion of *set-4* alone led to less but still significant rescue (Fig. 7A), indicating a genetic requirement for these factors in dosage compensation. However, SIR-2.1 rescued males at levels similar to vector RNAi (0.78%; Fig. 7A), indicating that SIR-2.1 depletion is not sufficient to disrupt dosage compensation. Since these chromatin factors affect histone modifications globally and are not specific to dosage compensation, low levels of male rescue should not be interpreted as representing a lack of a dosage compensation role. *set-1* RNAi singly caused 95% lethality among progeny; however, male rescue of 3.6% was seen using a shortened RNAi treatment beginning with stage L4 worms (data not shown).

FIG 7 Chromatin regulators function in dosage compensation. (A) Male rescue assay for dosage compensation function. Knockdown of the DCC component *dpy-27* rescued 54% of the expected number of male progeny. Sequential *set-4* and *set-1* RNAi rescued about 21% of the expected number of males, and *set-4* RNAi singly rescued 4.5%. Sample sizes are listed at the bottom of each bar in the graph and represent a combination of the total numbers of embryos laid in three independent trials conducted simultaneously. (B) Proposed model of chromatin regulation of transcription during *C. elegans* dosage compensation. A graphical cartoon illustrates DCC, SET-1, SET-4, and SIR-2.1 effects on X-linked transcription in hermaphrodites. DCC function, through SET-1 and SET-4, leads to an enrichment of H4K20me1 on the X chromosome, which, in turn, through SIR-2.1 function, reduces H4K16ac and creates an environment repressive to transcription.

DISCUSSION

In this study, we searched for evidence of X chromosome histone modification differences in *C. elegans*. We established that H4K16ac levels are reduced, but H4K20me1 levels are enriched, on the dosage-compensated X chromosomes in *C. elegans* hermaphrodites (Fig. 1). These changes depend both on DCC function (Fig. 2 and 3) and the function of the chromatin modifiers SET-1, SET-4, and SIR-2.1 (Fig. 5 and 6).

DCC-mediated chromatin changes. Sex chromosome dosage compensation in worms is thought to involve two mechanisms. First, X chromosome expression is upregulated in both sexes compared to that of autosomes by an unknown mechanism (14, 24), and the upregulation is followed by hermaphrodite-specific downregulation of both X chromosomes (11, 50). Our data indicate that in the absence of DCC activity, H4K16ac is enriched on hermaphrodite X chromosomes, suggesting that this mark may be involved in the X chromosome upregulation process. However, our data in males show no X chromosome enrichment of H4K16ac, indicating that this mark is not responsible for general X chromosome upregulation. These observations suggest that other sex-specific, but not DCC-mediated, mechanisms may regulate the X:A gene dosage balance in worms. These additional processes may involve feedback mechanisms between sex determination- and chromosome dosage-regulatory mechanisms as reported previously (13, 23, 26, 28). Indeed, males carrying mutations in DCC genes have X chromosomes similar to those of wild-type males (not enriched for H4K16ac), while karyotypically male (XO) animals transformed into hermaphrodites by a genetic mutation and carrying mutations in DCC genes [*dpy-28(s939)*; *xol-1(y9)* and *her-1(e1520)*; *sdcc-3(y126)* *xol-1(y9)*] had X chromosomes enriched for H4K16ac, similar to DCC mutant hermaphrodites (data not shown).

A mechanistically better-understood aspect of the regulation of chromosome dosage effects is the 2-fold downregulation of the

X chromosomes in hermaphrodites. Extensive genetic studies have demonstrated that the DCC is essential for this process (8, 9, 29, 43, 50, 76, 78). Mutations in genes encoding DCC subunits lead to an increase in mRNA levels from the X chromosomes (32, 51). How the DCC regulates transcription is not known, but our results show that the mechanism of transcriptional downregulation by the DCC likely involves the decreased levels of H4K16ac and increased levels of H4K20me1 observed on X chromosomes. Based on our data, we propose the following model (Fig. 7B). First, DCC activity via the function of SET-1 and SET-4 leads to an enrichment of H4K20me1 on the X chromosome. How the DCC influences SET-1 and SET-4 function is unclear. One possibility is that DCC activity leads to an enrichment of the SET-1 protein on X chromosomes, leading to increased levels of H4K20me1. Alternatively, or in addition, DCC activity may lead (directly or indirectly) to enrichment of SET-4 on autosomes, causing reduced levels of H4K20me1 on autosomes. SET-1 and SET-4 may act in the same pathway and/or in parallel pathways to regulate H4K20me1 levels. Second, DCC activity, via the function of SIR-2.1, leads to a depletion of H4K16ac levels on X chromosomes. Our results suggest that H4K20me1 regulation is upstream of H4K16ac regulation (Fig. 5 and 6), indicating that H4K20me1 may antagonize H4K16ac. Previous work suggested that H4K20me1 and H4K16ac are mutually antagonistic (54), but other studies have found that this may not always be the case (55, 74). Our data suggest a similar dichotomy. In hermaphrodites, H4K20me1 antagonizes H4K16ac on X chromosomes, because we see loss of H4K16ac depletion when H4K20me1 is no longer X chromosome enriched (Fig. 5). In males (Fig. 1) and in *set-4* mutant hermaphrodites (Fig. 6), however, both marks coexist across the entire nucleus, suggesting that H4K16ac does not antagonize H4K20me1 in our system, at least at the level of whole chromosomes. Furthermore, H4K16ac is distributed uniformly in the nucleus both in the absence (*set-1* mutants) and the presence (*set-4*

mutants) of uniformly high levels of H4K20me1, indicating a more complex relationship. The possibility remains that additional parallel chromatin pathways regulate H4K16ac and H4K20me1, perhaps through regulation of H4K16 acetyltransferase(s).

It is worth noting that loss of DPY-21 had the greatest effect on both H4K16ac and H4K20me1 levels on hermaphrodite X chromosomes (Fig. 2 and 3A). To date, the mechanistic contribution of DPY-21 to dosage compensation in *C. elegans* has not been well characterized. Previous studies indicated that DPY-21 is enriched on X chromosomes in a DCC-dependent manner and regulates gene expression both inside and outside the context of the DCC (48, 49, 78). However, severe loss-of-function mutations in *dpy-21* do not lead to hermaphrodite-specific lethality, while mutations in most other DCC components do (48, 78). Taken together, these observations suggest that other mechanisms beyond modulation of H4K20me1 and H4K16ac levels contribute to *C. elegans* dosage compensation. It should be interesting to determine whether these or other histone modifications contribute to gene expression changes on the X chromosomes.

Our results indicate that modulation of H4K16ac is a conserved feature of fly and worm dosage compensation. Enrichment of H4K16ac on the X chromosome in male flies leads to increased transcriptional output (1, 6, 69, 79), while depletion of H4K16ac on the X chromosomes in hermaphrodite worms leads to a chromatin environment repressive to transcription. H4K16ac can inhibit formation of the 30-nm fiber without recruiting accessory chromatin proteins (67), and perhaps this feature makes it well suited for 2-fold modulation of gene expression. Both the fly and the worm dosage compensation chromatin regulation mechanisms appear largely different from the chromatin profile associated with X chromosome inactivation in mammals. While fly and worm dosage compensation leads to a 2-fold adjustment in gene expression levels, X chromosome inactivation leads to complete silencing of many genes on the affected chromosome(s). The inactive mammalian X chromosome is enriched for many repressive histone marks and is depleted of many activating histone marks (27). In contrast, activating chromatin marks are still present on each of the two dosage-compensated X chromosomes in worms, and the repressive H3K27me3 modification is not enriched on the dosage-compensated X chromosomes (data not shown). This work demonstrates that a mechanism similar to but opposite of that observed in the fly for transcriptional control during dosage compensation is at work in *C. elegans* and that modulation of the histone H4 chromatin state is uniquely well suited to schemes of 2-fold gene regulation.

Transcriptional regulation. Changes in chromatin structure may affect RNA production, including different stages of transcription as well as co- and posttranscriptional processing, to achieve dosage compensation in several ways. During mammalian X chromosome inactivation, RNA polymerase II (Pol II) is almost completely excluded from the inactive X chromosome territory early in the X chromosome inactivation process, leading to transcriptional silencing (7, 57). In *Drosophila*, upregulation of the male X chromosomes is thought to be achieved by increased transcriptional elongation facilitated by H4K16ac enriched in gene bodies (22, 31, 37, 39, 40, 68, 70). The most compelling evidence for increased elongation comes from data from a recent study gathered using global run-on sequencing to map active transcription across the *Drosophila* genome (39). The results support a

model in which the MSL complex facilitates transcriptional elongation through gene bodies.

Studies in other systems have revealed a link between H4K16ac and regulation of transcriptional elongation by RNA polymerase II. H4K16ac, together with H3S10ph, creates a binding site for the bromodomain protein BRD4, which in turn leads to recruitment of P-TEFb and productive elongation (31, 79). Consistent with this, SIR silencing at the yeast mating type loci has been linked to H4K16 deacetylation and Pol II stalling (18, 63). It is tempting to speculate that regulation of transcription elongation may be involved in *C. elegans* dosage compensation as well. H4K20me1 has been previously correlated with transcriptional repression in other contexts (3, 35, 38).

Comparison of high-resolution chromatin profiles of X-linked and autosomal genes (Fig. 4) (45) provided additional insight into potential dosage compensation mechanisms. H4K16ac levels on X-linked genes peak near the transcription start site (TSS) and are very low around the transcription termination site (TTS). In contrast, autosomal genes have higher H4K16ac levels across the TSS and much higher H4K16ac levels near the TTS (45), consistent with increased elongation on autosomes compared to the X chromosome. However, the greatest H4K16ac signal is near the TSS, which is different from the gene body enrichment that has been observed in flies (20, 37). In addition, the greatest difference in H4K16ac between DC and non-DC genes is in the promoter region, suggesting that regulation of transcription initiation is another possibility.

It is important to acknowledge the strengths and weaknesses of the assays we employed in this study. The often low standard deviations seen in our fluorescence intensity quantification suggest that the technique and our raw data are quite reproducible, and the large changes in the X chromosome/nucleus signal ratio (0.76 to 1.29, comparing H4K16ac in the WT and the *dpy-21* mutants; Fig. 2A) indicate the power of this assay to detect differences. However, due to the nature of the technique, this quantification method is best suited to making relative comparisons rather than absolute quantification of protein or modification occupancy.

Condensin and chromatin regulation. The exact mechanism by which DCC function leads to the observed chromatin changes remains unknown. We did not observe a physical interaction between the DCC subunits and SIR-2.1, SET-1, or SET-4 by proteomics approaches (9). However, the interaction may be weak and need not be direct. DPY-21 interacts only weakly with other members of the DCC (78) but may represent a link between SET-1, SET-4, SIR-2.1, and the DCC. It is also possible that other linker proteins are involved or that DCC action influences SET-1, SET-4, and SIR-2.1 localization or activity indirectly by modulating the higher-order structure of the X chromosomes. Condensin is thought to influence higher-order chromosome architecture, and it is possible that condensin-mediated changes in the folding path of the chromatin fiber affect the binding or the activity of other chromosomal proteins. It is worth noting that condensin II recognizes H4K20me1 for chromosomal binding during mitosis in HeLa cells (46), suggesting a connection between condensin and this chromatin modification.

Condensin regulation of Sir2 and transcription has been previously documented in other systems. Condensin regulates Sir2 localization and Sir2-mediated ribosomal DNA (rDNA) silencing in budding yeast (47). Beyond worms, condensin has been implicated in transcriptional regulation in budding yeast and *Drosophila*.

ila (4, 12). Therefore, our studies of *C. elegans* condensin I^{DC} function may shed further light on the mechanism of gene repression by condensin in other organisms as well.

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REFERENCES

- Akhtar A, Becker PB. 2000. Activation of transcription through histone H4 acetylation by MOF, an acetyltransferase essential for dosage compensation in *Drosophila*. *Mol. Cell* 5:367–375.
- Andersen EC, Horvitz HR. 2007. Two *C. elegans* histone methyltransferases repress lin-3 EGF transcription to inhibit vulval development. *Development* 134:2991–2999.
- Balakrishnan L, Milavetz B. 2010. Decoding the histone H4 lysine 20 methylation mark. *Crit. Rev. Biochem. Mol. Biol.* 45:440–452.
- Bhalla N, Biggins S, Murray AW. 2002. Mutation of YCS4, a budding yeast condensin subunit, affects mitotic and nonmitotic chromosome behavior. *Mol. Biol. Cell* 13:632–645.
- Blauwkamp TA, Csankovszki G. 2009. Two classes of dosage compensation complex binding elements along *Caenorhabditis elegans* X chromosomes. *Mol. Cell. Biol.* 29:2023–2031.
- Bone JR, et al. 1994. Acetylated histone H4 on the male X chromosome is associated with dosage compensation in *Drosophila*. *Genes Dev.* 8:96–104.
- Chaumeil J, Le Baccon P, Wutz A, Heard E. 2006. A novel role for Xist RNA in the formation of a repressive nuclear compartment into which genes are recruited when silenced. *Genes Dev.* 20:2223–2237.
- Chuang PT, Albertson DG, Meyer BJ. 1994. Dpy-27—a chromosome condensation protein homolog that regulates *C. elegans* dosage compensation through association with the X-chromosome. *Cell* 79:459–474.
- Csankovszki G, et al. 2009. Three distinct condensin complexes control *C. elegans* chromosome dynamics. *Curr. Biol.* 19:9–19.
- Csankovszki G, McDonel P, Meyer BJ. 2004. Recruitment and spreading of the *C. elegans* dosage compensation complex along X chromosomes. *Science* 303:1182–1185.
- Csankovszki G, Petty EL, Collette KS. 2009. The worm solution: a chromosome-full of condensin helps gene expression go down. *Chromosome Res.* 17:621–635.
- Dej KJ, Ahn C, Orr-Weaver TL. 2004. Mutations in the *Drosophila* condensin subunit dCAP-G: defining the role of condensin for chromosome condensation in mitosis and gene expression in interphase. *Genetics* 168:895–906.
- DeLong L, Plenefisch JD, Klein RD, Meyer BJ. 1993. Feedback control of sex determination by dosage compensation revealed through *Caenorhabditis elegans* Sdc-3 mutations. *Genetics* 133:875–896.
- Deng X, et al. 2011. Evidence for compensatory upregulation of expressed X-linked genes in mammals, *Caenorhabditis elegans* and *Drosophila melanogaster*. *Nat. Genet.* 43:1179–1185.
- Ercan S, Dick LL, Lieb JD. 2009. The *C. elegans* dosage compensation complex propagates dynamically and independently of X chromosome sequence. *Curr. Biol.* 19:1777–1787.
- Ercan S, Lubling Y, Segal E, Lieb JD. 2011. High nucleosome occupancy is encoded at X-linked gene promoters in *C. elegans*. *Genome Res.* 21:237–244.
- Fang J, et al. 2002. Purification and functional characterization of SET8, a nucleosomal histone H4-lysine 20-specific methyltransferase. *Curr. Biol.* 12:1086–1099.
- Gao L, Gross DS. 2008. Sir2 silences gene transcription by targeting the transition between RNA polymerase II initiation and elongation. *Mol. Cell. Biol.* 28:3979–3994.
- Gelbart ME, Kuroda MI. 2009. *Drosophila* dosage compensation: a complex voyage to the X chromosome. *Development* 136:1399–1410.
- Gelbart ME, Larschan E, Peng S, Park PJ, Kuroda MI. 2009. *Drosophila* MSL complex globally acetylates H4K16 on the male X chromosome for dosage compensation. *Nat. Struct. Mol. Biol.* 16:825–832.
- Gerstein MB, et al. 2010. Integrative analysis of the *Caenorhabditis elegans* genome by the modENCODE Project. *Science* 330:1775–1787.
- Greenberg AJ, Yanowitz JL, Schedl P. 2004. The *Drosophila* GAGA factor is required for dosage compensation in males and for the formation of the male-specific-lethal complex chromatin entry site at 12DE. *Genetics* 166:279–289.
- Grote P, Conradt B. 2006. The PLZF-like protein TRA-4 cooperates with the Gli-like transcription factor TRA-1 to promote female development in *C. elegans*. *Dev. Cell* 11:561–573.
- Gupta V, et al. 2006. Global analysis of X-chromosome dosage compensation. *J. Biol.* 5:3.
- Han Z, Saam JR, Adams HP, Mango SE, Schumacher JM. 2003. The *C. elegans* Toslud-like kinase (TLK-1) has an essential role in transcription. *Curr. Biol.* 13:1921–1929.
- Hargitai B, et al. 2009. *xol-1*, the master sex-switch gene in *C. elegans*, is a transcriptional target of the terminal sex-determining factor TRA-1. *Development* 136:3881–3887.
- Heard E, Distche CM. 2006. Dosage compensation in mammals: fine-tuning the expression of the X chromosome. *Genes Dev.* 20:1848–1867.
- Hodgkin J. 2002. Exploring the envelope: systematic alteration in the sex-determination system of the nematode *Caenorhabditis elegans*. *Genetics* 162:767–780.
- Hsu DR, Meyer BJ. 1994. The Dpy-30 gene encodes an essential component of the *Caenorhabditis elegans* dosage compensation machinery. *Genetics* 137:999–1018.
- Imai S, Armstrong CM, Kaeberlein M, Guarente L. 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403:795–800.
- Ivaldi MS, Karam CS, Corces VG. 2007. Phosphorylation of histone H3 at Ser10 facilitates RNA polymerase II release from promoter-proximal pausing in *Drosophila*. *Genes Dev.* 21:2818–2831.
- Jans J, et al. 2009. A condensin-like dosage compensation complex acts at a distance to control expression throughout the genome. *Genes Dev.* 23:602–618.
- Jin Y, Wang Y, Johansen J, Johansen KM. 2000. JIL-1, a chromosomal kinase implicated in regulation of chromatin structure, associates with the male specific lethal (MSL) dosage compensation complex. *J. Cell Biol.* 149:1005–1010.
- Kamath RS, Ahringer J. 2003. Genome-wide RNAi screening in *Caenorhabditis elegans*. *Methods* 30:313–321.
- Karachentsev D, Sarma K, Reinberg D, Steward R. 2005. PR-Set7-dependent methylation of histone H4 Lys 20 functions in repression of gene expression and is essential for mitosis. *Genes Dev.* 19:431–435.
- Kelley RL, et al. 1995. Expression of *msl-2* causes assembly of dosage compensation regulators on the X chromosomes and female lethality in *Drosophila*. *Cell* 81:867–877.
- Kind J, et al. 2008. Genome-wide analysis reveals MOF as a key regulator of dosage compensation and gene expression in *Drosophila*. *Cell* 133:813–828.
- Kohlmaier A, et al. 2004. A chromosomal memory triggered by Xist regulates histone methylation in X inactivation. *PLoS Biol.* 2:E171.
- Larschan E, et al. 2011. X chromosome dosage compensation via enhanced transcriptional elongation in *Drosophila*. *Nature* 471:115–118.
- Lee C, et al. 2008. NELF and GAGA factor are linked to promoter-proximal pausing at many genes in *Drosophila*. *Mol. Cell. Biol.* 28:3290–3300.
- Leeb M, Wutz A. 2010. Mechanistic concepts in X inactivation underlying dosage compensation in mammals. *Heredity* 105:64–70.
- Lerach S, et al. 2005. JIL-1 kinase, a member of the male-specific lethal (MSL) complex, is necessary for proper dosage compensation of eye pigmentation in *Drosophila*. *Genesis* 43:213–215.
- Lieb JD, Capowski EE, Meneely P, Meyer BJ. 1996. DPY-26, a link

- between dosage compensation and meiotic chromosome segregation in the nematode. *Science* 274:1732–1736.
44. Liu T, et al. 2011. Cistrome: an integrative platform for transcriptional regulation studies. *Genome Biol.* 12:R83.
 45. Liu T, et al. 2011. Broad chromosomal domains of histone modification patterns in *C. elegans*. *Genome Res.* 21:227–236.
 46. Liu W, et al. 2010. PHF8 mediates histone H4 lysine 20 demethylation events involved in cell cycle progression. *Nature* 466:508–512.
 47. Machín F, et al. 2004. Condensin regulates rDNA silencing by modulating nucleolar Sir2p. *Curr. Biol.* 14:125–130.
 48. Meneely PM, Wood WB. 1984. An autosomal gene that affects X chromosome expression and sex determination in *Caenorhabditis elegans*. *Genetics* 106:29–44.
 49. Meneely PM, Wood WB. 1987. Genetic analysis of X-chromosome dosage compensation in *Caenorhabditis elegans*. *Genetics* 117:25–41.
 50. Meyer BJ. 2010. Targeting X chromosomes for repression. *Curr. Opin. Genet. Dev.* 20:179–189.
 51. Meyer BJ, Casson LP. 1986. *Caenorhabditis elegans* compensates for the difference in X-chromosome dosage between the sexes by regulating transcript levels. *Cell* 47:871–881.
 52. Mourón S, et al. 2010. RINGO C is required to sustain the spindle-assembly checkpoint. *J. Cell Sci.* 123:2586–2595.
 53. Nguyen DK, Distèche CM. 2006. Dosage compensation of the active X chromosome in mammals. *Nat. Genet.* 38:47–53.
 54. Nishioka K, et al. 2002. PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. *Mol. Cell* 9:1201–1213.
 55. Oda H, et al. 2009. Monomethylation of histone H4-lysine 20 is involved in chromosome structure and stability and is essential for mouse development. *Mol. Cell. Biol.* 29:2278–2295.
 56. Ohno S. 1967. Sex chromosomes and sex-linked genes. Springer-Verlag, Berlin, Germany.
 57. Okamoto I, Otte AP, Allis CD, Reinberg D, Heard E. 2004. Epigenetic dynamics of imprinted X inactivation during early mouse development. *Science* 303:644–649.
 58. Payer B, Lee JT. 2008. X chromosome dosage compensation: how mammals keep the balance. *Annu. Rev. Genet.* 42:733–772.
 59. Petty E, Laughlin E, Csankovszki G. 2011. Regulation of DCC localization by HTZ-1/HTZ-2 and DPY-30 does not correlate with H3K4 methylation levels. *PLoS One* 6:e25973.
 60. Petty EL, Collette KS, Cohen AJ, Snyder MJ, Csankovszki G. 2009. Restricting dosage compensation complex binding to the X chromosomes by H2A.Z/HTZ-1. *PLoS Genet.* 5:e1000699.
 61. Rechtsteiner A, et al. 2010. The histone H3K36 methyltransferase MES-4 acts epigenetically to transmit the memory of germline gene expression to progeny. *PLoS Genet.* 6:e1001091.
 62. Regnard C, et al. 2011. Global analysis of the relationship between JIL-1 kinase and transcription. *PLoS Genet.* 7:e1001327.
 63. Rudner AD, Hall BE, Ellenberger T, Moazed D. 2005. A nonhistone protein-protein interaction required for assembly of the SIR complex and silent chromatin. *Mol. Cell. Biol.* 25:4514–4528.
 64. Sakaguchi A, Karachentsev D, Seth-Pasricha M, Druzhinina M, Steward R. 2008. Functional characterization of the *Drosophila* Hmt4-20/Suv4-20 histone methyltransferase. *Genetics* 179:317–322.
 65. Shapira M, et al. 2006. A conserved role for a GATA transcription factor in regulating epithelial innate immune responses. *Proc. Natl. Acad. Sci. U. S. A.* 103:14086–14091.
 66. Shin H, Liu T, Manrai AK, Liu XS. 2009. CEAS: cis-regulatory element annotation system. *Bioinformatics* 25:2605–2606.
 67. Shogren-Knaak M, et al. 2006. Histone H4–K16 acetylation controls chromatin structure and protein interactions. *Science* 311:844–847.
 68. Smith ER, Allis CD, Lucchesi JC. 2001. Linking global histone acetylation to the transcription enhancement of X-chromosomal genes in *Drosophila* males. *J. Biol. Chem.* 276:31483–31486.
 69. Smith ER, et al. 2000. The *Drosophila* MSL complex acetylates histone H4 at lysine 16, a chromatin modification linked to dosage compensation. *Mol. Cell. Biol.* 20:312–318.
 70. Smith ER, Winter B, Eissenberg JC, Shilatifard A. 2008. Regulation of the transcriptional activity of poised RNA polymerase II by the elongation factor ELL. *Proc. Natl. Acad. Sci. U. S. A.* 105:8575–8579.
 71. Song JS, et al. 2007. Model-based analysis of two-color arrays (MA2C). *Genome Biol.* 8:R178.
 72. Straub T, Becker PB. 2007. Dosage compensation: the beginning and end of generalization. *Nat. Rev. Genet.* 8:47–57.
 73. Strukov YG, Sural TH, Kuroda MI, Sedat JW. 2011. Evidence of activity-specific, radial organization of mitotic chromosomes in *Drosophila*. *PLoS Biol.* 9:e1000574.
 74. Talasz H, Lindner HH, Sarg B, Helliger W. 2005. Histone H4-lysine 20 monomethylation is increased in promoter and coding regions of active genes and correlates with hyperacetylation. *J. Biol. Chem.* 280:38814–38822.
 75. Tissenbaum HA, Guarente L. 2001. Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature* 410:227–230.
 76. Tsai CJ, et al. 2008. Meiotic crossover number and distribution are regulated by a dosage compensation protein that resembles a condensin subunit. *Genes Dev.* 22:194–211.
 77. Whittle CM, et al. 2008. The genomic distribution and function of histone variant HTZ-1 during *C. elegans* embryogenesis. *PLoS Genet.* 4:e1000187.
 78. Yonker SA, Meyer BJ. 2003. Recruitment of *C. elegans* dosage compensation proteins for gene-specific versus chromosome-wide repression. *Development* 130:6519–6532.
 79. Zippo A, et al. 2009. Histone crosstalk between H3S10ph and H4K16ac generates a histone code that mediates transcription elongation. *Cell* 138:1122–1136.