INVESTIGATION

Opposing Activities of DRM and MES-4 Tune Gene Expression and X-Chromosome Repression in Caenorhabditis elegans Germ Cells

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ABSTRACT During animal development, gene transcription is tuned to tissue-appropriate levels. Here we uncover antagonistic regulation of transcript levels in the germline of Caenorhabditis elegans hermaphrodites. The histone methyltransferase MES-4 (Maternal Effect Sterile-4) marks genes expressed in the germ-line with methylated lysine on histone H3 (H3K36me) and promotes their transcription; MES-4 also represses genes normally expressed in somatic cells and genes on the X chromosome. The DRM transcription factor complex, named for its Dp/E2F, Retinoblastoma-like, and MuvB subunits, affects germline gene expression and prevents excessive repression of X-chromosome genes. Using genome-scale analyses of germline tissue, we show that common germline-expressed genes are activated by MES-4 and repressed by DRM, and that MES-4 and DRM co-bind many germline-expressed genes. Reciprocally, MES-4 represses and DRM activates a set of autosomal soma-expressed genes and overall X-chromosome gene expression. Mutations in mes-4 and the DRM subunit lin-54 oppositely skew the transcript levels of their common targets and cause sterility. A double mutant restores target gene transcript levels closer to wild type, and the concomitant loss of lin-54 suppresses the severe germline proliferation defect observed in mes-4 single mutants. Together, “yin-yang” regulation by MES-4 and DRM ensures transcript levels appropriate for germ-cell function, elicits robust but not excessive dampening of X-chromosome-wide transcription, and may poise genes for future expression changes. Our study reveals that conserved transcriptional regulators implicated in development and cancer counteract each other to fine-tune transcript dosage.

Proper development requires that genes be expressed at appropriate levels in appropriate tissues. Developmental gene regulation often is viewed as a series of all-or-none switches that turn genes on or off to promote cell identity and function. However, a gene that is “on” may only be expressed at moderate levels. Similarly, a gene that is “off” may not be completely or irreversibly inactivated but may instead be expressed at very low levels and poised for reactivation. Such fine-tuning is particularly important for genes for which a relatively small degree of transcriptional variability may have a profound influence on cell identity or function. For example, transcription of Oct3/4, which is critical for self-renewal, is precisely regulated in embryonic stem cells; either too much or too little Oct3/4 expression leads to differentiation (Niwa et al. 2000). How the transcriptional regulatory machinery precisely controls and maintains proper transcript levels is not well understood. In some cases, tuning is achieved through the combined action of factors that activate and factors that repress transcription (Reynolds et al. 2013). In this study, we investigated gene expression regulation in the germ cells of Caenorhabditis elegans and uncovered a system of transcriptional fine-tuning by antagonistic transcriptional regulators. This transcriptional fine-tuning system acts

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on sets of autosomal genes and on the X chromosomes and is essential for germ-cell development.

Germ cells give rise to gametes and the next generation of an organism. To serve this critical role, germ cells must express genes required for germline functions and silence genes that might interfere with germline development, including genes associated with somatic development. Key regulators of the transcriptional program in *C. elegans* germ cells are the MES histone methyltransferases (Capowski et al. 1991). MES-4 methylates histone H3 on lysine 36 (H3K36me), a mark associated with active gene expression (Bender et al. 2006; Rechtsteiner et al. 2010). MES-2, MES-3, and MES-6 form the worm version of polycomb repressive complex 2 and generate H3K27me3, which leads to gene repression (Bender et al. 2004; Ketel et al. 2005; Pengelly et al. 2013; Xu et al. 2001). Together, the MES proteins define domains of germline-expressed genes marked with MES-4 and H3K36me and mutually exclusive domains of germline-repressed genes marked with H3K27me3 (Gaydos et al. 2012). Loss of MES-4 or MES-2/3/6 results in down-regulation of germine-expressed genes and ectopic up-regulation of somatically expressed genes (Gaydos et al. 2012). These patterns of misexpression are thought to underlie the maternal-effect sterile phenotype displayed by mutants: worms that inherit mes(+) product from their mothers develop into fertile adults, whereas worms that do not inherit maternal mes(+) product develop into sterile adults (Capowski et al. 1991). Thus, the MES proteins cooperate to promote development of healthy germ cells by activating germline genes and repressing somatic genes.

Another feature of gene regulation in *C. elegans* hermaphrodite germ cells is the significant dampening of transcription from the X chromosomes. Somatic cells reduce X-linked gene expression by approximately twofold in XX worms (hermaphrodites) to match expression in XO worms (males) through a process called X-chromosome dosage compensation (Meyer 2010). Germ cells instead exhibit near-complete silencing of the single X in males and partial silencing of both Xs in hermaphrodites (Bean et al. 2004; Kelly et al. 2002; Strome and Kelly 2006). MES proteins serve pivotal roles in X-chromosome regulation in the germ cells of hermaphrodites. The MES-2/3/6 complex concentrates repressive H3K27me3 on the X chromosomes (Bender et al. 2004; Gaydos et al. 2012). MES-4 and H3K36me are concentrated on the autosomes, antagonize methylation of H3K27 and help focus MES-2/3/6-generated H3K27me3 on the X chromosomes (Bender et al. 2004, 2006; Fong et al. 2002; Gaydos et al. 2012). Loss of MES-4 or MES-2/3/6 results in up-regulation of genes on the X chromosome (Bender et al. 2006; Gaydos et al. 2012). The sensitivity of the maternal-effect sterile mutant phenotype to X-chromosome dosage (Garvin et al. 1998) suggests that up-regulation of X-linked genes contributes to sterility and thus that repression of genes on the X is crucial for normal germline development.

A recent study implicated another player, the multiprotein DRM complex, in germline X-chromosome regulation and showed that DRM loss affects the X in an opposite manner to the MES proteins (Tabuchi et al. 2011). DRM is a conserved transcription factor complex that includes a retinoblastoma-related pocket protein (LIN-35), an E2F/DP heterodimer (EFL-1/DPL-1), and the Multi-va1a class B core subunits (LIN-9, LIN-37, LIN-52, LIN-53, and LIN-54) (Harrison et al. 2006; Sadasivam and Decaprio 2013; van den Heuvel and Dyson 2008). *C. elegans* DRM and its homologs in other species regulate genes involved in cell cycle and development, and its dysfunction is linked to sterility, developmental defects, and cancer (e.g., Chi and Reinke 2006; Dimova et al. 2003; Georlette et al. 2007; Korenjak et al. 2004; Kudron et al. 2013; Litovchick et al. 2007; Reichert et al. 2010; Sadasivam and Decaprio 2013; Tabuchi et al. 2011; Thomas et al. 2003). In *C. elegans* germ cells, DRM predominantly localizes to autosomes, yet loss of the DRM subunit LIN-54 leads to excessive repression of X-linked genes (Tabuchi et al. 2011). Autosomally concentrated LIN-54 affecting the expression of genes on the X is reminiscent of MES-4. Although DRM and MES-4 share the unique feature of acting on the X from a distance, it was not previously known whether DRM and MES-4 oppositely influence the same set of X-linked genes, and if they antagonistically regulate genes on the autosomes.

In this work, we show that MES-4 and DRM oppositely regulate a common set of genes to maintain proper transcript dosages for germ cells. We found that DRM counters activation of germline genes and repression of somatic genes and X-linked genes by MES-4. Loss of either factor oppositely skewed transcript levels of those genes, whereas loss of both restored their levels closer to wild type. Moreover, the maternal-effect sterile phenotype of mes-4 mutants was ameliorated by concomitant loss of DRM, highlighting the importance of the oppositely-acting gene regulatory activities of MES-4 and DRM for the development of germ cells. Such opposing regulation was particularly striking for genes located on the X chromosome, illustrating how the X chromosomes in *C. elegans* germ cells are not silenced but tuned to low levels of transcription. We propose that the combined action of MES-4 and DRM prevents transcripts from deviating toward excessive or insufficient levels incompatible with germ-cell function, achieves significant but not complete dampening of X chromosome-wide transcription, and perhaps lowers the barrier to future gene expression changes in the soma. This work illustrates how the action of antagonistic transcriptional regulators on common target genes can control the precise levels of transcripts in a tissue during development.

## MATERIALS AND METHODS

### Strains

All strains were cultured at 20°C, using standard methods. The following strains were used: N2 (Bristol) as wild type, lin-54(n3423) IV/nT1[qIS51] (IV;V) and lin-54(n2990) IV/nT1[qIS51] (IV;V), mes-4(ok2326) V/nT1 [qIS1] (IV;V), dpy-11(e224) mes-4(bn23) unc-76(e911) V/DS(st[unc (n754)let] (IV;V), and dpy-11(e224) mes-4(bn58) V/DS(st[unc(n754)let] (IV;V). See Supporting Information, File S1 for descriptions of alleles.

### Microarray analysis of dissected germelines

A total of 50–70 germlines were dissected from wild-type and mutant (M+Z-generation) young adult hermaphrodites (24 hr after L4 stage) in 1× egg buffer containing 0.1% Tween20, and cut germ lines extending from the mitotic tip through meiotic late pachytene were transferred to a tube containing Trizol (Invitrogen) for RNA isolation. The MessageAmp II aRNA Amplification Kit (Ambion) was used to create cDNA, to amplify antisense RNA (aRNA), and to label aRNA with biotinylated UTP. This differs from the labeled amplified RNA preparation method in Tabuchi et al. (2011), in which an additional linear amplification step was included. Fragmentation of biotin-labeled aRNA, hybridization to Affymetrix GeneChip *C. elegans* genome arrays, and scanning were performed at the Genomics Core Facility at University of Massachusetts Medical School. Three biological replicates were performed for each strain. Germlines from each genotype were collected and analyzed in parallel to facilitate comparison and are not the same samples used for germline microarray analysis in previous studies (Bender et al. 2006; Gaydos et al. 2012; Tabuchi et al. 2011). We compared our data with these previous microarray data sets and found high reproducibility, despite differences in amplification methods, germline regions harvested, microarray platforms, and mes-4 alleles used. Correlation coefficients for significantly changed
genes in (Tabuchi et al. 2011) and our current lin-54 vs. wild type are 
$R = 0.92$ for autosomal genes and $R = 0.76$ for X-linked genes; com-
paring (Gaydos et al. 2012) and our current mes-4 vs. wild type, the 
values are $R = 0.91$ for autosomal genes, $R = 0.27$ for significantly 
changed X-linked genes, and $R = 0.60$ for all X-linked genes.

Statistical analyses were performed with the use of custom scripts 
and packages in R (http://www.r-project.org) (Ihaka and Gentleman 
1996) and Bioconductor (Gentleman et al. 2004). The affy package 
(Gautier et al. 2004) was used to quantile normalize the data across 
replicates (Bolstad et al. 2003) and the robust multichip average algo-
rithm was used to obtain probe-set expression values (Bolstad et 
al. 2003). Log$_2$-transformed data were used in subsequent analysis and 
plotting. Statistical analysis for misexpression was performed using the 
moderated t-test from the Bioconductor package limma (Smyth 2004).
Statistical significance of misexpression (false discovery rate q) was 
obtained with the qvalue package (Storey and Tibshirani 2003). Genes 
with q $\leq 0.05$ were called significant for all comparisons. Microarray 
data were deposited in National Center for Biotechnology Informa-
tion’s Gene Expression Omnibus and are accessible through GEO 
series accession number GSE52064.

For box-and-whisker plots in Figure 1, Figure 2, Figure S1, A and 
B, and Figure S4, the transcript level of each gene within the set was 
represented by its normalized probe set log$_2$ intensity (or the average 
of multiple probe sets corresponding to one gene). For the box-and-
whisker plot in Figure S1C, log$_2$(normalized read depth per tran-
script + 1) were plotted (data can be accessed at http://intermine.
modencode.org/ under accession ID 4006). Log$_2$-fold change expres-
sion values compared with the wild type were calculated for each gene 
in each strain. Boxes extend from the 25th to the 75th percentile, with 
the median indicated by a horizontal line; whiskers extend to the 2.5th 
and 97.5th percentiles. The Student’s t-test was used to calculate 
statistical significance.

To define expressed genes in Figure 1F, we used the present/marginal/
absent call generated by the masculin algorithm in the Bioconductor 
affy package. We required two or more present calls, or at least one present 
call and at least one marginal call among three biological replicates. To define 
expressed genes in Figure S4A, the top 720 highly expressed X-linked 
genes in wild-type soma (L1 larvae) were selected (Petrella et al. 2011).

Except where noted, if multiple probe sets correspond to the same 
genome annotation, the probe set with the most statistical significance of 
misexpression was used. The area-proportional Venn diagrams were 
created using the VennDiagram package, and the statistical signifi-
cance of overlap was calculated using the hypergeometric test in R.

**Phenotypic analysis of germlines in single and double mutants**

L4 stage wild-type or homozygous mutants from heterozygous mothers 
(M+Z- generation) were transferred to plates, and embryos laid within 
24 hr were raised to adults (the M-Z- generation) and analyzed. Young 
adults were fixed with Carnoy’s solution and stained with the DNA dye 
DAPI. Images were acquired on a Zeiss Axioskop and processed with 
Image J and Adobe Photoshop. To facilitate quantification of germ cells in 
wild type, lin-54(n2990) single mutant, and dpy-11(e224) mutant animals, 
gonads were dissected and germ cells in one of the two gonad arms were 
counted and multiplied by two (Figure 3B). For mes-4 single and lin-54; 
mes-4 double mutant animals, germ cells in intact worms were counted.

**Chromatin immunoprecipitation (ChIP) data**

ChIP-chip data for MES-4, H3K36me3, and H3K27me3 (in early em-
byros) can be obtained from GEO under the accession ID GSE38180, 
and H3K36me2 can be obtained under GSE22717 (Gaydos et al. 2012; 
Rechtsteiner et al. 2010). MES-4–bound genes were determined as 
described in Rechtsteiner et al. (2010). ChIP-chip data for LIN-54 
(in mixed staged animals) were obtained from GEO under the accession 
ID GSE28852 (Tabuchi et al. 2011); LIN-54–bound genes were 
determined as described in Tabuchi et al. (2011). The LIN-54 genome 
broader tracks in Figure 4 C display the ratio of IP/input channel inten-
sities. Intensity ratios were scaled to a median absolute deviation of 
1, and the median was set to 1. All ChIP-chip data except LIN-54 were 
obtained on platforms based on genome assembly WS170. LIN-54 was 
shifted over from WS120 to WS170 using the UCSC Genome Browser 
liftover utility (http://genome.ucsc.edu’util.html). Ppie-1:EFL-1:GFP 
ChIP-seq data (EFL-1 expressed under the pie-1 germline promoter; 
ChIP performed from young adults) were obtained from GEO under 
the accession ID GSE30246 (Kudron et al. 2013). Raw reads were 
mapped to WS170 using bowtie with default parameters (Langmead 
2009). MACS1.4 (Zhang et al. 2008) was used to call peaks for the 
mapped data using a bandwidth parameter of 300 and P-value of $10^{-3}$.
Final peak calls for EFL-1 retained only peaks that overlapped in two 
biological replicates. A gene was called promoter bound by EFL-1 if 
a peak overlapped at least 200 bp with the region 500 bp upstream 
from the transcript start site to 500 bp downstream from the transcript 
start site as obtained from Wormbase.

**RESULTS**

In germ cells, MES-4 and LIN-54 antagonistically 
regulate X-linked genes, and a double mutant restores more normal X expression

X chromosomes in many species and tissues are subject to special 
chromosome-wide forms of gene regulation. In C. elegans hermaph-
rodites, gene expression from the two X chromosomes is dampened in 
somatic and germ cells, but demasking occurs by different mecha-
nisms in the two cell types. In the hermaphrodite soma, a “dosage 
compensation complex” related to chromosome condensation factors 
binds the two X chromosomes and down-regulates their expression to 
equal that of the single X in males (Meyer 2010). In the hermaphro-
dite germline, different factors including the MES proteins repress X-
chromosome gene expression, and the X chromosome produces lower 
overall transcript levels than an average autosome (Figure S1A, also 
compare Figure S2, A–C, to Figure S2, D–F) (Bender et al. 2006; 
Gaydos et al. 2012; Kelly et al. 2002; Reinke et al. 2000; Wang et 
al. 2009). In germline tissue, genes with germline expression that reside 
on the X chromosome exhibit lower expression than those that reside 
on the autosomes (Figure S1A) (Reinke et al. 2004; Reinke et al. 2000).
In contrast, in somatic tissues, genes with somatic expression that 
reside on the X chromosome show expression similar to those that reside 
on autosomes (Figure S1, B and C) (Deng et al. 2011; Gupta et 
al. 2006). Perhaps as an evolutionary consequence of germline X-
chromosome repression, fewer germline-expressed genes are located 
on the X chromosome compared with autosomes (Figure S1D) (Piano 
et al. 2002; Reinke et al. 2000; Petrella et al. 2011; Gupta et 
al. 2004; Reinke et al. 2000).

Previous studies implicated the MES proteins in repressing the X 
chromosomes in the germline, and the DRM complex in preventing 
excessive X-chromosome repression, but did not link the two systems 
in this process (Bender et al. 2006; Gaydos et al. 2012; Tabuchi et 
al. 2011). In other cellular contexts, such as vulva development, MES-4 
and DRM mutants exhibit genetic antagonism (e.g., Cui et al. 2006; 
Petrella et al. 2011; Wang et al. 2005; Wu et al. 2012). We therefore 
hyposthesized that MES-4 and DRM counteract each other to promote 
proper germline gene expression, in particular by maintaining

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X-chromosome gene regulation

**Figure 1** In germ cells, MES-4 and LIN-54 antagonistically regulate X-linked genes, and a double mutant restores more normal X expression. (A-F) Microarray analysis of dissected hermaphrodite germlines showing only X-linked transcripts. (A-B) Volcano plots with x-axis values showing log₂ of the fold change in transcript level (A) between mes-4(ok2326) vs. wild type (WT) and lin-54(n3423) vs. WT and (B) between the double mutant vs. lin-54 or the double mutant vs. mes-4 single mutant. The y-axis values indicate the statistical significance (−log₁₀ q-value) of misexpression of X-linked genes. The gray line marks the significance cutoff of q = 0.05. The numbers of genes significantly up- or down-regulated (q ≤ 0.05) are indicated in the top corners. Genes with −log₁₀ q ≥ 3.5 are shown as 3.5. (C) The overlap of X-linked genes significantly up-regulated in mes-4 vs. WT and significantly down-regulated in lin-54; mes-4 vs. mes-4 defines 203 genes named “X-up” for their behavior in the mes-4 mutant (overlap P < 10⁻⁹⁹, hypergeometric test). (D) Scatterplot of X-linked transcripts significantly changed in mes-4 vs. WT (q ≤ 0.05) and in the double vs. mes-4 (q ≤ 0.05). (E-F) Transcript levels (log₂ fold change) of X-up genes (E) or all germline-expressed X-linked genes (F) in mes-4(ok2326) (blue), lin-54(n3423) (yellow), and the double mutant (green) relative to WT (red). Each box extends from the 25th to the 75th percentile, with the median indicated by the horizontal line; whiskers extend from the 2.5th to the 97.5th percentile. All differences are statistically significant (Student’s t-test, P < 0.001), except for WT vs. lin-54; mes-4 in E and F. See also Table S1 and Figure S2 and Figure S3.
Figure 2 In germ cells, MES-4 and LIN-54 antagonistically regulate two classes of autosomal genes. (A-H) Microarray analysis of dissected hermaphrodite germlines showing only autosomal transcripts. (A and B) Volcano plots with x-axis values showing log2 of the fold change in autosomal transcript levels between each single mutant and wild type (WT) or between the double mutant and single mutant, and y-axis values showing statistical significance (−log10 q-value) of all autosomal genes on the microarray. The gray line marks the significance cut-off of q = 0.05. The numbers of genes significantly up- or down-regulated (q ≤ 0.05) are in the top corners. Genes with −log10 q ≥ 3.5 are shown as 3.5. (C) The overlap of autosomal genes significantly up-regulated in mes-4 vs. WT and down-regulated in lin-54; mes-4 vs. mes-4 defines 178 genes, named “A-up” for their behavior in the mes-4 mutant (overlap P < 10−154, hypergeometric test). The opposite direction of transcript changes defines 101 “A-down” genes (overlap P < 10−6). (D) Scatter plot of 296 autosomal genes significantly changed in mes-4 vs. WT (q ≤ 0.05) and in the double vs. mes-4 (q ≤ 0.05); only 17 do not fall into the A-up or A-down categories. This illustrates the mostly opposite directions of transcript fold changes caused by loss of MES-4 and LIN-54. Linear regression analysis yields a slope of −0.93, R = 0.85, indicating strong anti-correlation (P < 0.001). (E and H) Expected (gray) and observed (black) numbers of A-up genes (F) or A-down genes (H) in different expression categories defined in (Gaydos et al. 2012). Significant enrichment (+) or depletion (−) over expected are indicated (P < 0.05, **P < 0.001, ***P < 10−10 by hypergeometric test). See also Table S1, Figure S2, and Figure S3.
mes-4 double mutant compared with the mes-4 single mutant. We named this set of genes “A-up” for its behavior in the mes-4 mutant; these genes in wild-type germlines are repressed by MES-4 and their repression is antagonized by LIN-54 (178 genes, Figure 2, C and D, Table S1F, Figure S3B, overlap significance $P < 10^{-146}$). The second gene set is also antagonistically regulated by MES-4 and LIN-54, but reciprocally: down-regulated in the mes-4 mutant and up-regulated in lin-54; mes-4 compared to mes-4. We named this gene set “A-down” because of its behavior in the mes-4 mutant; these genes in wild-type germlines are activated by MES-4, and their activation is antagonized by LIN-54 (101 genes, Figure 2, C and D, Table S1G, Figure S3C, overlap significance $P < 10^{-61}$). The scatter plot in Figure 2D compares expression changes of all genes significantly misexpressed both in the mes-4 single mutant vs. wild type (effect of the mes-4 mutation) and in the double mutant vs. mes-4 single mutant (effect of the lin-54 mutation). Of 296 autosomal genes with significantly altered expression in both experiments, 279 (94%) are antagonistically regulated. The line of best fit has a slope of $-0.93$ and a correlation coefficient $R$-value of 0.85 ($P < 0.001$), indicating a strong negative correlation between the two mutants and thus similar degrees of gene misregulation but in opposite directions in the two mutants.

For both sets of autosomal genes, we found a restoration of more wild-type expression levels in the absence of both factors. Expression of A-up genes was increased in the mes-4 mutant compared with the wild type ($P < 0.001$), decreased in the lin-54 mutant compared with the wild type ($P < 0.001$), and returned to near-normal levels in the lin-54; mes-4 double mutant (no significant difference from wild type, Figure 2E). Reciprocally, expression of A-down genes was decreased in the mes-4 mutant compared with the wild type ($P < 0.001$), increased in the lin-54 mutant compared with the wild type ($P < 0.001$), and returned to near-normal levels in the double mutant (no significant difference from wild type, Figure 2F, Figure S2F). These results demonstrate that MES-4 and LIN-54 antagonistically modulate transcription of two sets of autosomal genes, in reciprocal manners, and that removing both factors restores target autosomal gene expression to nearer-to-normal levels.

**MES-4 and DRM and antagonistic regulation of genes expressed in germline or soma**

One role of the DRM complex is to prevent the ectopic activation of germline genes in the soma of *C. elegans*, and this function is antagonized by MES-4 and other germline chromatin factors (Cui et al. 2006; Petrella et al. 2011; Tabuchi et al. 2011; Wang et al. 2005; Wu et al. 2012). Reciprocally, one role of MES-4 is to prevent expression of somatic genes in the *C. elegans* hermaphrodite germline, protecting pluripotent germ cells from differentiating into somatic cell types (Gaydos et al. 2012; Patel et al. 2012). These findings prompted us to investigate what types of genes (germline-expressed, somatically expressed, or ubiquitously expressed) are antagonistically regulated by MES-4 and DRM in germline tissue.

First we examined X-up genes. The X-up genes are X-linked genes that go up in the mes-4 mutant and back down in the double mutant, implying that wild-type MES-4 represses these genes and LIN-54 antagonizes their repression. We found that X-up genes include more genes with ubiquitous, germline-enriched, and germline-specific expression than expected by chance (Figure S4B). Consistently, X-up genes show greater transcript levels than all X-linked genes in wild type, because the latter category includes genes not expressed in the germline (Figure S4C). Next, we considered A-up genes, the 178 autosomal genes that go up in the mes-4 mutant and back down in the double mutant, which we infer are repressed by wild-type MES-4 and whose repression is antagonized by LIN-54. A-up genes include more genes with ubiquitous and soma-specific expression and fewer genes with germline-enriched expression than expected by chance (Figure 2F). Thus, as in a prior study (Gaydos et al. 2012), we find that MES-4 represses somatic genes to prevent their ectopic expression in germline tissue, and we now show that LIN-54 antagonizes this activity. Finally, we considered A-down genes, the 101 autosomal genes whose expression goes down in the mes-4 mutant and back up in the double mutant, implying that wild-type MES-4 activates these genes and LIN-54 antagonizes their activation. A-down genes include more genes with germline-enriched and germline-specific expression than expected by chance (Figure 2H). Consistently, transcript levels from A-down genes in wild-type germline tissue are higher than those from all autosomal genes, since the latter category includes genes not expressed in the germline (Figure S4D). This result supports previous reports that MES-4 activates germline-expressed genes (Gaydos et al. 2012), many of which are on autosomes, and we now show that LIN-54 antagonizes this activity.

Together, our findings show that MES-4 and LIN-54 each have both activating and repressing capability, but act oppositely on common target gene sets. LIN-54 antagonizes the repressive role of MES-4 on the X chromosomes (X-up) and on somatic genes (A-up), and antagonizes the activating role of MES-4 on germline genes (A-down). Removal of either factor skew s levels of target transcripts, while removal of both factors restores more wild-type expression levels for these transcripts. We propose that together DRM and MES-4 tune gene expression to levels that are appropriate for germ-cell function.

**Loss of LIN-54 suppresses germ-cell defects caused by loss of MES-4**

Disruption of either MES-4 or LIN-54 causes germ-cell defects (Capowski et al. 1991; Garvin et al. 1998; Tabuchi et al. 2011), presumably as the result of to gene misexpression (Bender et al. 2006; Gaydos et al. 2012; Tabuchi et al. 2011). lin-54 mutants have well-proliferated germlines but produce endomitotic oocytes (Figure 3A) (Tabuchi et al. 2011). mes-4 mutants contain drastically stunted germlines due to death of nascent germ cells (Figure 3A) (Capowski et al. 1991; Garvin et al. 1998). We wondered whether the more normal patterns of gene expression restored to the germlines of lin-54; mes-4 double mutants would restore germ-cell development. We therefore scored germline proliferation in single and double mutants by quantifying the number of germ cells per worm and their ability to produce gametes (allele choices explained in File S1).

Wild-type adult hermaphrodite germlines, which proliferate in two gonad arms, contain ~1500 germ cells per worm and include gametes (Figure 3, A and B). Germlines of homozygous lin-54(n2990) mutants from homozygous mothers (the M-Z- generation, which lacks both maternal and zygotic gene product) proliferate similarly well (~1300 germ cells/worm) and produce gametes, although many oocytes are endomitotic with excess DNA (Figure 3, A and B; and endomitotic oocyte indicated by arrow). mes-4(bn23) and mes-4(bn58) M-Z- mutant germlines contain very few germ cells (Figure 3, A and B; median of 8 and 34 germ cells per worm, respectively). The weakest mes-4 allele, mes-4(bn58), occasionally produces gametes and embryos (Bender et al. 2006; Capowski et al. 1991; Garvin et al. 1998; Rechtsteiner et al. 2010). Strikingly, in lin-54(n2990); mes-4(bn23) and lin-54(n2990); mes-4(bn58) double mutants, the number of germ cells was significantly increased (median of 203 and 169 germ cells per worm, respectively) compared with mes-4 single mutants (Figure 3, A and B; Wilcoxon signed-rank test $P < 10^{-4}$). Moreover, some double
mutant animals produced gametes (sperm, oocytes, or endomitotic oocytes) (Figure 3, A and B; endomitotic oocyte indicated by arrow). Despite the improvement in germline proliferation and gamete production, the double mutant did not restore the production of viable progeny. Of 50–100 animals tested per genotype, only 2% of lin-54; mes-4(bn23) and 1% of lin-54; mes-4(bn58) were fertile, compared with 0–1% for mes-4 alone and 68% for lin-54 alone (data not shown).

Together, our results indicate that the germ-cell proliferation and gamete production defects caused by disruption of mes-4 are suppressed when both mes-4 and lin-54 function are disrupted. The improvement in germline formation and function in lin-54; mes-4 double mutants likely reflects the restoration of gene expression patterns and levels closer to those appropriate for germ cells.

**MES-4 and the DRM complex cobind germline-expressed genes**

To begin to address how MES-4 and DRM oppositely regulate gene expression, we assessed the overlap between antagonistically regulated genes and genes bound by MES-4, DRM, and key histone modifications. On genes expressed in the germline, MES-4 binds and catalyzes the active mark H3K36me2/3 and repels the repressive mark H3K27me3, helping to keep H3K27me3 concentrated on the X chromosomes and somatic genes (Bender et al. 2006; Gaydos et al. 2012; Rechtsteiner et al. 2010). We therefore analyzed MES-4, DRM, H3K36me3, and H3K27me3, using available ChIP data. To assess germline patterns of MES-4 and histone marks, we analyzed ChIP data from early embryos, which retain germline chromatin signatures (Gaydos et al. 2012; Rechtsteiner et al. 2010). To assess germline binding of the DRM complex, we analyzed ChIP data from EFL-1 (E2F) obtained from adults carrying tagged EFL-1 expressed from a germline-specific promoter (Kudron et al. 2013). In addition, we analyzed LIN-54 ChIP data from mixed-stage worms, which contain but are not limited to germline tissue (Tabuchi et al. 2011).

Our analyses indicate that MES-4 and DRM cobind many germline-expressed genes. Of the 1549 genes bound by LIN-54 and the 1884 genes bound by germline-specific EFL-1, 1171 and 1365 also are bound by MES-4, respectively (Figure 4A, P < 10^{-300} for both Venn diagram overlaps). Accordingly, EFL-1–bound genes are enriched for binding of MES-4 and H3K36me3 in wild-type animals, an

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**Figure 3** DNA staining of adult hermaphrodites in wild type (WT) and in mutants at the M-Z generation with the following genotypes: lin-54(n2990), mes-4(bn23), mes-4(bn58), lin-54(n2990); mes-4(bn23), and lin-54(n2990); mes-4(bn58). The images show a region including germ cells in one of the two gonad arms (indicated by the box in the illustration), with the distal end of each gonad arm oriented to the top right and the vulva at the bottom right. Gonad arms are outlined with dashed white lines to show the extent of germline proliferation. The mes-4(bn23) or mes-4(bn58) worms have no or few germ cells, whereas the double mutants have expanded germlines and sometimes contain gametes. Arrows point to endomitotic oocytes in lin-54 and lin-54; mes-4(bn58). Because the mes-4(bn23) and mes-4(bn58) alleles are linked to the phenotypic marker dpy-11(e224), this mutant was included as a control; dpy-11 hermaphrodites contained close-to-normal numbers of germ cells (~1200) and gametes (not shown). (B) Germ-cell number per worm in each strain. Germ-cell counts were significantly greater in the lin-54; mes-4 double mutants than in the mes-4 single mutants (Wilcoxon signed-rank test, P < 10^{-5}). Worms that contained gametes (sperm, oocytes, or endomitotic oocytes) are indicated with red dots. Scale bar = 50 μm.
Figure 4  MES-4 and the DRM complex co-bind germline-expressed genes. (A) Overlap of gene bodies bound by MES-4 in embryos (Gaydos et al. 2012; Rechtsteiner et al. 2010) with gene promoters bound by LIN-54 in mixed-stages (Tabuchi et al. 2011) or by EFL-1 in the germline [determined from raw data of (Kudron et al. 2013), see Materials and Methods]. Overlap significance for both Venn diagrams is $P < 10^{-300}$. (B) Expected (gray) and observed (black) numbers of genes co-bound by MES-4 and LIN-54 (left) or by MES-4 and EFL-1 (right) in different gene expression categories defined in (Gaydos et al. 2012). (C) View of ChIP binding data at representative genes expressed in the germline, which are
enrichment that is lost in MES-4-depleted animals (Figure S5, A and B). Genes cobound by MES-4 and DRM subunits are enriched for genes normally expressed in the germline, and depleted for genes with soma-specific expression (Figure 4B). Figure 4C illustrates representative genes expressed in the germline, with promoters occupied by the DRM subunits LIN-54 and EFL-1, and gene bodies enriched for MES-4 and H3K36me2/3 and depleted for H3K27me3.

Next, we examined MES-4 and DRM binding enrichment on the three categories of antagonistically regulated genes. The A-up genes show significantly fewer MES-4, LIN-54, and EFL-1 binding peaks than expected by chance (Figure 4D). These results imply that A-up genes, which include soma-expressed genes repressed by MES-4 and whose repression is antagonized by DRM (Figure 2, E and F), may be regulated indirectly (also see Gaydos et al. 2012). We predicted that in contrast, the A-down category, which includes autosomal genes activated by MES-4 and whose activation is antagonized by DRM, might be directly bound targets, since this category was enriched for germline-expressed genes (Figure 2, G and H) (Gaydos et al. 2012), and because genes cobound by MES-4 and DRM tend to be germline-expressed (Figure 4B). We found that A-down genes are statistically significantly enriched for MES-4, H3K36me3, and EFL-1 binding, but not for LIN-54 binding (Figure 4D and Figure S5C), making it difficult to conclude whether these genes are regulated directly by bound MES-4 and DRM. Similarly, X-up genes show some enrichment for MES-4, LIN-54, and EFL-1 binding (Figure 4D), but the numbers of X-up genes bound by these factors is small, making it difficult to infer direct or indirect regulation. We envision two explanations for our observations concerning binding and gene regulation. First, MES-4 and DRM may in fact co-bind and directly regulate germline-expressed genes. Although this would predict strong binding enrichment on A-down genes, this may be difficult to observe because the A-down gene set is small (101 genes), and because the analysis demands intersections between multiple sets of independent microarray and ChIP data, each adding some noise to the analysis. Alternatively, MES-4 and DRM may tune most germline gene expression patterns indirectly. MES-4 and DRM might both bind and antagonistically regulate only one or a few key targets, which are then responsible for the many altered germline gene expression patterns we observe. Or, MES-4 and DRM may influence gene expression at sites distant from where they are bound, for example by altering long-range chromatin organization.

DISCUSSION

We show that the DRM transcription factor complex and the histone methyltransferase MES-4 regulate common genes, in opposite directions, to tune transcript levels in the C. elegans germline. MES-4 promotes, and DRM limits, transcription of germline-expressed genes. In contrast, MES-4 represses and DRM promotes expression of somatic and X-linked genes. Our findings show that conserved transcriptional regulators implicated in development and cancer provide antagonizing activities that together ensure normal germline transcript levels and germline proliferation, and tune chromosome-wide transcript dosage from the X chromosomes.

Here, we place DRM in a common pathway with MES-4, but with opposite action. The current model for MES-4 function is that MES-4 repels H3K27me3, catalyzed by the polycomb repressive complex 2-like MES-2/3/6 complex, from germline-expressed genes, focusing H3K27me3 on somatic and X-linked genes (Bender et al. 2004, 2006; Fong et al. 2002; Gaydos et al. 2012). Because DRM acts oppositely to MES-4, one model is that DRM may encourage H3K27me3 on germline-expressed genes. A potential link between DRM and H3K27me is suggested by the finding that certain Drosophila genes require both DRM and H3K27me2 for silencing (Lee et al. 2010). Perhaps in lin-54 mutants, MES-4 activity is unchecked, causing higher H3K36 methylation of germline genes and more effective repulsion of H3K27 methylation; this could lead to greater levels of repressive H3K27me3 on somatic genes and the X chromosomes, causing their observed down-regulation. A second model is that DRM and MES-4 have antagonistic effects on MRG-1, whose homologs bind H3K36me2/3 and recruit histone modifiers, and whose mutant phenotype resembles that of mes-4 (Takasaki et al. 2007). Perhaps MRG-1 binds and reinforces MES-4-mediated H3K36me2/3, and DRM limits MRG-1 at germline-expressed genes. Future tests of these models will require ChIP of candidate proteins and chromatin marks in DRM mutants with the use of techniques that assess patterns in germline tissue.

MES-4 serves critical germline functions, so why use DRM to restrict its activity? We propose that the mutual antagonism of MES-4 and DRM in the germline serves three purposes. First, it ensures tissue-appropriate transcript doses and prevents them from veering toward excessive or insufficient levels that would be detrimental to germline function. Such tuning mechanisms are critical during development, going beyond simple on/off control to produce varied transcriptional outputs that can achieve different developmental consequences (Reynolds et al. 2013). Second, DRM/MES-4 antagonism contributes to the specialized transcript dosage regulation of the sex chromosomes. Together, MES-4 and DRM ensure transcript levels from the X chromosomes that are lower, but not excessively lower, than those from an average pair of autosomes. An interesting question for future study is whether MES-4 and DRM act like established X dosage compensation mechanisms to balance X transcript levels between the sexes. Third, DRM/MES-4 antagonism may specify tissue-specific expression programs yet poised target genes for future changes in transcription. In C. elegans, chromatin states of pluripotent germ cells are propagated into early embryos, which divide to form primordial germ cells that retain those states, and somatic cells that reprogram those states (Furuhashi et al. 2010; Gaydos et al. 2012; Petrella et al. 2011; Rechtsteiner et al. 2010). We speculate that DRM limits MES-4-mediated H3K36me2/3 to make germline-expressed genes amenable to reprogramming and repression during embryonic somatic differentiation. Consistent with this idea, in DRM mutant animals, germline genes are not properly repressed in the soma, a defect that is suppressed in double mutants also lacking germline chromatin regulators like MES-4 (Cai et al. 2006; Petrella et al. 2011; Wang et al. 2005; Wu et al. 2012). Similarly, disruption of the Drosophila DRM-associated factor L(3)MBT causes germline gene activation in somatic tumors, and tumorigenesis is suppressed in double mutants also lacking germline specifiers (Janic et al. 2010). Reciprocally, in the worm germline MES-4 disruption causes ectopic somatic gene expression,

enriched for MES-4 and H3K36me2/3 over the gene bodies (blue and green, respectively), depleted for H3K27me3 (brown), and bound by LIN-54 and EFL-1 (red) at the promoters. (D) Expected (gray) and observed (black) numbers of genes bound by MES-4, LIN-54, EFL-1, and both MES-4 and LIN-54 or MES-4 and EFL-1, among X-up, A-up, and A-down genes defined in Figures 1 and 2. Significant enrichment (*) or depletion (†) is indicated (* or † P < 0.05, ** or †† P < 0.001, *** or ††† P < 10−10 by hypergeometric test). See also Figure S5.
which is suppressed in double mutants also lacking DRM (this study and Gaydos et al. 2012), and MES-4 disruption allows introduced somatic transcription factors to reprogram germline to somatic fates (Patel et al. 2012). Analogous to antagonism between chromatin modifiers and transcription factors on mammalian stem cell pluripotency genes (Hu and Wade 2012; Reynolds et al. 2013), the antagonism of MES-4 and DRM may maintain cell fate distinctions while also keeping chromatin states flexibly poised to go down other fate paths upon receiving developmental cues.

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LITERATURE CITED
Bean, C. J., C. E. Schaner, and W. G. Kelly, 2004 Meiotic pairing and imprinted X chromatin assembly in Caenorhabditis elegans. Nat. Genet. 36: 100–105.
Bender, L. B., R. Cao, Y. Zhang, and S. Strome, 2004 The MES-2/MES-3/MES-6 complex and regulation of histone H3 methylation in C. elegans. Curr. Biol. 14: 1639–1643.
Bender, L. B., J. Suh, C. R. Carroll, Y. Fong, I. M. Fingerman et al., 2006 MES-4: an autosomal-associated histone methyltransferase that participates in silencing the X chromosomes in the C. elegans germ line. Development 133: 3907–3917.
Bolstad, B. M., R. A. Irizarry, M. Astrand, and T. P. Speed, 2003 A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19: 185–193.
Capowski, E. E., P. Martin, C. Garvin, and S. Strome, 1991 Identification of grandchildless loci whose products are required for normal germ-line development in the nematode Caenorhabditis elegans. Genetics 129: 1061–1072.
Chi, W., and V. Reinke, 2006 Promotion of oogenesis and embryogenesis in the C. elegans gonad by EFL-1/DPL-1 (E2F) does not require LIN-35 (pRB). Development 133: 3147–3157.
Cui, M., E. B. Kim, and M. Han, 2006 Diverse chromatin remodeling genes antagonize the Rb-involved SynMuv pathways in C. elegans. PLoS Genet. 2: e74.
Deng, X., J. B. Hiatt, D. K. Nguyen, S. Ercan, D. Sturgill et al., 2011 Evidence for compensatory upregulation of expressed X-linked genes in mammals, Caenorhabditis elegans and Drosophila melanogaster. Nat. Genet. 43: 1179–1185.
Dimova, D. K., O. Stevaks, M. V. Frolov, and N. J. Dyson, 2003 Cell cycle-dependent and cell cycle-independent control of transcription by the Drosophila E2F/RB pathway. Genes Dev. 17: 2308–2320.
Fong, Y., L. Bender, W. Wang, and S. Strome, 2002 Regulation of the different chromatin states of autosomes and X chromosomes in the germ line of C. elegans. Science 296: 2235–2238.
Furushashi, H., T. Takasaki, A. Rechsteiner, T. Li, H. Kimura et al., 2010 Trans-generational epigenetic regulation of C. elegans primordial germ cells. Epigenetics Chromatin 3: 15.
Garvin, C., R. Holdeman, and S. Strome, 1998 The phenotype of mes-2, mes-3, mes-4 and mes-6, maternal-effect genes required for survival of the germline in Caenorhabditis elegans, is sensitive to chromosome dosage. Genetics 148: 167–185.
Gautier, L., L. Cope, B. M. Bolstad, and R. A. Irizarry, 2004 afyff—analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 20: 307–315.
Gaydos, L., J. A. Rechsteiner, T. A. Egelhofer, C. R. Carroll, and S. Strome, 2012 Antagonism between MES-4 and Polycrome repressive complex 2 promotes appropriate gene expression in C. elegans germ cells. Cell Reports 2: 1169–1177.
Gentleman, R. C., V. J. Carey, D. M. Bates, B. Bolstad, M. Dettling et al., 2004 Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 5: R80.
Reinke, V., H. E. Smith, J. Nance, J. Wang, C. Van Doren et al., 2000 A global profile of germline gene expression in C. elegans. Mol. Cell 6: 605–616.

Reinke, V., I. S. Gil, S. Ward, and K. Kazmer, 2004 Genome-wide germline-enriched and sex-biased expression profiles in Caenorhabditis elegans. Development 131: 311–323.

Reynolds, N., A. O’Shaughnessy, and B. Hendrich, 2013 Transcriptional repressors: multifaceted regulators of gene expression. Development 140: 505–512.

Sadasivam, S., and J. A. DeCaprio, 2013 The DREAM complex: master coordinator of cell cycle-dependent gene expression. Nat. Rev. Cancer 13: 585–595.

Smyth, G. K., 2004 Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3: Article3.

Storey, J. D., and R. Tibshirani, 2003 Statistical significance for genomewide studies. Proc. Natl. Acad. Sci. USA 100: 9440–9445.

Strome, S., and W. Kelly, 2006 Epigenetic regulation of the X chromosome in C. elegans, pp. 291–305 in Epigenetics, edited by D. C. Allis, D. Jenuwein, and D. Reinberg, Cold Spring Harbor Press, Cold Spring Harbor, NY.

Tabuchi, T. M., B. Deplancke, N. Osato, L. J. Zhu, M. I. Barrasa et al., 2011 Chromosome-biased binding and gene regulation by the Caenorhabditis elegans DRM complex. PLoS Genet. 7: e1002074.

Takasaki, T., Z. Liu, Y. Habara, K. Nishiwaki, J. Nakayama et al., 2007 MRG-1, an autosome-associated protein, silences X-linked genes and protects germline immortality in Caenorhabditis elegans. Development 134: 757–767.

Thomas, J. H., C. J. Ceol, H. T. Schwartz, and H. R. Horvitz, 2003 New genes that interact with lin-35 Rb to negatively regulate the let-60 ras pathway in Caenorhabditis elegans. Genetics 164: 135–151.

van den Heuvel, S., and N. J. Dyson, 2008 Conserved functions of the pRB and E2F families. Nat. Rev. Mol. Cell Biol. 9: 713–724.

Wang, D., S. Kennedy, D. Conte, Jr., J. K. Kim, H. W. Gabel et al., 2005 Somatic misexpression of germline P granules and enhanced RNA interference in retinoblastoma pathway mutants. Nature 436: 593–597.

Wang, X., Y. Zhao, K. Wong, P. Ehlers, Y. Kohara et al., 2009 Identification of genes expressed in the hermaphrodite germ line of C. elegans using SAGE. BMC Genomics 10: 213.

Wu, X., Z. Shi, M. Cui, M. Han, and G. Ruvkun, 2012 Repression of germline RNAi pathways in somatic cells by retinoblastoma pathway chromatin complexes. PLoS Genet. 8: e1002542.

Xu, L., Y. Fong, and S. Strome, 2001 The Caenorhabditis elegans maternal-effect sterile proteins, MES-2, MES-3, and MES-6, are associated in a complex in embryos. Proc. Natl. Acad. Sci. USA 98: 5061–5066.

Yildirim, O., R. Li, J. H. Hung, P. B. Chen, X. Dong et al., 2011 Mbd3/NURD complex regulates expression of 5-hydroxymethylcytosine marked genes in embryonic stem cells. Cell 147: 1498–1510.

Zhang, Y., T. Liu, C. A. Meyer, J. Eckhoute, D. S. Johnson et al., 2008 Model-based analysis of ChIP-Seq (MACS). Genome Biol. 9: R137.

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