OXFORD GENETICS

GENETICS, 2026, 232(3), iyag008
https://doi.org/10.1093/genetics/iyag008
Advance Access Publication Date: 16 January 2026
Investigation

The DREAM and MEC NuRD complexes reinforce SPR-5/MET-2 maternal reprogramming to maintain the germline–soma distinction

Sindy R. Chavez,[1,†] Jazmin Dozier,[2,†] Saahj P. Gosrani,[1] Sandra K. Nguyen,[2] Jovan S. Brockett ,[1] Sarah D. Blancher,[2] Sydney L. Morgan-Benitez,[2] Juan D. Rodriguez,[1] Onur Birol,[3] Monica N. Reeves,[1] Karen L. Schmeichel ,[4] David J. Katz ,[1,*] Brandon S. Carpenter [2,*]

[1]Department of Cell Biology, Emory University School of Medicine, Emory University, Atlanta, GA 30322, United States
[2]Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA 30144, United States
[3]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA 30332, United States
[4]Natural Sciences Division, Oglethorpe University, Atlanta, GA 30319, United States

*Corresponding authors: David J. Katz, Department of Cell Biology, Emory University School of Medicine, Emory University, Atlanta, GA 30322, United States. Email: djkatz@emory.edu; Brandon S. Carpenter, Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA 30144, United States. Email: bscarpe18@kennesaw.edu
†These authors contributed equally to this work.

The proper coordination of transcription factors, ATP dependent chromatin remodelers, and histone modifications is essential for tissue-specific gene expression, but how gene expression is regulated at these different levels is not well understood. In *Caenorhabditis elegans*, H3K4 methylation that is acquired in the germline is reprogrammed at fertilization by the H3K4me1/2 demethylase SPR-5/LSD1/KDM1A and the H3K9 methyltransferase MET-2/SETDB1/KMT2E. SPR-5/MET-2 maternal reprogramming is required to help establish the germline–soma distinction and prevent developmental delay by preventing inherited H3K4 methylation from inappropriately maintaining germline gene expression in somatic tissues. To determine if the DREAM transcriptional repressor complex and the MEC NuRD ATP dependent nucleosome remodeling and histone deacetylase complex function to reinforce SPR-5/MET-2 maternal reprogramming, we asked if loss of these complexes affects the ectopic germline transcription and developmental delay in *spr-5*; *met-2* double mutants. We find that knocking down the DREAM or MEC NuRD complexes specifically exacerbates the developmental delay and the ectopic expression of germline genes in the soma caused by loss of SPR-5 and MET-2. In addition, the DREAM and MEC NuRD complexes bind together at SPR-5/MET-2 reprogramming targets. These data suggest that the transcriptional repression of DREAM and the ATP dependent chromatin remodeling and deacetylation activities of the MEC NuRD complex are required somatically to reinforce maternal histone reprogramming by SPR-5/MET-2. Thus, these data provide a novel example of how gene regulation is coordinated at multiple levels to maintain the germline–soma distinction and ensure proper development.

Keywords: histone methylation; developmental delay; maternal reprogramming; transgenerational inheritance; epigenetics; DREAM complex; MEC complex; MEP-1; LIN-35; WormBase

Introduction

Gene transcription must be coordinately regulated to establish tissue-specific gene expression and ensure normal development. To accomplish this, genes must be regulated at multiple levels, including by transcription factors, ATP dependent chromatin remodelers, and histone modifications. However, how tissue-specific gene expression is coordinately regulated at these different levels is not well understood.

In *Caenorhabditis elegans*, the specification of germline vs soma is partially established by the coordinate regulation of inherited histone modifications. During gametogenesis, sperm and oocyte-specific genes acquire transcription-coupled histone methylation which helps sustain germline gene expression by maintaining DNA access to transcription factors and RNA polymerase (Hirabayashi and Gotoh 2010; Burton and Torres-Padilla 2014; Jambhekar et al. 2019). Transcription acquired histone methylation can be inherited through mitotic cell divisions and between generations via both the sperm and oocyte. For this reason, the newly formed zygote must reset, or "reprogram", the inherited histone methylation to prevent the inappropriate maintenance of germline gene expression (Gaydos et al. 2014; Öst et al. 2014; Siklenka et al. 2015; Zheng et al. 2016; Tabuchi et al. 2018; Jambhekar et al. 2019; Kaneshiro et al. 2019). To do this, maternally deposited histone modifying enzymes add and remove methyl groups on the N-terminal tails of histone core proteins to limit

Received on 22 July 2025; accepted on 15 December 2025

DNA accessibility and reset the chromatin landscape. For example, in *C. elegans* and mice, the histone demethylase SPR-5/LSD1, removes mono- and di-methylation of lysine 4 on histone H3 (H3K4me1/2) at fertilization to prevent the inappropriate maintenance of germline gene expression in the embryo (Stewart et al. 2015; Kim et al. 2015a; Ancelin et al. 2016; Wasson et al. 2016). SPR-5/LSD1 functions together with the H3K9me2 methyltransferase, MET-2/SETDB1, during reprogramming at fertilization and this reprogramming is also conserved in mice (Katz et al. 2009; Greer et al. 2014; Kerr et al. 2014; Kim et al. 2016). In *C. elegans,* progeny of mutants lacking both SPR-5 and MET-2 are developmentally delayed and exhibit maternal effect sterility (Greer et al. 2014; Kerr et al. 2014; Carpenter et al. 2021). SPR-5/MET-2 maternal reprogramming is antagonized by the H3K36 methyltransferase MES-4, which transgenerationally maintains H3K36 methylation that is acquired in the germline of the previous generation and inherited in the embryo of the progeny (Furuhashi et al. 2010; Rechtsteiner et al. 2010). Hereafter, the set of germline genes that are bound by MES-4 and in which H3K36 methylation is transgenerationally maintained will be referred to as MES-4 germline genes. In progeny lacking SPR-5/MET-2 maternal reprogramming, the MES-4 germline genes are inappropriately expressed in somatic tissues. This inappropriate somatic expression causes a severe developmental delay, since knocking down *mes-4* in *spr-5*; *met-2* mutants rescues the developmental delay (Carpenter et al. 2021).

Because the specification of germline vs soma is partially established by the coordinate regulation of inherited histone modifications, it provides the perfect opportunity to investigate how the regulation of transcription factors and chromatin remodeling complexes coordinate with histone modifications to regulate tissue-specific transcription. In *C. elegans,* loss of either the DREAM or MEC **Nu**cloesome **R**emo**d**eling (NuRD) complexes also leads to somatic expression of germline genes in the soma (Unhavaithaya et al. 2002; Wang et al. 2005; Cui et al. 2006; Petrella et al. 2011; Wu et al. 2012; Goetsch et al. 2017; Costello and Petrella 2019; Rechtsteiner et al. 2019; Gal et al. 2021). As a result, we considered the possibility that these complexes may be functioning coordinately with SPR-5/MET-2 maternal reprogramming to help specify germline vs soma. The DREAM complex, which includes LIN-35 (homolog of human retinoblastoma (rb)protein), LIN-9, LIN-37, and LIN-52, is a transcriptional repressor complex most known for its roles in repressing cell-cycle re-entry genes (Lu and Horvitz 1998; Dick and Rubin 2013). At high temperatures, loss of DREAM complex members lead to an early larval arrest at the L1 stage and this L1 arrest can be rescued by knocking down *mes-4* (Rechtsteiner et al. 2019).

The NuRD complex is thought to primarily function as a repressor. It accomplishes this via two different activities: (i) ATP-dependent nucleosome remodeling, which represses chromatin by limiting the spacing between nucleosomes, and (ii) histone deacetylation, which primarily tightens the association between nucleosomes and DNA (Xue et al. 1998). In *C. elegans*, two Mi-2 orthologues, LET-418 and CHD-3, are members of three distinct histone deacetylation NuRD-like complexes, including the NuRD-like MEC complex (hereafter referred to as the MEC NuRD complex) which contains MEP-1, LET-418, and HDA-1 (Passannante et al. 2010). Similar to loss of DREAM, loss of the MEC NuRD complex component MEP-1 in *C. elegans* causes germline gene expression in somatic tissues (Unhavaithaya et al. 2002; Wang et al. 2005). This leads to a developmental delay that phenocopies *spr-5*; *met-2* mutants and can be rescued by knocking down *mes-4* (Unhavaithaya et al. 2002).

Here, we examined the synergism between SPR-5/MET-2 reprogramming and the highly conserved DREAM and MEC NuRD complexes and demonstrate that loss of either the DREAM or MEC NuRD complex in *spr-5*; *met-2* mutants causes an L1 larval arrest that is more severe than the L2 larval delay that we observe in *spr-5*; *met-2* mutants. We also find that similar genes that are misregulated in the soma of *spr-5*; *met-2* are further exacerbated upon loss of either DREAM or MEC NuRD complex members. Finally, using published chromatin immunoprecipitation (ChIP)-seq data sets we confirm that MEP-1 and LIN-35 directly bind the genes that are misregulated in the soma of *spr-5*; *met-2* mutants. Together, our findings suggest that SPR-5, MET-2, the DREAM complex and the MEC NuRD complex cooperate to maintain the germline-soma distinction in *C. elegans*.

Materials and methods

Strains

All *C. elegans* strains were grown and maintained at 20 °C under standard conditions, as previously described (Brenner 1974). Strains used were: N2: Bristol wild type strain provided by the *Caenorhabditis* Genetics Center; the *C. elegans spr-5 (by101)(I)* strain was provided by R. Baumeister (Albert Ludwig University of Freiburg, Germany); MT13293: *met-2 (n4256)(III) strain* was provided by R. Horvitz (Massachusetts Institute of Technology, MA, USA); the *spr-5 (by101)(I)/tmC27[unc-75(tmIs1239)](I); met-2 (n4256) (III)*/qC1 [qIs26 (lag2::gfp + rol-6(su1006))](III) strain was created to maintain *spr-5 (by101)(I); met-2 (n4256)(III)* double-mutant animals as balanced heterozygotes (Carpenter et al. 2021).

Scoring developmental delay

C. elegans adult hermaphrodites were allowed to lay embryos for 2 to 4 h and then removed in order to synchronize the development of progeny. Progeny were then imaged and scored for developmental progression at 72 h after the synchronized lay.

RNA sequencing and analysis

Total RNA was isolated using TRIzol reagent (Invitrogen) from 500 to 1,000 starved L1 larvae born at 20 °C overnight on unseeded NGM plates. Total RNA was sent to Georgia Genomics and Bioinformatics Core (University of Georgia, Athens, Georgia) for standard Poly-A RNA-seq services (Illumina Nextseq, 50 bp paired-end reads). Raw sequencing reads were checked for quality using FastQC (Wingett and Andrews 2018), filtered using Trimmomatic (Bolger et al. 2014), and remapped to the *C. elegans* transcriptome (ce11, WBcel235) using HISAT2 (Kim et al. 2015b). Read count by gene was obtained by FeatureCounts (Liao et al. 2014). Differentially expressed transcripts (significance threshold, Wald test, P-adj < 0.05 with a log2 fold change > 1 for upregulated gene and < -1 for downregulated) were determined using DESEQ2 (v.2.11.40.2) (Love et al. 2014). Transcripts per million (TPM) values were calculated from raw data obtained from FeatureCounts output. Subsequent downstream analysis was performed using R with normalized counts and P-values from DESEQ2 (v.2.11.40.2). Heatmaps were produced using the ComplexHeatmap R Package (Gu et al. 2016). Data was scaled and hierarchical clustering was performed using the complete linkage algorithm. In the linkage algorithm, distance was measured by calculating pairwise distance. Volcano plots were produced using the EnhancedVolcano package (v.1.20.0). Raw and processed RNAseq files have been deposited into Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo)

under accession code GSE303062 (*lin-35* RNAi RNAseq) and GSE303085 (*mep-1* RNAi RNAseq). Gene Ontology (GO) pathway analysis was performed using the online platform WormEnrichr (Chen et al. 2013; Kuleshov et al. 2016).

Chromatin immunoprecipitation analysis

LIN-35 and MEP-1 ChIP data were retrieved from the publicly available ENCODE datasets (LIN-35: https://www.encodeproject.org/experiments/ENCSR472HTK/; MEP-1: https://www.encodeproject.org/experiments/ENCSR048MPV/). Single and paired files for each replicate were used for downstream analysis. Quality co ntrol using FastQC was performed before and after adapter trimming using Trimmomatic to verify appropriate trimming. Reads were aligned to the ce11 *C. elegans* genome using Bowtie2. Peak calling was performed using macs2. To visualize peaks, bigWig files were generated from the ChIP data (bin size 20, normalized using RPKM). Heatmap matrices were prepared by combining sco re files (bigWig) and region files (BED) obtained from RNAseq datasets. Heatmaps were generated using the corresponding matrix.

RNA interference methods

RNA interference (RNAi) by feeding was carried out using clones from the Ahringer library (Kamath and Ahringer 2003). Feeding experiments were performed on RNAi plates (NGM plates containing 100 µg/ml ampicillin, 0.4 mM IPTG, and 12.5 µg/ml tetracycline). F0 worms were placed on RNAi plates as L2 larvae and then moved to fresh RNAi plates 48 h later where they were allowed to lay embryos for 2 to 4 h. F0 worms were then removed from plates and sacrificed or placed on unseeded RNAi plates overnight so that starved L1 progeny could be isolated for RNAseq experiments. To score L1 larval arrest 72 h after the synchronized lay, F1 progeny remained on freshly seeded RNAi plates to knockdown the target both maternally and zygotically. For each RNAi experiment, *pos-1* RNAi was used as a positive control. Each RNAi experiment reported here *pos-1* RNAi resulted in >95% embryonic lethality, indicating that RNAi plates were optimal.

Differential interference contrast microscopy

Worms were immobilized in 0.1% levamisole and placed on a 2% agarose pad for differential interference contrast (DIC) imaging at 10× magnification.

Results

Loss of the DREAM or MEC NuRD complexes enhances the developmental delay of *spr-5*; *met-2* mutants

Previously we found that progeny of *spr-5*; *met-2* double mutants have a severe L2 developmental delay, with a small number of progeny becoming sterile adults approximately 1 wk after being laid (Carpenter et al. 2021). To determine if the DREAM and MEC NuRD complexes are functioning in the SPR-5/MET-2 maternal reprogramming pathway, we asked whether knocking down DREAM and MEC NuRD complex components by RNAi in *spr-5*; *met-2* mutants affects the severe L2 developmental delay. After 72 h, all wild type (N2) worms fed empty vector control (L4440) RNAi reached adult stages (Fig. 1a and k). Likewise, 72 h after hatching, 82% of progeny from *spr-5*; *met-2* mutants fed control RNAi proceeded past the L1 stage (Fig. 1b and k). But as we previously reported, almost all of these worms were delayed as L2 larvae (Carpenter et al. 2021). In controls, after 72 h 100% of progeny from wild type hermaphrodites fed *lin-35*, *lin-9*, *lin-37* or *lin-52* RNAi proceeded past the L1 stage (Fig. 1c, e, g, i, and k), with most of these progeny reaching the adult stage. Thus, knockdown of the DREAM complex alone is not sufficient to result in developmental delay under these conditions. In contrast, after 72 h 100% of progeny from *spr-5*; *met-2* mutants fed RNAi against DREAM complex components *lin-35*, *lin-9*, *lin-37* or *lin-52* remain at the L1 larval stage (Fig. 1d, f, h, j, and k), with almost all of these progeny being permanently arrested and never proceeding past the L1 stage. These data suggests that DREAM functions with SPR-5 and MET-2 to help development proceed normally.

To address the interaction between the MEC NuRD complex and SPR-5/MET-2 maternal reprogramming, we also performed RNAi against *mep-1* in *spr-5*; *met-2* mutants. We chose to perform RNAi against *mep-1* because amongst the *C. elegans* NuRD-like complexes, MEP-1 is unique to the MEC NuRD complex and represses germline gene expression in the soma (Passannante et al. 2010). Though in the future, it might also be interesting to knockdown the other NuRD-like complexes, including those containing CHD-3 (the other *C. elegans* Mi2 ortholgue) in *spr-5*; *met-2* mutants. Similar to what we observe with DREAM, knocking down the MEC NuRD complex components also exacerbated the developmental delay caused by the loss of SPR-5 and MET-2. After 72 h, all wild-type progeny from hermaphrodites fed control RNAi reached adult stages (Fig. 2a and e). Likewise, 98.8% of progeny from *spr-5*; *met-2* mutants fed control RNAi proceeded past the L1 stage (Fig. 2b and e), but were subsequently delayed at the L2 stage. The 98.8% of *spr-5*; *met-2* mutants on control RNAi that proceeded past the L1 stage is higher than the 82% that we observed in the DREAM experiments (Fig. 1k). It is possible that the remaining 18% of *spr-5*; *met-2* progeny in the DREAM experiments that arrested as L1 s may be caused by a transgenerational build-up of H3K4me2 due to maintaining the balanced *spr-5*; *met-2* mutant strain for multiple generations prior to selecting double homozygous mutants. As a control, 99.9% of progeny from wild-type hermaphrodites fed *mep-1* RNAi proceeded past the L1 stage, with most of these progeny reaching the adult stage after 72 h (Fig. 2c and e). This finding suggests that knocking down the MEC NuRD complex alone is not sufficient to cause developmental delay under these conditions. In contrast, 91% of progeny from *spr-5*; *met-2* mutants fed *mep-1* RNAi remain at the L1 larval stage after 72 h (Fig. 2d and e). As with DREAM, almost all of these progeny are permanently arrested and do not proceed past the L1 stage. Taken together, these results suggest that the DREAM complex and the MEC NuRD complex function together with SPR-5/MET-2 maternal reprogramming to ensure normal development.

Loss of the DREAM or MEC NuRD complexes specifically exacerbates *spr-5*; *met-2* gene expression changes

Since knocking down both DREAM and MEC NuRD complex members causes an earlier developmental arrest in *spr-5*; *met-2* mutants, it raised the possibility that the DREAM and MEC NuRD complexes may specifically reinforce SPR-5/MET-2 maternal reprogramming. To determine if these functional interactions are specific, we performed RNA sequencing (RNAseq) on *spr-5*; *met-2* mutants fed RNAi against either DREAM or MEC NuRD complex components. Previously, we found that the developmental delay of *spr-5*; *met-2* mutants is caused by the expression of germline genes in somatic tissues (Carpenter et al. 2021). If the DREAM and MEC NuRD complexes are functioning to reinforce SPR-5/MET-2 maternal reprogramming, we would expect the ectopic expression of germline genes in the somatic tissues of *spr-5*; *met-2* mutants to be specifically exacerbated. To address this possibility, we performed RNAseq on starved L1 larvae. Starved L1 larvae

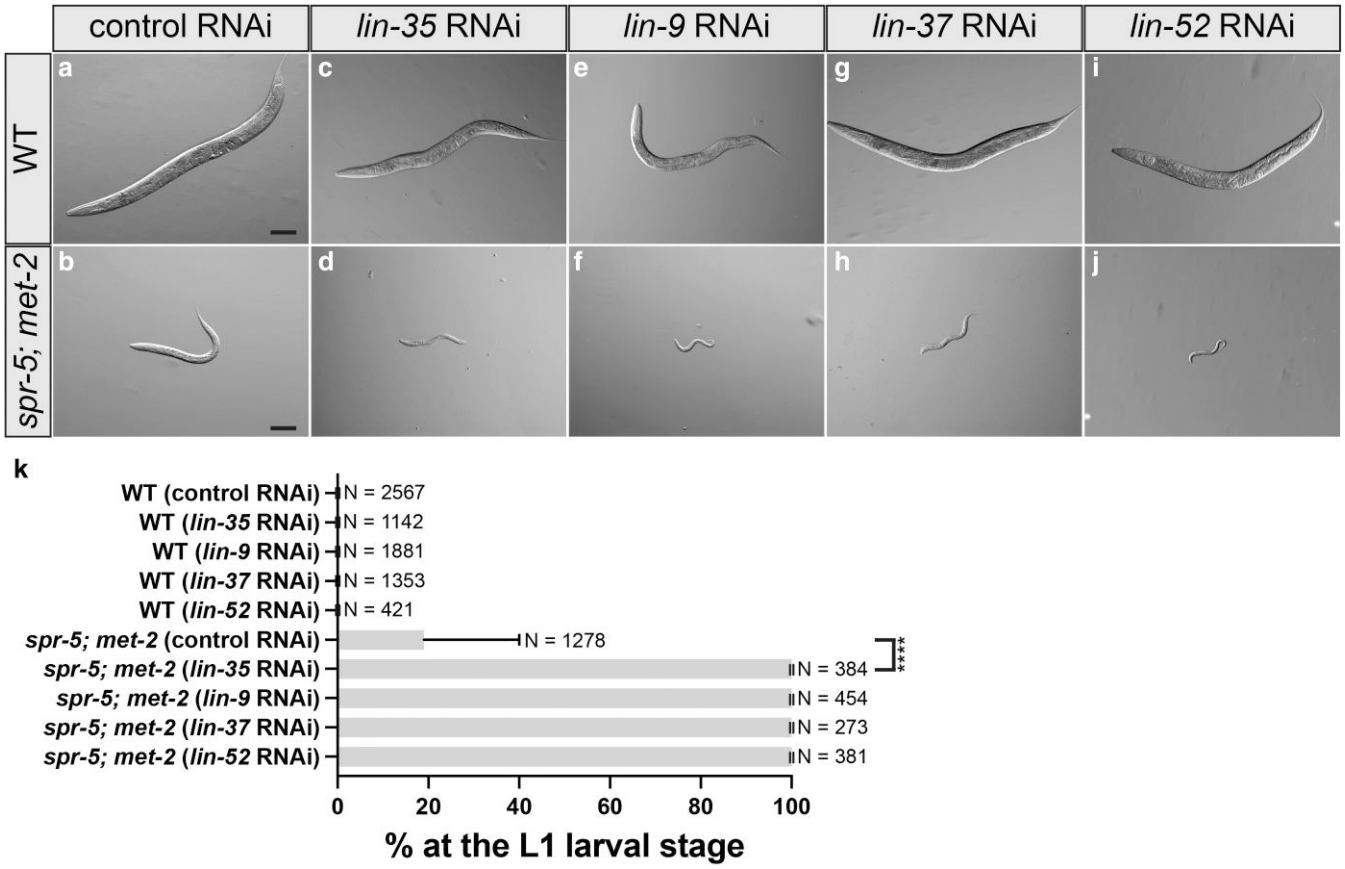

Fig. 1. Knockdown of the DREAM complex causes L1 arrest in *spr-5*; *met-2* mutants. a to j) Ten times DIC images taken of progeny 72 h after synchronized lay. wild type (WT) fed either control (L4440) RNAi (a), *lin-35* RNAi (c), *lin-9* RNAi (e), *lin-37* RNAi (g), or *lin-52* RNAi (i) and *spr-5*; *met-2* mutants fed either control RNAi (b), *lin-35* RNAi (d), *lin-9* RNAi (f), *lin-37* RNAi (h), or *lin-52* RNAi (j). k) Quantification of (a) to (d) scoring the percentage of L1 arrested progeny in WT and *spr-5*; *met-2* mutants after 72 h of synchronized lay. Data are mean ± SD. *N* = the total number of progeny from three or more replicates. **** denotes *P*-value < 0.0001 (two-tailed unpaired *t*-test). Scale bars: 100 μm.

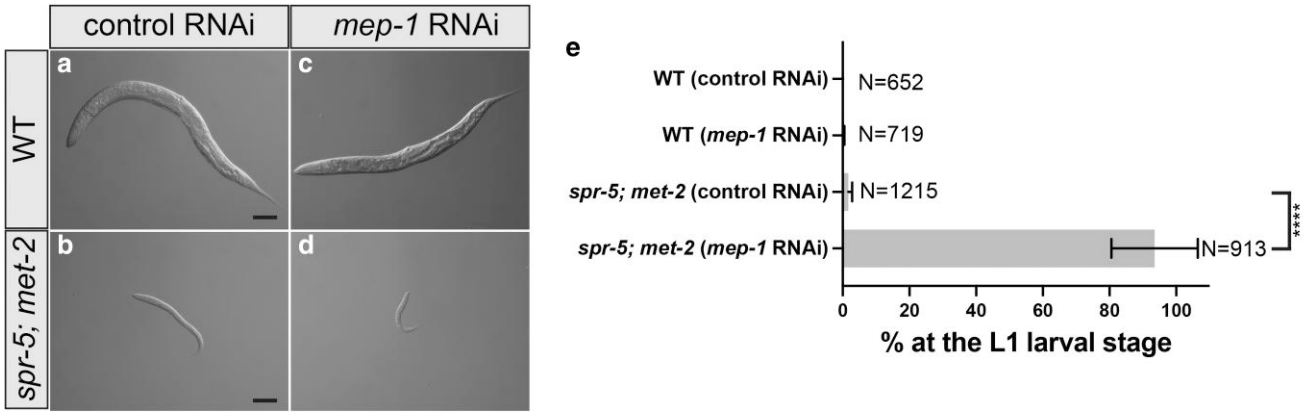

Fig. 2. Knockdown of MEP-1 causes L1 arrest in *spr-5*; *met-2* mutants. a to d) Ten times DIC images of progeny 72 h after synchronized lay. Wild type (WT) fed either control RNAi (a) or *mep-1* RNAi (c) and *spr-5*; *met-2* mutants fed either control RNAi (b) or *mep-1* RNAi (d). e) Quantification of (a) to (d) scoring the percentage of L1 arrested progeny in wild type and *spr-5*; *met-2* mutants after 72 h of synchronized lay. Data are mean ± SD. *N* = the total number of progeny from three or more replicates. **** denotes *P*-value < 0.0001 (two-tailed unpaired *t*-test). Scale bars: 100 μm.

have 550 somatic cells and only two germ cells, which have not begun proliferating or initiated germline expression. As a result, performing the RNAseq analysis on starved L1 larvae enables us to examine the ectopic expression of germline genes in somatic tissues.

To determine whether the ectopic expression of genes in *spr-5*; *met-2* mutants is exacerbated by the loss of the DREAM complex, we performed RNAseq on wild type L1 larvae compared to *spr-5*; *met-2* L1 larvae from hermaphrodites fed either control or *lin-35* RNAi (Supplementary Figs. 1 and 2). Compared to wild type, we identified 1,806 genes that are significantly upregulated in *spr-5*; *met-2* mutants with an average log2 fold change of 2.5 (Fig. 3a). Upon knockdown of *lin-35* in *spr-5*; *met-2* mutants, the average log2 fold change of these 1,806 genes increases from 2.5 to 3.0

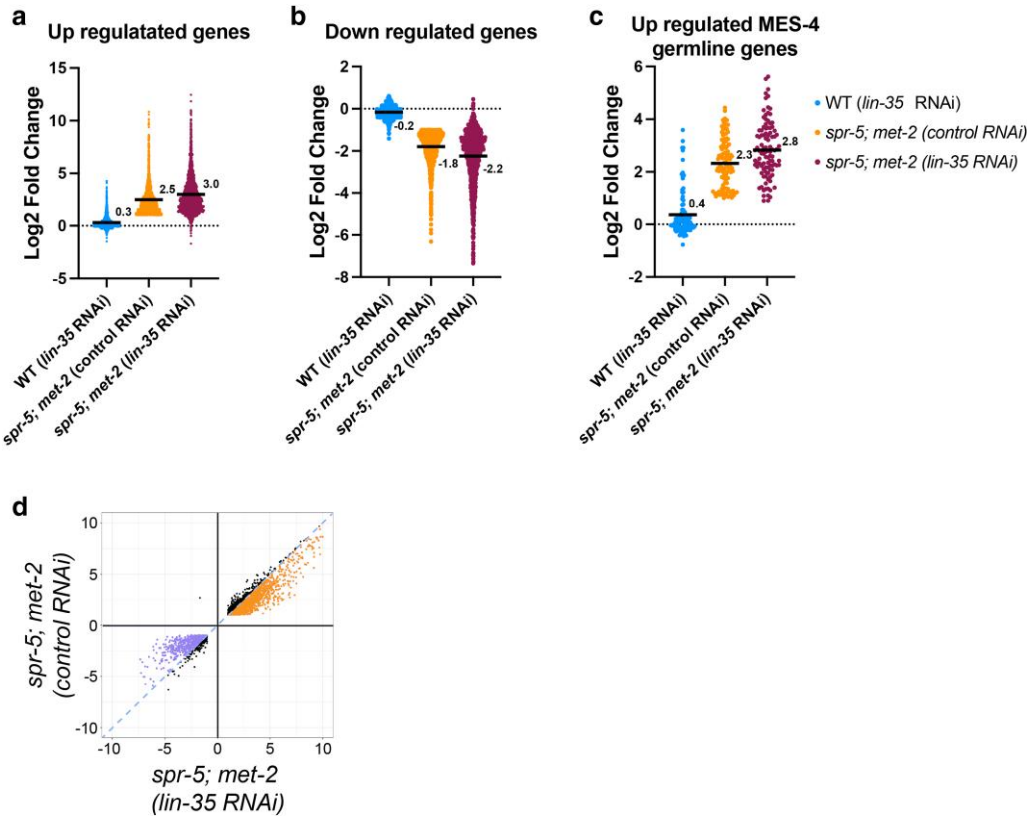

Fig. 3. Knocking down *lin-35* further exacerbates differentially expressed genes in the soma of *spr-5*; *met-2* mutants. Scatter dot plots displaying the log2 fold change of the 1,665 upregulated genes (a), 752 downregulated genes (b), and 101 upregulated MES-4 germline genes (c) in *spr-5*; *met-2* mutants fed control RNAi (orange). The average log2 fold change of these same genes were examined in WT hermaphrodites fed *lin-35* RNAi (blue) and *spr-5*; *met-2* mutants fed *lin-35* RNAi (maroon). a to c) Numbers and solid black lines represent the mean log2 fold change. d) Scatter plot displaying the correlation in log2 fold change of the all overlapping DEGs between *spr-5*; *met-2* mutants fed control RNAi and *spr-5*; *met-2* mutants *lin-35* RNAi L1 progeny. Dotted-blue line represents a one to one correlation in log2 fold change. Genes that are further upregulated and further downregulated compared to *spr-5*; *met-2* mutants fed control RNAi are denoted by orange and purple data points, respectively.

(Fig. 3a). This finding suggests that the DREAM complex reinforces SPR-5/MET-2 gene repression. As a control, we also compared the gene expression changes induced by RNAi of *lin-35* alone. Knocking down *lin-35* resulted in 318 differentially expressed genes with 310 of these genes upregulated and only eight downregulated compared to wild type (Supplementary Fig. 2). Of these 318 differentially expressed genes, 200 of them overlap with the 2,701 genes differentially expressed in *spr-5*; *met-2* mutants (Supplementary Fig. 2; hypergeometric test, P-value < 1.95e−96). Although there are some genes significantly upregulated by RNAi of *lin-35* alone, the average log2 fold change of the 1,806 genes upregulated in *spr-5*; *met-2* mutants compared to wild type in *lin-35* RNAi alone is only 0.3 (Fig. 3a). Together, these findings suggest loss of DREAM complex alone causes upregulation of gene targets in the soma that are also upregulated in *spr-5*; *met-2* mutants, but that loss of the DREAM complex alone is generally not sufficient to upregulate the majority of genes that are ectopically expressed in the absence of SPR-5/MET-2 maternal reprogramming.

Knocking down *lin-35* also further decreases the expression of genes that are downregulated in *spr-5*; *met-2* mutants. Of the 895 genes that are significantly downregulated in *spr-5*; *met-2* mutants compared to wild type, the average log2 fold change is −1.8 (Fig. 3b). When *lin-35* is knocked down in *spr-5*; *met-2* mutants, the average log2 fold change is −2.2 (Fig. 3b). This finding suggests that the DREAM complex is reinforcing SPR-5/MET-2 gene expression changes. Knocking down *lin-35* alone caused eight genes to be significantly downregulated compared to wild type (Supplementary Fig. 2). Of these eight downregulated genes, three are also significantly downregulated in *spr-5*; *met-2* mutants. Similar to what we observe with the upregulated genes, RNAi of *lin-35* alone causes a few of the 895 genes downregulated in *spr-5*; *met-2* mutants compared to wild type to be significantly downregulated. However, the average log2 fold change of these 895 downregulated genes in *lin-35* RNAi alone is only −0.2, demonstrating that loss of the DREAM complex alone is generally not sufficient to downregulate these genes (Fig. 3b).

To determine whether knocking down of *lin-35* specifically increases the ectopic expression of MES-4 germline genes, we also examined the gene expression changes of the 176 germline genes targeted by MES-4 (Rechtsteiner et al. 2010; Carpenter et al. 2021). There are 103 MES-4 germline genes that are significantly misregulated in *spr-5*; *met-2* mutants compared to wild type. Of these 103 MES-4 germline genes, 102 are upregulated with an average log2 fold change of 2.3, confirming that MES-4 germline genes are ectopically expressed in *spr-5*; *met-2* mutants (Fig. 3c). Upon knockdown of *lin-35* in *spr-5*; *met-2* mutants, this log2 fold change increases from 2.3 to 2.8 (Fig. 3c). These data suggest that the DREAM complex is reinforcing SPR-5/MET-2 repression of MES-4 germline genes in the soma. As a control, we also examined the expression of the MES-4 germline genes upon RNAi of *lin-35* alone. There are some MES-4 germline genes that are significantly upregulated by RNAi of *lin-35* alone. However, the average log2 fold change of the MES-4 germline genes in wild-type animals fed

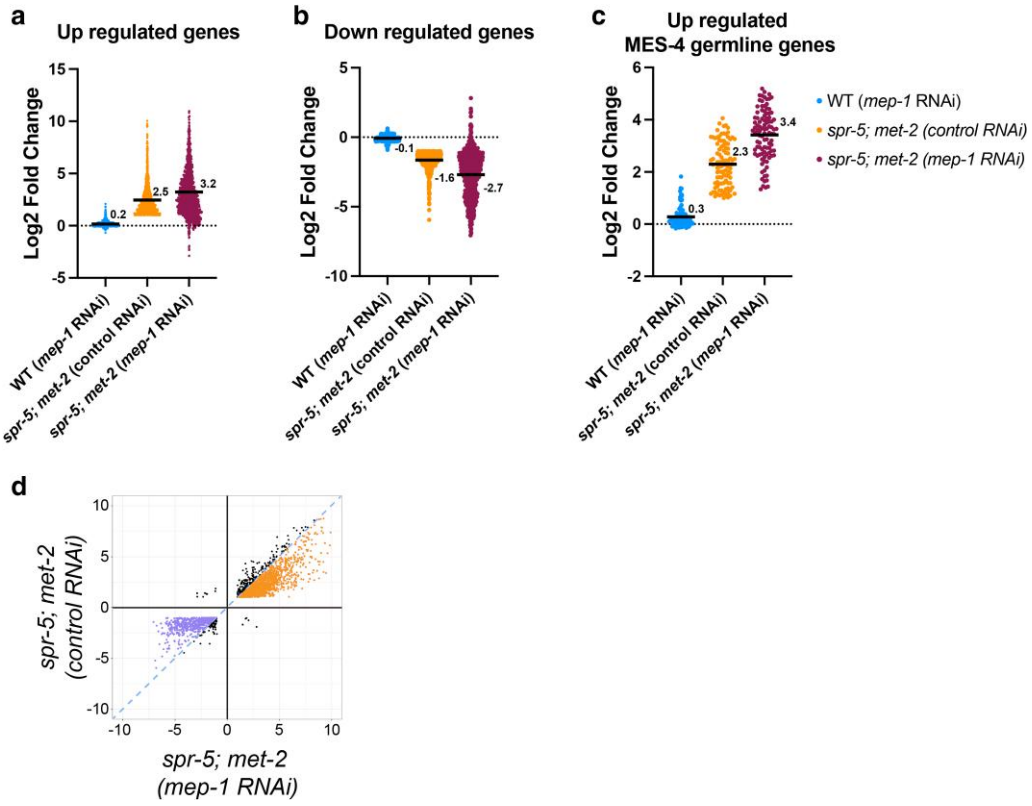

Fig. 4. Knocking down *mep-1* further exacerbates differentially expressed genes in the soma of *spr-5*; *met-2* mutants. Scatter dot plots displaying the log2 fold change of the 1,806 upregulated genes (a), 895 downregulated genes (b), and 102 upregulated MES-4 germline genes (c) in *spr-5*; *met-2* mutants fed control RNAi (orange). The average log2 fold change of these same genes were examined in WT hermaphrodites fed *mep-1* RNAi (blue) and *spr-5*; *met-2* mutants fed *mep-1* RNAi (maroon). a–c) Numbers and solid black lines represent the mean log2 fold change. d) Scatter plot displaying the correlation in log2 fold change of the all overlapping DEGs between *spr-5*; *met-2* mutants fed control RNAi and *spr-5*; *met-2* mutants *mep-1* RNAi L1 progeny. Dotted-blue line represents a one to one correlation in log2 fold change. Genes that are further upregulated and further downregulated compared to *spr-5*; *met-2* mutants fed control RNAi are denoted by orange and purple data points, respectively.

lin-35 RNAi is only 0.4, suggesting that loss of the DREAM complex alone is generally not sufficient to cause ectopic expression of MES-4 germline genes (Fig. 3c).

To examine the overall specificity of the interaction between DREAM and SPR-5/MET-2 reprogramming, we also examined the gene expression changes of all 2,389 genes that were changed in both *spr-5*; *met-2* mutants and *spr-5*; *met-2* mutants fed *lin-35* RNAi. 99.5% were changed in the same direction and of these 78.1% were exacerbated in *spr-5*; *met-2* mutants fed *lin-35* RNAi compared to *spr-5*; *met-2* mutants fed control RNAi. Only one gene was in an anti-correlated quadrant (Fig. 3d). Overall, these results suggest that the DREAM complex is functioning specifically to reinforce genes normally repressed by SPR-5 and MET-2.

To determine whether the ectopic expression of germline genes is exacerbated by the loss of the MEC NuRD complex, we also performed RNAseq on starved wild type L1 larvae compared to starved *spr-5*; *met-2* L1 larvae from hermaphrodites fed either control or *mep-1* RNAi (Supplementary Figs. 3 and 4). Compared to wild type, we identified 1,665 genes that are significantly upregulated in *spr-5*; *met-2* mutants with an average log2 fold change of 2.5 (Fig. 4a). Knocking down *mep-1* in *spr-5*; *met-2* mutants caused the log2 fold change of these 1,665 genes to increase from 2.5 to 3.2, suggesting that the MEC NuRD complex is reinforcing SPR-5/MET-2 gene repression (Fig. 4a). As a control, we also compared the gene expression changes induced by RNAi of *mep-1* alone. Knocking down *mep-1* resulted in 38 differentially expressed genes with all 38 of these genes upregulated and none of them downregulated compared to wild type (Supplementary Fig. 4). Of these 38 differentially expressed genes, 37 of them overlap with the 2,417 genes differentially expressed in *spr-5*; *met-2* mutants (Supplementary Fig. 4; hypergeometric test, *P*-value < 1.28e−33). Knocking down *mep-1* also decreases the expression of genes that are down egulated in *spr-5*; *met-2* mutants compared to wild-type animals. Of the 752 genes that are significantly downregulated in *spr-5*; *met-2* mutants compared to wild type, the average log2 fold change is −1.6 (Fig. 4b). When *mep-1* is knocked down in *spr-5*; *met-2* mutants, the average log2 fold is −2.7, suggesting that the MEC NuRD complex is reinforcing SPR-5/MET-2 gene expression changes (Fig. 4b). These changes induced by the loss of *mep-1* in *spr-5*; *met-2* mutants are similar to what we observe when *lin-35* is knocked down in *spr-5*; *met-2* mutants. Also, similar to what we observed with knocking down *lin-35*, knocking down *mep-1* in *spr-5*; *met-2* mutants appears to be synergistic because knockdown of *mep-1* by itself only results in a 0.2-fold increase and a −0.1-fold decrease in the genes that are significantly misregulated in *spr-5*; *met-2* mutants (Fig. 4a, b).

To determine if knocking down *mep-1* specifically increases the ectopic expression of germline genes, we also compared the gene expression changes specially in the 176 MES-4 germline genes. The average log2 fold change of upregulated MES-4 germline genes in *spr-5*; *met-2* mutants compared to wild type is 2.3 (Fig. 4c). When *mep-1* is knocked down in *spr-5*; *met-2* mutants, this log2 fold change increases from 2.3 to 3.4, suggesting that the MEC NuRD complex is also reinforcing SPR-5/MET-2

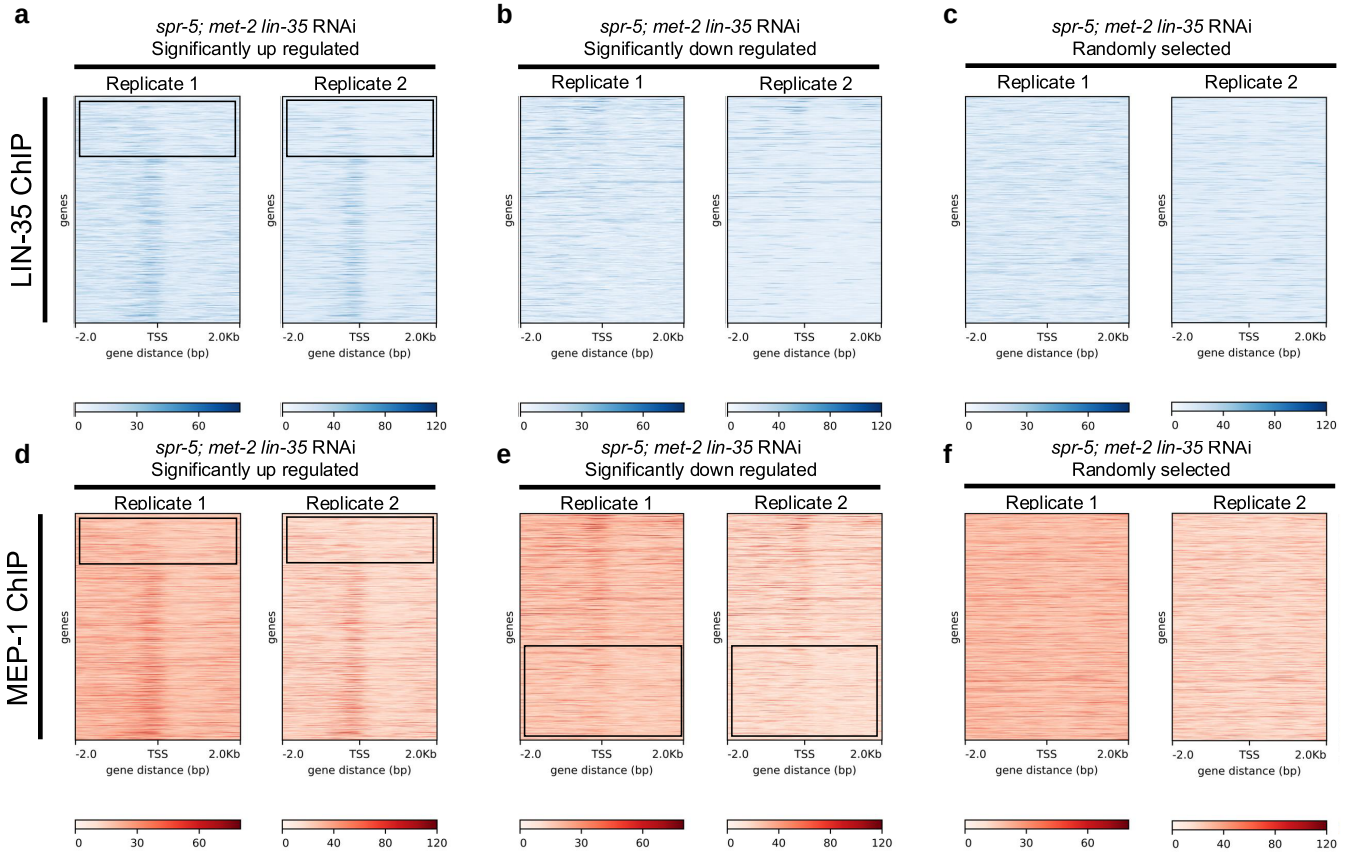

Fig. 5. The DREAM and MEC NuRD complexes directly bind to SPR-5/MET-2 gene targets. Re-analysis of LIN-35 and MEP-1 ChIP-seq data performed on genes misregulated in *spr-5*; *met-2* mutants plus *lin-35* RNAi. a to f) Two replicate heat maps aligned from −2.0 to +2.0 kb with respect to the transcriptional start site (TSS) of LIN-35 (a to c) and MEP-1 binding (d to f) show a similar enrichment (darker blue or darker red in the heat maps) of binding around the TSS at genes that are significantly upregulated (a, d) upon knockdown of *lin-35* in *spr-5*; *met-2* mutants (from Fig. 3a), compared to no enrichment at an identical number of randomly chosen genes (c, f). The enrichment of LIN-35 binding is absent at genes downregulated upon knockdown of *lin-35* in *spr-5*; *met-2* mutants (b), suggesting that LIN-35 acts predominantly as a repressor. In contrast, the enrichment of MEP-1 at genes downregulated upon knockdown of *lin-35* in *spr-5*; *met-2* mutants (from Fig. 3b) is reduced but not completely eliminated, suggesting that MEP-1 may sometimes function as an activator. In (a) to (c) the genes are listed from most highly up regulated at the top to least highly upregulated at the bottom. In (d) to (f), the genes are listed from least highly downregulated at the top to most highly downregulated at the bottom. The boxes indicate the most highly upregulated (a, d) or downregulated genes (e) which do not bind LIN-35 (a) or MEP-1 (d, e). Similar results were obtained for analysis of genes upregulated and downregulated upon knockdown of *mep-1* in *spr-5*; *met-2* mutants (see Supplementary Fig. 3).

repression of MES-4 germline genes in the soma (Fig. 4c). The changes in MES-4 germline gene expression appear to be synergistic because knocking down *mep-1* by itself only results in a 0.3-fold increase of the MES-4 germline genes.

To examine the overall specificity of the interaction between the MEC NuRD complex and SPR-5/MET-2 reprogramming, we also examined the gene expression changes of all 2,150 number of genes that were changed in both *spr-5*; *met-2* mutants and *spr-5*; *met-2* mutants fed *mep-1* RNAi. 99.4% were changed in the same direction and of these, 86.8% were exacerbated in *spr-5*; *met-2* mutants fed *mep-1* RNAi compared to *spr-5*; *met-2* mutants alone. Only 13 genes were in an anti-correlated quadrant (Fig. 4d). Taken together, these results suggest that, like we observe for the DREAM complex, the MEC NuRD complex is functioning specifically to reinforce SPR-5/MET-2 maternal reprogramming.

The DREAM and MEC NuRD complexes directly bind to SPR-5/MET-2 targets

Our combined developmental delay and transcriptomics results are consistent with a model where the DREAM and MEC NuRD complexes function in the soma to reinforce SPR-5/MET-2 germline reprogramming. To determine whether the DREAM and MEC NuRD complexes bind directly to SPR-5/MET-2 germline reprogramming targets, we reanalyzed published LIN-35 and MEP-1 ENCODE ChIP datasets (see Materials and Methods). As with our RNAseq experiments, these ChIP experiments were performed on L1 larvae that only have two germ cells, enabling us to examine binding in the soma. Initially, we chose to examine the 2,698 genes that are significantly upregulated and the 1,556 genes that are significantly downregulated in *spr-5*; *met-2* mutants with *lin-35* RNAi compared to wild type. LIN-35 ChIP analysis of these targets demonstrated binding of LIN-35 just upstream of the transcription start site of genes significantly upregulated in *spr-5*; *met-2* mutants with *lin-35* RNAi compared to wild type (Fig. 5a). This enrichment is greatly reduced in genes that are significantly downregulated in *spr-5*; *met-2* mutants with *lin-35* RNAi compared to wild type, suggesting that the DREAM complex predominantly acts as a repressor (Fig. 5b). The binding of LIN-35 to misregulated targets appears to be specific, since we do not detect any enrichment in a set of 2,699 randomly chosen genes (Fig. 5c). Surprisingly, amongst the binding of LIN-35 to the 2,698 significantly upregulated genes, LIN-35 does not seem to bind to the genes that are most highly upregulated (boxed in Fig. 5a). It is unclear why this is the case, but it suggests that these most highly

upregulated genes are indirectly affected. Alternatively, this could reflect some type of tissue specific binding that cannot be detected in the bulk L1 ChIP data.

To determine whether the MEC NuRD complex binds directly to SPR-5/MET-2 germline reprogramming targets in the soma, we also reanalyzed published MEP-1 ChIP data performed on L1 larvae (see Materials and Methods). Examination of the 2,698 genes that are significantly up regulated in *spr-5*; *met-2* mutants with *lin-35* RNAi compared to wild type demonstrated an enrichment for binding of MEP-1 just upstream of the transcription start site (Fig. 5d). Since the MEC NuRD complex is thought to predominantly act as a repressor, we expected to see much less enrichment of MEP-1 binding to genes that are significantly downregulated in *spr-5*; *met-2* mutants with *lin-35* RNAi compared to wild type. We do observe less enrichment of MEP-1 at downregulated genes, but we still detect MEP-1 binding just upstream of the transcriptional start site in genes that are downregulated in *spr-5*; *met-2* mutants fed *lin-35* RNAi (Fig. 5e). This suggests that the MEC NuRD complex may sometimes be functioning as an activator in *C. elegans*. As with DREAM, the binding of MEP-1 appears to be specific, since we do not detect any enrichment in a set of 2,698 randomly chosen genes (Fig. 5f). Similar to what we observed with DREAM, MEP-1 also does not seem to bind to the genes that are most highly upregulated nor to the genes that are the most highly downregulated (boxed in Fig. 5d and e). It is unclear why this is the case, but it is consistent with these most highly changed genes being indirect. As with the case for LIN-35 binding, this could alternatively reflect some type of tissue-specific MEP-1 binding that cannot be detected in the bulk L1 ChIP data.

To confirm that LIN-35 and MEP-1 specifically reinforce SPR-5/MET-2 reprogramming, we also examined the binding of LIN-35 and MEP-1 to genes that are misregulated in *spr-5*; *met-2* mutants. The binding of LIN-35 and MEP-1 to these *spr-5*; *met-2* targets is highly similar to the binding that we observed to genes that are misregulated in *spr-5*; *met-2* mutants with either *lin-35* or *mep-1* RNAi (Supplementary Fig. 6). This suggests that the binding of LIN-35 and MEP-1 to genes that are misregulated in *spr-5*; *met-2* mutants with either *lin-35* or *mep-1* RNAi is not just due to genes misregulated by loss of *lin-35* or *mep-1*.

It is possible that the DREAM and MEC NuRD complexes predominantly function together at SPR-5/MET-2 germline reprogramming targets. Alternatively, the DREAM and MEC NuRD complexes may be binding and repressing distinct subsets of SPR-5/MET-2 germline reprogramming targets. To distinguish between these possibilities, we compared the LIN-35 (Fig. 5a) and MEP-1 (Fig. 5d) ChIP data for the genes significantly upregulated in *spr-5*; *met-2* mutants with *lin-35* RNAi. The pattern of LIN-35 and MEP-1 binding to these genes is strikingly similar, suggesting that the DREAM and MEC NuRD complexes predominantly work together to repress SPR-5/MET-2 germline reprogramming targets. To confirm this overlap, we also examined LIN-35 and MEP-1 binding to the 2,857 genes that are significantly upregulated and the 2,317 genes that are significantly downregulated in *spr-5*; *met-2* mutants with *mep-1* RNAi compared to wild type. This analysis revealed one slight difference in the binding of LIN-35 to downregulated MEC NuRD targets (Supplementary Fig. 5b) vs the binding of LIN-35 to downregulated DREAM targets (Fig. 5b). Similar to what we observe for MEP-1 binding (Fig. 5e and Supplementary Fig. 5e), we detect slight LIN-35 binding to the least downregulated MEC NuRD targets (Supplementary Fig. 5b). This hints that, like the MEC NuRD complex, DREAM may also occasionally function as an activator. Nevertheless, overall the binding of both LIN-35 and MEP-1 to MEC NuRD targets is highly similar to each other (Supplementary Fig. 5) and highly similar to the binding of LIN-35 and MEP-1 to DREAM targets (Fig. 5 vs Supplementary Fig. 5). This further suggests that the DREAM and MEC complexes predominantly work together to repress SPR-5/MET-2 germline reprogramming targets.

Consistent with this possibility, when we compare the binding of LIN-35 and MEP-1 to genes that are affected only by the loss of LIN-35, we observe clear binding just upstream of the transcription start site at the genes upregulated by the loss of LIN-35 (Supplementary Fig. 7a and b), but not at the eight genes downregulated by the loss of LIN-35 (Supplementary Fig. 7c and d). When we perform the opposite comparison, examining the binding of LIN-35 to genes that are upregulated by the loss of only MEP-1, the binding is much less clear (Supplementary Fig. 7e). Based on this, it is possible that LIN-35 does not bind to targets only affected by the loss of MEP-1. However, we also do not detect the binding of MEP-1 to targets upregulated by the loss of MEP-1 (Supplementary Fig. 7f). Thus, it is possible that the lack of binding is simply due to their being only 38 genes affected by the loss of MEP-1 alone. There are zero genes downregulated by the loss of MEP-1, so binding could not be examined.

Discussion

Tissue-specific gene expression can be influenced at a number of different levels. Of particular interest is how transcriptional repressor complexes, ATP-dependent chromatin remodeling complexes and histone modifications are coordinately regulated to achieve the desired tissue-specific gene expression. However, a mechanistic understanding of how these three levels of gene regulation are coordinated has been difficult to decipher. To better understand this interaction, we examined how these gene regulatory mechanisms function together to help specify the germline vs soma in *C. elegans*.

Previously we showed that when maternal epigenetic reprogramming is compromised in an *spr-5*; *met-2* double mutant, there is a developmental delay at the L2 larval stage in the progeny caused by the inappropriate expression of germline genes in the soma (Carpenter et al. 2021). To determine how the DREAM transcriptional repressor complex and the MEC NuRD ATP-dependent remodeling and histone deacetylation complex interact with maternal SPR-5/MET-2 epigenetic reprogramming, we asked how knocking down members of each of these complexes affects the developmental delay and ectopic germline gene expression induced by the loss of SPR-5 and MET-2. We find that loss of either DREAM or the MEC NuRD complex converts the *spr-5*; *met-2* L2 larval delay into an L1 larval arrest. This suggests a genetic interaction in which DREAM and the MEC NuRD complex reinforce SPR-5/MET-2 maternal epigenetic reprogramming. To determine whether the genetic interaction between these different levels of gene regulation is specific, we performed RNAseq on L1 larvae to examine the ectopic expression of germline genes in somatic tissues. This analysis demonstrated that loss of either the DREAM or MEC NuRD complexes specifically increases the ectopic expression of germline genes in somatic tissues caused by loss of SPR-5 and MET-2. In addition, we find that genes upregulated by the knockdown of LIN-35 or MEP-1 alone significantly overlap with the genes upregulated in *spr-5*; *met-2* mutants. This suggests that the DREAM and MEC NuRD complexes specifically reinforce SPR-5/MET-2 maternal epigenetic reprogramming in the soma. The increase that we observe upon knockdown of *mep-1* is greater than what we observe for knockdown of *lin-35*. This could suggest that MEP-1 has a larger role in repressing *spr-5*; *met-2* targets. But it could also just reflect some technical aspect of the knockdown.

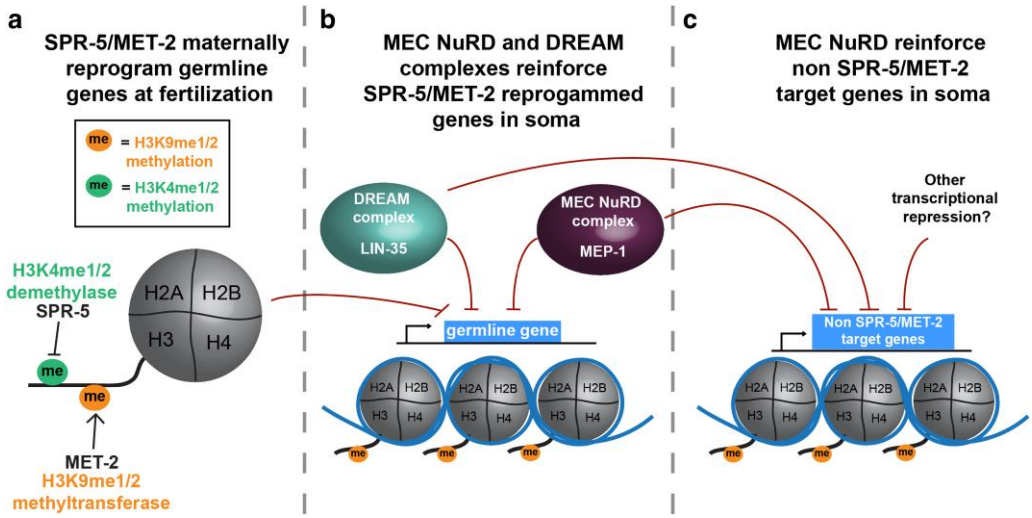

Fig. 6. MEC NuRD and DREAM complexes reinforce SPR-5/MET-2 reprogramming in the soma. a) SPR-5 and MET-2 maternally reprogram germline genes at fertilization by removing H3K4me1/2 (green) and H3K9me1/2 (orange). b) MEC NuRD and DREAM complexes reinforce SPR-5/MET-2 reprogamming at germline genes in the soma. c) In addition to reinforcing SPR-5/MET-2 reprogamming at germline genes, MEC NuRD and DREAM complexes reinforce non-SPR-5/MET-2 target genes in the soma.

The DREAM and MEC NuRD complexes may function directly at SPR-5/MET-2 maternal reprogramming targets or indirectly through other genes. To distinguish between these possibilities, we re-analyzed previously published DREAM and MEC NuRD complex ChIP data. Both the DREAM and MEC NuRD complexes are enriched at SPR-5/MET-2 maternal reprogramming targets, suggesting they act directly on SPR-5/MET-2 maternal reprogramming targets. It is possible that the Dream and MEC NuRD complexes are individually acting on mutually exclusive subsets of SPR-5/MET-2 maternal reprogramming targets. Alternatively, the DREAM and MEC NuRD complexes may both be functioning together at SPR-5/MET-2 maternal reprogramming targets. To distinguish between these possibilities, we compared the ChIP binding profiles of the DREAM and MEC NuRD complex members to one another at SPR-5/MET-2 maternal reprogramming targets. The binding profiles of the DREAM and MEC NuRD complex members to SPR-5/MET-2 maternal reprogramming targets are remarkably similar, suggesting that they act together directly at SPR-5/MET-2 maternal reprogramming targets. Consistent with this, we find that MEP-1 binds to the genes that are upregulated by the loss of LIN-35 alone. The high degree of overlap in binding of LIN-35 and MEP-1 at SPR-5/MET-2 maternal reprogramming targets also raises the possibility that one of these complexes may be recruiting the other. For example, the DREAM complex may predominantly function to recruit the MEC NuRD complex. However, the function of the DREAM and MEC NuRD complexes at these targets does not appear to be redundant because loss of either the DREAM or MEC NuRD complex members is sufficient on their own to exacerbate the developmental delay phenotype.

A model for how the maternal reprogramming of histone methylation is reinforced by transcriptional repression and ATP-dependent chromatin remodeling

We propose that the DREAM and MEC NuRD complexes reinforce SPR-5/MET-2 maternal reprogramming through the following model (Fig. 6). H3K4me2 is acquired during transcription in the germline and functions as a transcriptional memory helping to maintain transcription of germline genes (Lee and Katz 2020). SPR-5 and MET-2 function at fertilization as a maternal epigenetic reprogramming switch to remove the H3K4me2 transcriptional memory and replace it with H3k9me2 to further repress these genes (Andersen and Horvitz 2007; Katz et al. 2009; Greer et al. 2014; Kerr et al. 2014). SPR-5/MET-2 maternal reprogramming is necessary to reset the entire genome to a ground state and prevent the H3K4me2 transcriptional memory from being inappropriately propagated to the next generation where it could result in the ectopic expression of germline genes in the embryo.

In the embryo of the next generation, the germline blastomere P4 is specified after just four cell divisions. H3K36me3, which is acquired during transcription in the germline via the transcription dependent H3K36 methyltransferase MET-1, is maintained in the embryo by a transcription independent H3K36 methyltransferase MES-4 (Furuhashi et al. 2010; Rechtsteiner et al. 2010; Kreher et al. 2018). This maintained H3K36me3 "bookmark" prevents the chromatin at germline genes from being repressed by maternal SPR-5/MET-2 reprogramming and facilitates the re-initiation of germline transcription to help specify the germline. The SPR-5/MET-2 maternal reprogramming and MES-4 bookmarking systems are mutually antagonistic, because in the absence of SPR-5/MET-2 maternal reprogramming, MES-4 inappropriately maintains H3K36me3 at germline genes in the soma. This results in the expression of germline genes in the soma (Carpenter et al. 2021).

Our work here suggests that the initiation phase of germline vs soma distinction, facilitated by SPR-5/MET-2 and MES-4, is then reinforced in the soma by the DREAM and MEC NuRD complexes. As the soma differentiates during late embryonic and larval development, transcription factors (TFs) drive tissue specific gene expression. To prevent cross-talk between these tissue-specific TFs and germline genes which could result in the inappropriate expression of germline genes in somatic tissues, the repression of chromatin at germline genes that was initiated by SPR-5/MET-2 reprogramming in the initiation phase is reinforced in the soma during a maintenance phase by the DREAM and MEC NuRD complexes. This function of the DREAM and MEC NuRD complexes during the maintenance phase is consistent with previous data showing that MEP-1 and LIN-35 have defects in germline vs

soma specification (Unhavaithaya et al. 2002; Wang et al. 2005; Wu et al. 2012; Rechtsteiner et al. 2019). The DREAM and MEC NuRD complexes also clearly bind in the soma to non-SPR-5/MET-2 germline targets. However, our RNAseq analysis between *spr-5*; *met-2* mutants and *spr-5*; *met-2* mutants upon knockdown of either *lin-35* or *mep-1* demonstrates that only *spr-5*; *met-2* target genes are affected by further loss of *lin-35* or *mep-1*. This suggest that even if LIN-35 and MEP-1 bind to non-SPR-5/MET-2 target genes, loss of LIN-35 or MEP-1 does not transcriptionally depress these non-SPR-5/MET-2 target genes. It is possible that this is due to overlap with another type of somatic transcriptional repression.

Why is transcriptional repression and ATP-dependent chromatin remodeling required in the soma to reinforce SPR-5/MET-2 maternal epigenetic reprogramming?

In the very early *C. elegans* embryo, there is no transcription (Edgar et al. 1994; Seydoux and Dunn 1997; Baugh et al. 2003). Even after transcription begins in the *C. elegans* embryo at the 8 to 16 cell stage, the bulk of transcriptional elongation does not occur until the ~60 cell stage. As a result, the *C. elegans* embryos can survive until the 100-cell stage without RNA polymerase II (RNA pol II) because embryogenesis runs on maternally contributed factors (Powell-Coffman et al. 1996). Because of this lack of transcription in the early *C. elegans* embryo, the loss of H3K4me2 and addition of H3K9me2 may be sufficient in the early embryo to establish a repressed state genome wide. However, during later embryogenesis and the larval stages, somatic tissues differentiate and have significant tissue specific gene expression driven by tissue specific transcription factors (Murray et al. 2008). Because the tissue-specific TF binding sites may overlap slightly with the promoters of germline genes, there may be a more stringent requirement to block gene expression at multiple levels at these later stages; including via ATP dependent chromatin remodeling, histone deacetylation and transcriptional repression. This could potentially be why the DREAM and MEC NuRD are required in the soma to reinforce SPR-5/MET-2 maternal epigenetic reprogramming during the maintenance phase of germline vs soma specification.

Our data demonstrate that SPR-5/MET-2 maternal reprogramming is not sufficient to fully repress germline transcription in the soma. But how do the DREAM and MEC NuRD complexes provide additional gene repression that is distinct from loss of H3K4me2 and acquisition of H3K9me2? DREAM is thought to function as a transcriptional repressor, but does not have any known enzymatic activity associated with the complex (Hoareau et al. 2024). Thus it may function by binding to promoters and specifically competing against TF binding and RNA Pol II binding to prevent transcriptional initiation. Alternatively, or in addition, the DREAM complex may recruit the MEC NuRD complex, but this remains to be tested. The MEC NuRD complex has both ATP-dependent chromatin remodeling activity and HDAC activity associated with it (Xue et al. 1998). The ATP-dependent chromatin remodeling activity of NuRD and the HDAC activity associated with the NuRD complex are thought to function by restricting nucleosome spacing and tightening the association between nucleosomes and DNA (Denslow and Wade 2007). It is not entirely clear why ATP-dependent chromatin remodeling, histone deacetylation and transcriptional repression are needed on top of the addition of the loss of H3K4me2 and addition of H3K9me2. However, our finding that loss of either DREAM or the MEC NuRD complex exacerbates the transcriptional changes and developmental delay of *spr-5*; *met-2* double mutants indicates that the loss of H3K4me2 and addition of H3K9me2 are not functionally equivalent to gene repression via ATP dependent chromatin remodeling, histone deacetylation and transcriptional repression. Thus, our work provides insight into the relationship between histone modifications, nucleosome remodeling, and transcriptional repressors. Specifically, it suggests that to fully repress transcription, it may be necessary to block transcription at each of these levels.

Data availability

Raw and processed RNAseq files have been deposited into Gene Expression Omnibus (www.ncbi.nlm.nih.gov/geo) under accession code GSE303062 (*lin-35* RNAi RNAseq) and GSE303085 (*mep-1* RNAi RNAseq).

Supplemental material available at GENETICS online.

Acknowledgments

NuRD and DREAM complex RNAi enhancer screens were piloted by undergraduate students at Oglethorpe University as part of the *C. elegans* Pipeline CURE embedded in their biology major. The research cohort included 114 students (68 students from Developmental Biology [BIO 313—SP 2018, SP 2020 and SP2022] and 46 students from Research in Epigenetics [BIO 455—FA 2018, FA 20219, FA 2021 and FA 2023]). The Pipeline CURE supported and continues to support the Broader Impact goals of NSF Grants IOS1354998, IOS 1931697, and IOS2418128. We thank the *Caenorhabditis* Genetics Center (funded by NIH P40 OD010440) for strains and WormBase (WS292).

Funding

This work was funded by a grant to BC (NIH-1R15GM148887), Kennesaw State University (Jazmin Dozier NIH U-RISE T34GM140948), MR (NIH-T32GM008490-28 and T32GM149422-01), JR (NIH-F31HD100145-03S1 and T32GM008490-21), OB (NIH-K12GM00680-15 and 5F32HD097920-03), SG (NIH-T32NS096050, P30 AG066511, and T32AG087922-01), and DK (NSF IOS1354998).

Conflicts of interest

None declared.

Literature cited

Ancelin K *et al.* 2016. Maternal LSD1/KDM1A is an essential regulator of chromatin and transcription landscapes during zygotic genome activation. Elife. 5:e08851. https://doi.org/10.7554/eLife.08851.

Andersen EC, Horvitz HR. 2007. Two C. elegans histone methyltransferases repress lin-3EGF transcription to inhibit vulval development. Development. 134:2991–2999. https://doi.org/10.1242/dev.009373.

Baugh LR, Hill AA, Slonim DK, Brown EL, Hunter CP. 2003. Composition and dynamics of the Caenorhabditis elegans early embryonic transcriptome. Development. 130:889–900. https://doi.org/10.1242/dev.00302.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Brenner S. 1974. The genetics of caenorhabditis elegans. Genetics. 77:71–94. https://doi.org/10.1093/genetics/77.1.71.

Burton A, Torres-Padilla M-E. 2014. Chromatin dynamics in the regulation of cell fate allocation during early embryogenesis. Nat Rev Mol Cell Biol. 15:723–735. https://doi.org/10.1038/nrm3885.

Carpenter BS *et al.* 2021. Caenorhabditis elegans establishes germline versus soma by balancing inherited histone methylation. Development. 148:dev196600. https://doi.org/10.1242/dev.196600.

Chen EY *et al.* 2013. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinform. 14:128. https://doi.org/10.1186/1471-2105-14-128.

Costello ME, Petrella LN. 2019. C. elegans synMuv B proteins regulate spatial and temporal chromatin compaction during development. Development. 146:dev174383. https://doi.org/10.1242/dev.174383.

Cui M, Kim EB, Han M. 2006. Diverse chromatin remodeling genes antagonize the Rb-involved SynMuv pathways in C. elegans. PLoS Genet. 2:e74. https://doi.org/10.1371/journal.pgen.0020074.

Denslow SA, Wade PA. 2007. The human Mi-2/NuRD complex and gene regulation. Oncogene. 26:5433–5438. https://doi.org/10.1038/sj.onc.1210611.

Dick FA, Rubin SM. 2013. Molecular mechanisms underlying RB protein function. Nat Rev Mol Cell Biol. 14:297–306. https://doi.org/10.1038/nrm3567.

Edgar LG, Wolf N, Wood WB. 1994. Early transcription in Caenorhabditis elegans embryos. Development. 120:443–451. https://doi.org/10.1242/dev.120.2.443.

Furuhashi H *et al.* 2010. Trans-generational epigenetic regulation of C. elegans primordial germ cells. Epigenet Chromatin. 3:15. https://doi.org/10.1186/1756-8935-3-15.

Gal C *et al.* 2021. DREAM represses distinct targets by cooperating with different THAP domain proteins. Cell Rep. 37:109835. https://doi.org/10.1016/j.celrep.2021.109835.

Gaydos LJ, Wang W, Strome S. 2014. H3K27me and PRC2 transmit a memory of repression across generations and during development. Science. 345:1515–1518. https://doi.org/10.1126/science.1255023.

Goetsch PD, Garrigues JM, Strome S. 2017. Loss of the Caenorhabditis elegans pocket protein LIN-35 reveals MuvB's innate function as the repressor of DREAM target genes. PLoS Genet. 13:e1007088. https://doi.org/10.1371/journal.pgen.1007088.

Greer EL *et al.* 2014. A histone methylation network regulates transgenerational epigenetic memory in C. elegans. Cell Rep. 7:113–126. https://doi.org/10.1016/j.celrep.2014.02.044.

Gu Z, Eils R, Schlesner M. 2016. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics. 32:2847–2849. https://doi.org/10.1093/bioinformatics/btw313.

Hirabayashi Y, Gotoh Y. 2010. Epigenetic control of neural precursor cell fate during development. Nat Rev Neurosci. 11:377–388. https://doi.org/10.1038/nrn2810.

Hoareau M, Rincheval-Arnold A, Gaumer S, Guénal I. 2024. DREAM a little dREAM of DRM: model organisms and conservation of DREAM-like complexes. BioEssays. 46:e2300125. https://doi.org/10.1002/bies.202300125.

Jambhekar A, Dhall A, Shi Y. 2019. Roles and regulation of histone methylation in animal development. Nat Rev Mol Cell Bio. 20:625–641. https://doi.org/10.1038/s41580-019-0151-1.

Kamath RS, Ahringer J. 2003. Genome-wide RNAi screening in Caenorhabditis elegans. Methods. 30:313–321. https://doi.org/10.1016/S1046-2023(03)00050-1.

Kaneshiro KR, Rechtsteiner A, Strome S. 2019. Sperm-inherited H3K27me3 impacts offspring transcription and development in C. elegans. Nat Commun. 10:1271. https://doi.org/10.1038/s41467-019-09141-w.

Katz DJ, Edwards TM, Reinke V, Kelly WG. 2009. A C. elegans LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. Cell. 137:308–320. https://doi.org/10.1016/j.cell.2009.02.015.

Kerr SC, Ruppersburg CC, Francis JW, Katz DJ. 2014. SPR-5 and MET-2 function cooperatively to reestablish an epigenetic ground state during passage through the germ line. Proc National Acad Sci. 111:9509–9514. https://doi.org/10.1073/pnas.1321843111.

Kim D, Langmead B, Salzberg SL. 2015b. HISAT: a fast spliced aligner with low memory requirements. Nat Methods. 12:357–360. https://doi.org/10.1038/nmeth.3317.

Kim J *et al.* 2015a. LSD1 is essential for oocyte meiotic progression by regulating CDC25B expression in mice. Nat Commun. 6:10116. https://doi.org/10.1038/ncomms10116.

Kim J *et al.* 2016. Maternal Setdb1 is required for meiotic progression and preimplantation development in mouse. Plos Genet. 12:e1005970. https://doi.org/10.1371/journal.pgen.1005970.

Kreher J *et al.* 2018. Distinct roles of two histone methyltransferases in transmitting H3K36me3-based epigenetic memory across generations in Caenorhabditis elegans. Genetics. 210:969–982. https://doi.org/10.1534/genetics.118.301353.

Kuleshov MV *et al.* 2016. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 44:W90–W97. https://doi.org/10.1093/nar/gkw377.

Lee TW, Katz DJ. 2020. Hansel, gretel, and the consequences of failing to remove histone methylation breadcrumbs. Trends Genet. 36:160–176. https://doi.org/10.1016/j.tig.2019.12.004.

Liao Y, Smyth GK, Shi W. 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. 30:923–930. https://doi.org/10.1093/bioinformatics/btt656.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15:550. https://doi.org/10.1186/s13059-014-0550-8.

Lu X, Horvitz HR. 1998. lin-35 and lin-53, two genes that antagonize a C. elegans Ras pathway, encode proteins similar to Rb and its binding protein RbAp48. Cell. 95:981–991. https://doi.org/10.1016/S0092-8674(00)81722-5.

Murray JI *et al.* 2008. Automated analysis of embryonic gene expression with cellular resolution in C. elegans. Nat Methods. 5:703–709. https://doi.org/10.1038/nmeth.1228.

Öst A *et al.* 2014. Paternal diet defines offspring chromatin state and intergenerational obesity. Cell. 159:1352–1364. https://doi.org/10.1016/j.cell.2014.11.005.

Passannante M *et al.* 2010. Different Mi-2 complexes for various developmental functions in caenorhabditis elegans. PLoS One. 5:e13681. https://doi.org/10.1371/journal.pone.0013681.

Petrella LN *et al.* 2011. synMuv B proteins antagonize germline fate in the intestine and ensure C. elegans survival. Development. 138:1069–1079. https://doi.org/10.1242/dev.059501.

Powell-Coffman JA, Knight J, Wood WB. 1996. Onset of C. elegans gastrulation is blocked by inhibition of embryonic transcription with an RNA polymerase antisense RNA. Dev Biol. 178:472–483. https://doi.org/10.1006/dbio.1996.0232.

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. https://doi.org/10.1371/journal.pgen.1001091.

Rechtsteiner A *et al.* 2019. Repression of germline genes in Caenorhabditis elegans somatic tissues by H3K9 dimethylation of their promoters. Genetics. 212:125–140. https://doi.org/10.1534/genetics.118.301878.

Seydoux G, Dunn MA. 1997. Transcriptionally repressed germ cells lack a subpopulation of phosphorylated RNA polymerase II in

early embryos of caenorhabditis elegans and Drosophila melanogaster. Development. 124:2191–2201. https://doi.org/10.1242/dev.124.11.2191.

Siklenka K *et al.* 2015. Disruption of histone methylation in developing sperm impairs offspring health transgenerationally. Science. 350: aab2006–aab2006. https://doi.org/10.1126/science.aab2006.

Stewart KR *et al.* 2015. Dynamic changes in histone modifications precede de novo DNA methylation in oocytes. Genes Dev. 29: 2449–2462. https://doi.org/10.1101/gad.271353.115.

Tabuchi TM *et al.* 2018. Caenorhabditis elegans sperm carry a histone-based epigenetic memory of both spermatogenesis and oogenesis. Nat Commun. 9:4310. https://doi.org/10.1038/s41467-018-06236-8.

Unhavaithaya Y *et al.* 2002. MEP-1 and a homolog of the NURD complex component Mi-2 act together to maintain germline-soma distinctions in C. elegans. Cell. 111:991–1002. https://doi.org/10.1016/S0092-8674(02)01202-3.

Wang D *et al.* 2005. Somatic misexpression of germline P granules and enhanced RNA interference in retinoblastoma pathway mutants. Nature. 436:593–597. https://doi.org/10.1038/nature04010.

Wasson JA *et al.* 2016. Maternally provided LSD1/KDM1A enables the maternal-to-zygotic transition and prevents defects that manifest postnatally. Elife. 5:e08848. https://doi.org/10.7554/eLife.08848.

Wingett SW, Andrews S. 2018. FastQ screen: a tool for multi-genome mapping and quality control. F1000Res. 7:1338. https://doi.org/10.12688/f1000research.15931.1.

Wu X, Shi Z, Cui M, Han M, Ruvkun G. 2012. Repression of germline RNAi pathways in somatic cells by retinoblastoma pathway chromatin complexes. PLoS Genet. 8:e1002542. https://doi.org/10.1371/journal.pgen.1002542.

Xue Y *et al.* 1998. NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. Mol Cell. 2:851–861. https://doi.org/10.1016/S1097-2765(00)80299-3.

Zheng H *et al.* 2016. Resetting epigenetic memory by reprogramming of histone modifications in mammals. Mol Cell. 63:1066–1079. https://doi.org/10.1016/j.molcel.2016.08.032.

Editor: G. Seydoux