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THE RELATIVE ROLES OF H3K9ME AND H3K27ME IN REPRESSION IN THE *CAENORHABDITIS ELEGANS* GERMLINE

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ABSTRACT

The Relative Roles of H3K9me and H3K27me in Repression in the *Caenorhabditis elegans* Germline

Braden John Larson

The germ lineage is unique among cell types in that it must maintain its own cellular identity while simultaneously maintaining totipotency, the ability to generate all other cell types. To achieve this, germ cells package their genome into domains of transcriptionally active euchromatin and transcriptionally inactive heterochromatin to maintain expression of germline-appropriate genes and repression of germline-inappropriate genes. Germ cells must also differentiate between heterochromatin domains that are relatively permanent (constitutive heterochromatin) and heterochromatin domains that are temporary (facultative heterochromatin) and are activated at another time or in a different cell type. Precisely how constitutive and facultative heterochromatin promote germline development and function is not known. In *C. elegans*, the constitutive heterochromatin mark H3K9me and the facultative heterochromatin mark H3K27me are required for germline development in subsequent generations and have been shown to redundantly repress the single X chromosome in the male germline and promote germline development in offspring. Surprisingly, domains of H3K9me and H3K27me display significant overlap in *C. elegans*, suggesting that constitutive and facultative heterochromatin may be less distinct in *C. elegans* than in other organisms. Together, these observations suggest that H3K9me and H3K27me have at least some redundant roles in repressing genes to promote germline development in *C. elegans*. I determined the relative roles of H3K9me and H3K27me in gene repression in adult *C. elegans* germlines to promote

germline development in offspring. I used RNA-sequencing to identify the gene targets of H3K9me alone, of H3K27me alone, and of the two marks redundantly in adult hermaphrodite and male germlines. I found that oogenesis genes on the X chromosome are a particular focus of repression in the germline and that repression of the X is primarily carried out by H3K27me alone in the hermaphrodite germline and primarily by H3K9me and H3K27me redundantly in the male germline. I utilized these differences in X repression by H3K9me and H3K27me between hermaphrodite and male germlines to identify a set of genes whose upregulation causes sterility in offspring. I found that the activity of the transcription factors LIN-15B, LIN-54, and possibly EFL-1 contribute to sterility in offspring from germlines that lack H3K9me and H3K27me. I synthesize my findings with those in the literature to present a model of X repression through germline development and how the roles of H3K9me and H3K27me in X repression differ between hermaphrodites and males.

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CHAPTER 1

Introduction

The germline and soma

Multicellular organisms contain hundreds of different cell types but only germ cells are capable of creating the next generation. While somatic cells perish with the organisms, gametes (oocyte and sperm) produced by the germline live on in the offspring they generate. When oocyte and sperm merge, they create a single totipotent cell capable of generating every cell type. During development, the germ lineage must maintain its own cellular identity while simultaneously maintaining totipotency (Figure 1). In adult gonads, germ cells proliferate, perform the complex process of meiosis and generate gametes. For germ cells to develop and carry out their various functions they must regulate gene expression by expressing genes important for germline development and function while repressing genes involved in the development and function of somatic cells. Loss of germline gene expression or failure to repress somatic genes can cause failures in germline development and function that lead to sterility and infertility. Precisely how germ cells carry out gene regulation is not fully understood. A primary mechanism employed by germ cells is the regulation of the accessibility of a gene to transcriptional machinery through the wrapping of DNA around histones to form chromatin.

Chromatin regulation and its role in development

The packaging of eukaryotic genomes into chromatin is inherently restrictive to transcription (Kornberg and Lorch 2020). The fundamental unit of chromatin, called a

nucleosome, consists of a wedge-shaped histone octamer core (containing 2 dimers each of H3-H4 and H2A-H2B) wrapped by ~ 147 bp of DNA (Figure 2B) (Luger *et al.* 1997). Nucleosomes are connected by linker DNA which is variable in length (10-70 bp) depending on the degree of chromatin compaction. While linker DNA is accessible to protein binding, nucleosomal DNA is occluded from recognition by other proteins. Thus nucleosomes in promoter regions inhibit formation of transcription initiation complexes (Lorch *et al.* 1987). As transcription proceeds, nucleosomes encountered by RNA polymerase are partially unwrapped or evicted if multiple polymerases pass in quick succession (Kulaeva *et al.* 2013).

Modifications to histone tails play important regulatory roles in nucleosome assembly/ disassembly and formation of higher order chromatin structures. The N-terminal tails of all four histones extend beyond the nucleosome core. Many of the amino acids in the histone tails are targets of post-translational modifications such as acetylation, methylation, phosphorylation, and ubiquitylation (Zhang *et al.* 2015). These histone 'marks' can alter the strength of the histone-DNA interaction and recruit proteins that effect changes to chromatin packaging (Morrison and Thakur 2021). Histone H3 binds at the center of the wrapped DNA (dyad) as well as the entering and exiting DNA and is a particular focus of regulatory modification .

Several H3 modifications have been associated with gene expression states and have been used to define transcriptionally active euchromatin and inactive heterochromatin (Zhang *et al.* 2015). Methylation of Lys4 and Lys36 on histone H3 (H3K4me, H3K36me) are canonical marks of transcriptionally active genes and used to define domains of euchromatin (Figure 2C). H3K4me is found at transcription start sites and promotes transcription by recruiting proteins involved in transcription

initiation (Shilatifard 2012). H3K36me is found in gene bodies and is thought to repress transcription at cryptic initiation sites and to repel marks of heterochromatin (Huang and Zhu 2018). Heterochromatin is broadly divided into two subtypes, each with its own signature histone marks (Figure 2A). Constitutive heterochromatin, marked by methylation of Lys9 on histone H3 (H3K9me), is considered a more permanent state of repression and often found at repeat-rich and gene-poor sequences like centromeres and telomeres (Figure 2C)(Padeken *et al.* 2022). Facultative heterochromatin, marked by methylation od Lys27 on histone H3, is associated with sequences that are temporarily transcriptionally repressed and may become expressed later such as lineage specific developmental genes (Figure 2C) (Laugesen *et al.* 2019). Generally, domains of euchromatin, constitutive heterochromatin and facultative heterochromatin, along with their associated marks, occupy separate and distinct regions. However, transcriptionally inactive “bivalent” chromatin domains containing the euchromatin mark H3K4me and the facultative heterochromatin mark H3K27me are prevalent in embryonic stem cells at developmentally regulated genes and are thought to be poised for rapid activation as cells differentiate (Kumar *et al.* 2021). Additionally, marks of constitutive (H3K9me) and facultative H3K27me) heterochromatin generally occupy separate domains but the degree to which they anticorrelate varies between species (Ho *et al.* 2014; Wiles and Selker 2017).

Loss of chromatin marks or changes in their localization often lead to severe phenotypes such as cancer, sterility, or death. Study of histone marks in many model organisms has proven difficult as there are often several enzymes that deposit a mark and/ or complete loss of a histone mark is fatal for the embryo or cell. However,

mutant *C. elegans* lacking H3K4me, H3K36me, H3K27me or H3K9me have been found to be healthy and fertile but produce sterile offspring in the next generation (loss of H3K36me or H3K27me) or over several generations (loss of H3K4me or H3K9me) (Capowski *et al.* 1991; Paulsen *et al.* 1995; Bender *et al.* 2004, 2006; Xiao *et al.* 2011; Zeller *et al.* 2016). This makes *C. elegans* an excellent model for studying the role of histone marks and chromatin regulation in germline development (Figure 3).

The *C. elegans* germline and Mes mutants

Mutations in the genes encoding the *C. elegans* H3K36me and H3K27me histone methyltransferases (HMTs) were identified in a forward genetic screen as *mes* for "maternal-effect sterile" (Capowski *et al.* 1991). The first generation of *mes* mutants develop normally and are fertile but their offspring are sterile due to degeneration of the nascent germline during early larval development (Paulsen *et al.* 1995). MES-4 deposits H3K36me and is homologous to mammalian NSD-1, NSD2, and NSD3 (Bender *et al.* 2006). MES-2, MES-3, and MES-6 form the *C. elegans* version of the conserved Polycomb Repressive Complex 2 (PRC2) which deposits H3K27me (Figure 4A)(Fong *et al.* 2002; Bender *et al.* 2004). Each of the three components of *C. elegans* PRC2 are necessary for H3K27me in the germline, and in vitro. However, another unidentified HMT generates H3K27me in somatic cells (Holdeman *et al.* 1998; Korf *et al.* 1998; Bender *et al.* 2004; Ketel *et al.* 2005). Loss of either H3K36me or H3K27me results in upregulation of many genes, especially X-linked genes, in adult germlines that produce sterile offspring suggesting repression of the

X in parental germlines is important for germline development in offspring (Gaydos *et al.* 2012).

While all *PRC2* hermaphrodite offspring are sterile, *PRC2* male offspring can be fertile in a manner that depends on the gamete origin of the X chromosome (Figure 4C)(Gaydos *et al.* 2014). There are two sexes in *C. elegans*: hermaphrodites with two X chromosomes (XX) and males with only a single (XO). Male *C. elegans* normally inherit their single X from the oocyte (X^{oo}) and no X from the sperm. However, hermaphrodites with mutations that cause errors in meiotic X chromosome segregation produce oocytes lacking an X chromosome and, when mated with a male, can produce male offspring that receive their single X from the sperm (X^{sp}) (Hodgkin *et al.* 1979). Male offspring of *PRC2* mutants that inherit X^{oo} are sterile while male offspring that inherit X^{sp} are usually fertile (Gaydos *et al.* 2014). Intriguingly, the fertility of these *PRC2* male offspring depends on H3K9me (Gaydos *et al.* 2014). Additionally, by immunofluorescence, RNA polymerase is not observed on the X chromosome in the germline *PRC2* mutant fathers suggesting H3K9me maintains repression of the single male X in the absence of H3K27me (Gaydos *et al.* 2014). Together, these observations indicate that H3K9me and H3K27me redundantly repress transcription of the X chromosome in male germlines to promote fertility in the next generation of males.

How do H3K9me and H3K27me regulate genes to ensure proper germline development in *C. elegans*?

H3K9me and H3K27me are canonical marks of constitutive and facultative heterochromatin respectively. Analyses of the genomic patterns of these marks by ChIP showed that H3K9me and H3K27me anticorrelate in species like humans and *N. crassa*, have no correlation in *D. melanogaster*, and have an unusual positive correlation in *C. elegans* (Ho *et al.* 2014; Wiles and Selker 2017). Together, with the evidence that H3K9me and H3K27me redundantly repress the X chromosome of males, these observations suggest that H3K9me and H3K27me have independent and redundant roles in repressing genes to promote germline development in *C. elegans*. In chapter 2, I determined the relative roles of H3K9me and H3K27me in gene repression in adult *C. elegans* germlines to promote germline development in offspring.

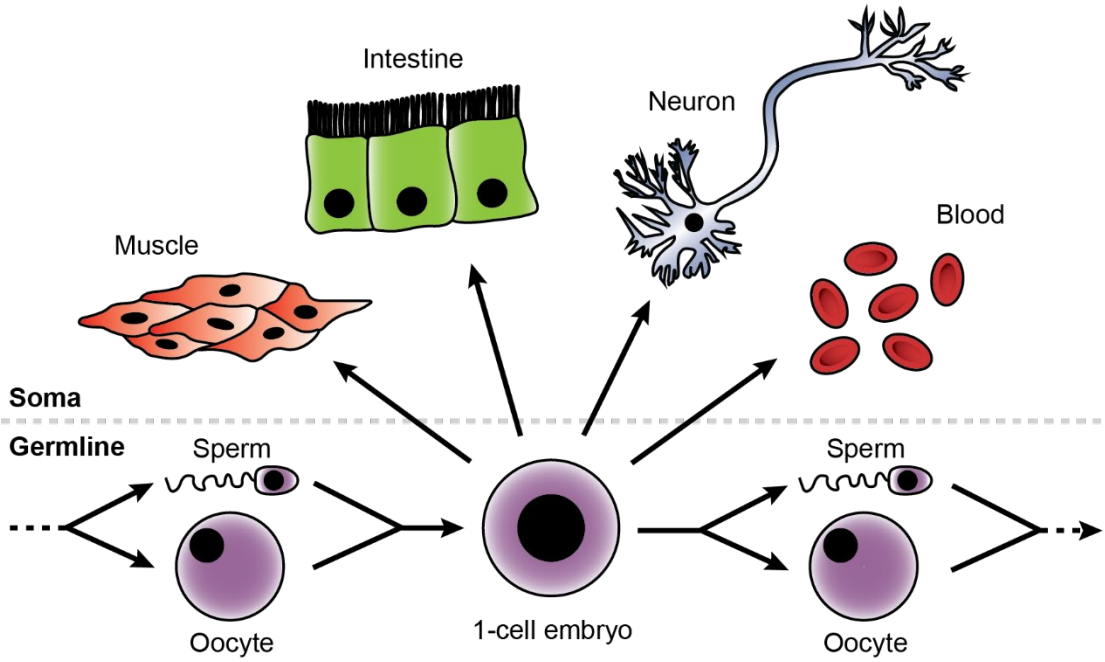

Figure 1. The germline and soma

Gametes (oocyte and sperm) combine to create a single cell with the ability to generate all cell types (totipotency). During development, the germ lineage must maintain its own cellular identity while also producing daughter cells that differentiate into somatic cells (e.g. muscle, intestine, neurons, blood). While somatic cells perish with each generation, the germ lineage is contiguous across generations as it creates the next generation through the production of gametes.

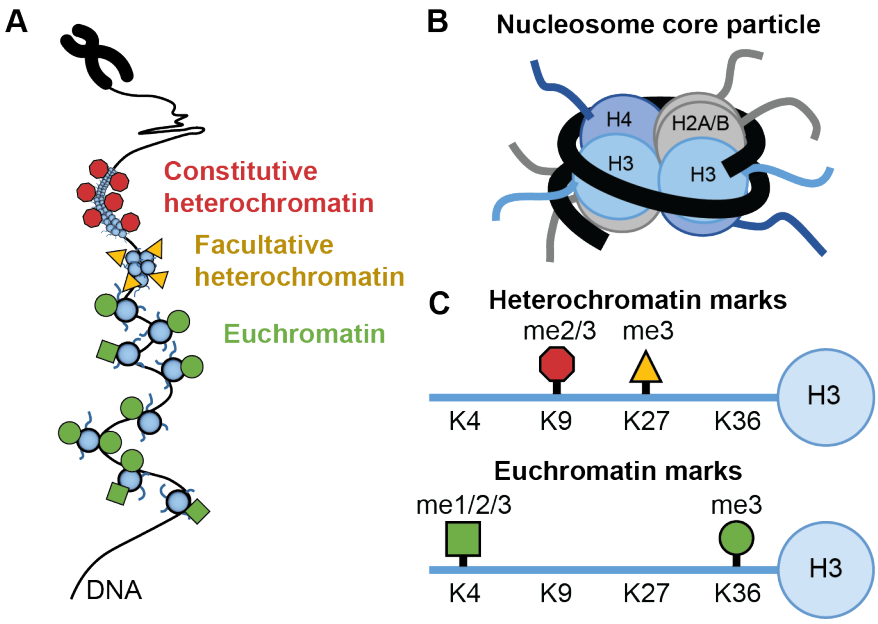

Figure 2. Types of chromatin and associated marks

(A) Diagram of DNA wrapped around histones to form chromatin. Different degrees of higher order chromatin packing are displayed and labeled. Euchromatin (green) is loosely packed and transcriptionally active. Facultative heterochromatin (yellow) is more tightly packed and transcriptionally repressed but transcription may be activated at another time. Constitutive heterochromatin (red) is tightly packed and maintains a relatively permanent state of transcriptional repression. (B) Diagram of the nucleosome core particle comprised of DNA wrapped around a histone octamer; two dimers each of H3-H4 and H2A-H2B. Histone tails extend beyond the nucleosome center and act as sites of chromatin regulation through post-translational modification of residues in the tails. (C) Post-translational modifications of the histone H3 tail and their association with euchromatin or heterochromatin. H3K9me (red octagon) is a mark of constitutive heterochromatin. H3K27me (yellow triangle) is a mark of facultative heterochromatin. H3K4me (green square) and H3K36me (green circle) are marks of euchromatin.

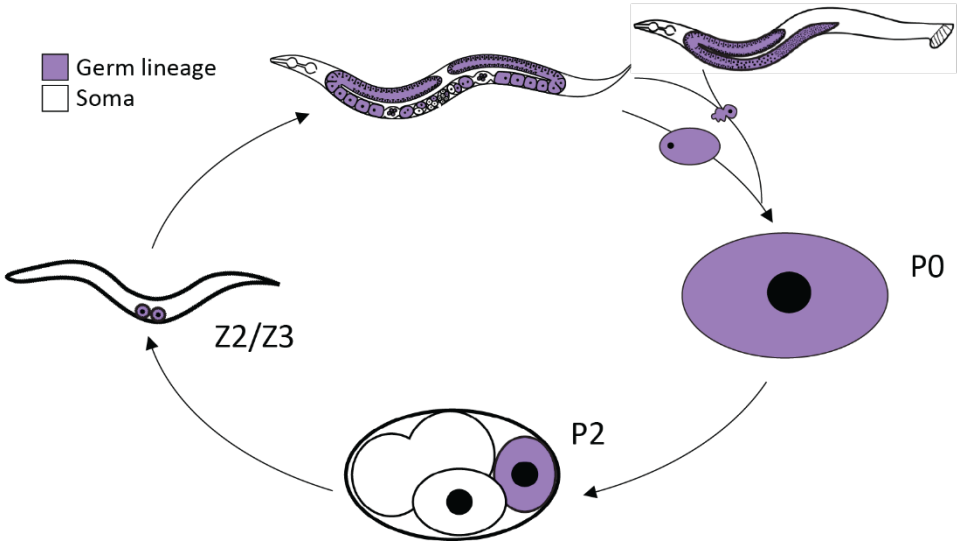

Figure 3. The life cycle of the C. elegans germ lineage.

The *C. elegans* germ lineage (purple) begins as a single cell (P0). For the first four cell divisions, germ identity is inherited by only the most posterior daughter cell: P1-P4 corresponding to the number of divisions. The fifth division of the germ lineage produces two germ cells (Z2/Z3) which remain quiescent until feeding during the first larval stage and generate all adult germ cells. *C. elegans* hermaphrodites produce both egg and sperm allowing for self-fertilization. Alternatively, hermaphrodites can be crossed to males whose sperm will be preferentially used for fertilization.

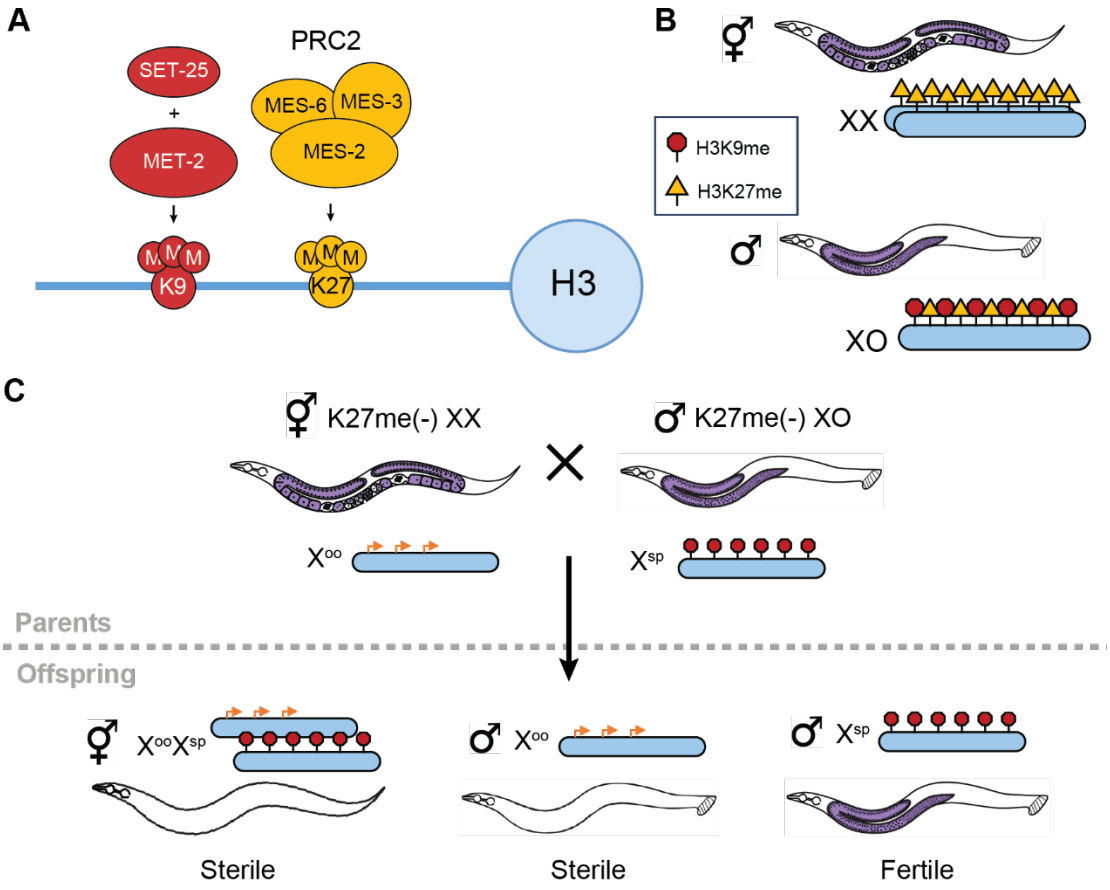

Figure 4. H3K9me and H3K27me redundantly repress the male X

(A) In *C. elegans*, all H3K9me is deposited by MET-2 and SET-25. H3K27me is deposited by MES-2, MES-3, and MES-6 which form the worm version of the conserved Polycomb Repressive Complex 2 (PRC2). (B) In the germline, the two hermaphrodite X chromosomes are enriched for only H3K27me while the single male X is enriched for both H3K9me and H3K27me. (C) Loss of H3K27me (K27me-) causes sterility in offspring that inherit a K27me(-) X from the oocyte (X^{oo}) while male offspring that inherit a K27me(-) X from the sperm (X^{sp}) are usually fertile.

CHAPTER 2

The Relative Roles of H3K9me and H3K27me in Repression in the *Caenorhabditis elegans* Germline

Abstract

Eukaryotes package transcriptionally repressed chromatin into two states that bear distinct marks on histone H3: constitutive heterochromatin is marked with methylated H3K9 (H3K9me), and facultative heterochromatin is marked with methylated H3K27 (H3K27me). In *C. elegans*, H3K9me and H3K27me domains display a greater degree of overlap than in most animals studied to date. This raises the possibilities that in worms, heterochromatin marked with H3K9me and H3K27me are not as distinct as in other organisms and that H3K9me and H3K27me cooperate to repress shared target genes. Indeed, at the cellular level, potential functional redundancy between H3K9me and H3K27me has been observed in X chromosome repression of male germlines which has consequences for offspring fertility. We sought to identify genes repressed by H3K9me, by H3K27me, and those redundantly repressed by both marks. To this end we measured differential expression of genes in oogenic and spermatogenic germlines lacking H3K9me, H3K27me, or both marks compared to wild type. We found that H3K27me represses many more genes than H3K9me in both oogenic and spermatogenic germlines. Genes repressed in germlines by H3K27me are enriched for genes that are typically expressed in somatic tissues, especially neurons, and X-linked genes that are repressed during germline mitosis and meiosis but become transcriptionally active during oogenesis. Compared to oogenic germlines, spermatogenic germlines employ H3K9me and H3K27me redundantly to a greater degree in repression of X-linked oogenesis genes. Two

situations lead to severely reduced proliferation of germ cells and sterility in offspring: inheritance of oocyte chromosomes lacking H3K27me or inheritance of sperm chromosomes lacking both H3K27me and H3K9me. The genes upregulated in the germlines of both mutant parents are dramatically enriched for X-linked oogenesis genes, including the THAP transcription factor gene *lin-15B*. We find that RNAi depletion of LIN-15B partially rescues fertility in offspring of mutant parents adding to a growing body of evidence that ectopic LIN-15B activity is a major contributor to sterility (Lee *et al.* 2017; Cockrum and Strome 2022). Altogether, our findings identify and characterize the uniquely and redundantly repressed gene targets of H3K27me and H3K9me in *C. elegans* germlines and identify genes on the X that normally turn on during oogenesis as major targets of repression in both oogenic and spermatogenic germlines.

Introduction

Organization of the genome into domains of transcriptionally active euchromatin and transcriptionally inactive heterochromatin is a conserved and essential feature of eukaryotes. These two types of chromatin are often distinguished by their associated histone tail post-translational modifications, often referred to as “marks” (Morrison and Thakur 2021). Heterochromatin is broadly divided into two subtypes, each with its own signature histone marks. Constitutive heterochromatin, marked by di- and trimethylation of Lys9 on histone H3 (H3K9me2/3), is generally associated with repeat-rich and gene-poor sequences (e.g. centromeres and telomeres) in the genome and is considered a more permanent state of repression (Padeken *et al.* 2022). Facultative heterochromatin, marked by trimethylation of Lys27 on histone H3

(H3K27me3), is associated with sequences that are temporarily transcriptionally repressed and may become expressed later such as lineage specific developmental genes (Laugesen *et al.* 2019). Based on chromatin immunoprecipitation (ChIP) analysis in diverse organisms, H3K9me2/3 and H3K27me3 generally occupy mutually exclusive regions of the genome, although the degree to which these marks anticorrelate varies between species (Ho *et al.* 2014; Wiles and Selker 2017). Surprisingly, in *C. elegans*, H3K9me and H3K27me significantly overlap, suggesting that constitutive and facultative heterochromatin are less distinct or that they are distinguished through different molecular mechanisms (Liu *et al.* 2011; Ho *et al.* 2014; Garrigues *et al.* 2015; Ahringer and Gasser 2018). This paper investigates the targets of H3K9me2/3 and H3K27me3 repression in the *C. elegans* genome, focusing on analysis of transcriptional changes in the germline of animals lacking H3K9me2/3, H3K27me3, or both.

In *C. elegans*, H3K9me2/3 is catalyzed by the HMTs MET-2 (homolog of SETDB1) and SET-25 (homolog of SUV39/G9a). MET-2 primarily generates H3K9me1 and me2, while SET-25 generates H3K9me3 (Towbin *et al.* 2012). Absence of both HMTs is tolerated at low-mid growth temperatures: *met-2 set-25* double mutants were viable and fertile when worms were grown at standard growth temperatures (15-20^{o}C)(Towbin *et al.* 2012; Garrigues *et al.* 2015; Zeller *et al.* 2016). At elevated temperature (25-26^{o}C), ~5% of M+Z- *met-2 set-25* double mutant hermaphrodites were sterile, while ~30% of M-Z- *met-2 set-25* double mutants were sterile (Garrigues *et al.* 2015). Upon propagation at elevated temperature, brood sizes were smaller and worms became completely sterile after two generations,

probably due to upregulation of repetitive elements and increased DNA damage and apoptosis in the germline (Zeller *et al.* 2016; McMurchy *et al.* 2017).

In *C. elegans*, H3K27me3 is catalyzed by the histone methyltransferase (HMT) polycomb repressive complex 2 (PRC2). Worm PRC2 is composed of MES-2 (the catalytic subunit and homolog of E(Z)/EZH2), MES-6 (homolog of ESC/EED), and MES-3 (novel protein)(Xu *et al.* 2001; Bender *et al.* 2004). The three subunits are necessary and sufficient to generate repressive H3K27me3 in the germline and embryos and *in vitro*; another unidentified HMT generates H3K27me3 in somatic cells in larvae and adults (Holdeman *et al.* 1998; Korf *et al.* 1998; Bender *et al.* 2004; Ketel *et al.* 2005). MES-2, MES-3, and MES-6 were discovered in a forward-genetic screen for maternal-effect sterile (mes) mutants (Capowski *et al.* 1991). *PRC2* mutant hermaphrodites that inherit a maternal load of enzyme but are unable to newly synthesize enzyme from the zygotic genome (called M+Z-) are fertile, while 100% of *PRC2* mutant hermaphrodites that lack both maternal and zygotically synthesized enzyme (called M-Z-) are sterile (diagrammed in Figure S1A), due to degeneration of the nascent germline early in larval development (Capowski *et al.* 1991; Paulsen *et al.* 1995). It was recently shown that degeneration of the nascent germline in M-Z- larvae is due to upregulation of X-linked genes, as discussed below (Cockrum and Strome 2022).

In comparative analyses of histone marks by ChIP in human cells, flies, and worms, H3K9me3 and H3K27me3 distributions anticorrelate in human cells, display no correlation in flies, and display a surprising degree of positive correlation in worms (Ho *et al.* 2014). In *C. elegans* embryos and L3 larvae, H3K9me2 and me3 are concentrated on the “arms” of the five autosomes (Liu *et al.* 2011; Ho *et al.* 2014;

Garrigues *et al.* 2015; Ahringer and Gasser 2018). Notably, the chromosomes of *C. elegans* are holocentric, with centromeres distributed intermittently along their lengths (Gassmann *et al.* 2012). The “arms” (~4 Mb at each end) are rich in repeats, preferentially associated with the nuclear lamina, and display lower gene expression and higher meiotic recombination than the chromosome “centers” (~8 Mb) (Ikegami *et al.* 2010; Liu *et al.* 2011; Garrigues *et al.* 2015; Ahringer and Gasser 2018). H3K27me3 is more widely distributed along the autosomes but shows some arm enrichment, where it often colocalizes with H3K9me3 (Liu *et al.* 2011; Garrigues *et al.* 2015; Ahringer and Gasser 2018). At the gene level, H3K9me2/3 associates preferentially with repetitive elements and silent tissue-specific genes (Zeller *et al.* 2016; Ahringer and Gasser 2018), and H3K27me3 associates with broad regulated domains containing genes that are not expressed in the germline but that turn on in a tissue-, stage- or condition-specific manner (Gassmann *et al.* 2012; Evans *et al.* 2016).

The X chromosome in *C. elegans* is a focus of chromosome-wide repression. Most of the X chromosome resembles an autosome “arm” in gene density and recombination rates but displays less enrichment of repeats and H3K9me2/3. Only a small region at the left end of the X is gene-rich like the center of an autosome. (Liu *et al.* 2011; Garrigues *et al.* 2015). Hermaphrodites, which are XX (diploids with two Xs), and males, which are XO (diploids with one X), display interesting differences in X repression in both soma and germline. In somatic tissues, the well-studied process of dosage compensation occurs in XX worms and reduces expression of genes on both Xs about two-fold, to equalize X-linked gene expression in animals with two Xs versus one X (Strome *et al.* 2014; Meyer 2022). The germline has a different

challenge. Because the single X in XO males lacks a pairing partner in meiosis, it undergoes “meiotic silencing of unpaired chromatin” (MSUC) via heterochromatinization (Kelly *et al.* 2002). Indeed, the single X in each male germline nucleus displays strong enrichment for H3K9me2 immunostaining (Kelly *et al.* 2002; Strome *et al.* 2014; Gaydos *et al.* 2014). Probably to match the situation in the male germline, both Xs in XX hermaphrodites are also globally repressed during the stages that hermaphrodite germlines share with male germlines, namely mitosis and early meiosis. In hermaphrodite germlines, genes on the X turn on during later meiosis, as oocytes form (Reinke *et al.* 2000, 2004). In contrast to the single X in male germline nuclei being enriched for H3K9me2, the paired Xs in hermaphrodite germline nuclei display enrichment for H3K27me3 immunostaining (Bender *et al.* 2004).

Initial insights into an interplay between repression by H3K27me3 and by H3K9me2/3 came from analyzing triple mutants defective in methylating H3K27 and H3K9. As noted above, 100% of M-Z- *PRC2* mutant hermaphrodites (lacking maternal and zygotic H3K27me3) are sterile, but their sibling M-Z- *PRC2* mutant males can be fertile (Gaydos *et al.* 2014). Remarkably, male fertility depends on the gamete source of the X: males that inherited their X from the oocyte are sterile, while males that inherited their X from the sperm are usually fertile (Gaydos *et al.* 2014). The fertility of these M-Z- *PRC2* mutant males depends on H3K9me, as evidenced by the sterility displayed by *PRC2; met-2 set-25* triple mutant males (lacking H3K27me3 and H3K9me2/3) (Gaydos *et al.* 2014)(diagrammed in Figure S1A). Thus, H3K27me3 and H3K9me2/3 collaborate to maintain repression of X-linked genes in the male germline and to ensure fertility in the next generation of males.

The present study sought to identify the gene targets of repression by H3K27me3, by H3K9me2/3, and redundantly by both marks, focusing on the germline of adult hermaphrodites and males. Our approach was to perform RNA-sequencing from germlines dissected from wild-type animals and fertile animals lacking H3K27me, lacking H3K9me, or lacking both marks and to perform differential expression analysis. Our main findings are: 1) H3K27me and H3K9me each repress more protein-coding genes than non-coding genes and transposable elements. 2) H3K27me represses many more genes than H3K9me in both hermaphrodite germlines and male germlines. 3) H3K27me-repressed genes are enriched for soma-specific genes, especially neuronal genes, as expected given that soma-specific genes should be kept silent in germline tissue. 4) Genes repressed by H3K27me alone and redundantly by H3K27me and H3K9me are enriched for X-linked oogenesis genes, which are normally repressed during the mitotic and early meiotic stages in the hermaphrodite germline and during all stages in the male germline. Compared to hermaphrodite germlines, X-linked genes in male germlines are more often redundantly repressed by H3K27me and H3K9me than by H3K27me alone. 5) Germlines that produce sterile offspring (hermaphrodite germlines lacking H3K27me and male germlines lacking both H3K27me and H3K9me) display upregulation of X-linked oogenesis genes, including *lin-15B*. Upregulation of *lin-15B* and to a lesser extent *lin-54* and *efl-1* contribute to sterility. These findings shed light on the relative roles of the two major repressive histone marks in *C. elegans* germ cells.

Materials and methods

Caenorhabditis elegans maintenance and genetics

C. elegans were maintained at 20°C on Nematode Growth Medium (NGM) plates seeded with *E. coli* OP50 as a food source (Brenner 1974). Males of each strain were initially generated by heat shocking L4 hermaphrodites at 30°C for 4 hours, and then maintained by mating hermaphrodites and males to generate male offspring.

Strains used in this study are listed below. Strains generated in this study (*) are indicated.

N2 - (Bristol) wild type

GW638 - *met-2(n4256) set-25(n5021) III*

JK2739 - *mcm-4(e1466) dpy-5(e61)* l/hT2 [*bli-4(e937) let-?(q782) qIs48] (I:III)*

SS1506* - *met-2(n4256) set-25(n5021) III/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III)*

SS0818 - *mes-3(bn35) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III)*

SS1507* - *mes-3(bn35) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III); met-2(n4256) set-25(n5021) III/hT2 [bli-4(e937) let-?(q782) qIs48] (I;III)*

PD2217 - *ccTi1594 [mex-5p::GFP::gpr-1::smu-1 3'UTR + Cbr-unc-119(+)] III: 680195] unc-119(ed3) III; hjSi20 [myo-2p::mCherry::unc-54 3'UTR] IV*

DUP64 - *glh-1(sams24 [glh-1::GFP::3xFLAG]) I*

SS1473 - *mes-3(bn35) I/hT2 [bli-4(e937) let-?(q782) qIs48] (I,III); ccTi1594 [mex-5p::GFP::gpr-1::smu-1 3'UTR + Cbr-unc-119(+)] III: 680195]/hT2 [bli-4(e937) let-?(q782) qIs48] (I,III); hjSi20 [myo-2p::mCherry::unc-54 3'UTR] IV*

SS1453 - *mes-3(bn35) glh-1::GFP I/hT2 [bli-4(e937) let-?(q782) qIs48] (I,III)*

SS1510* - *mes-3(bn-35) glh-1::GFP I/hT2 [bli-4(e937) let-?(q782) qIs48] (I,III); met-2(n4256) set-25(n5021) III/ hT2 [bli-4(e937) let-?(q782) qIs48] (I,III)*

Generation of non-Mendelian hermaphrodites and scoring germline proliferation

Non-Mendelian hermaphrodites were generated by crossing GPR-1 overexpressing hermaphrodites containing an mCherry pharyngeal marker (*gpr-1(OE)*; *myo2p*::*mCherry*) with males containing endogenously tagged *glh-1::GFP,* which labels germ cells. Non-Mendelian hermaphrodites with germlines lacking H3K27me were generated by crossing *mes*-3 M+Z-; *gpr-1(OE)*; *myo2p*::*mCherry* hermaphrodites with *mes*-3 M+Z- *glh-1::GFP* males. Non-Mendelian hermaphrodites with germlines lacking both H3K9me and H3K27me were generated by crossing *mes*-3 M+Z-; *gpr-1(OE)*; *myo2p*::*mCherry* hermaphrodites with *mes*-3 M+Z- *glh-1::GFP*; *met-2 set-2* males. Outcross offspring were identified by the presence of GLH-1::GFP in the germline, and non-Mendelian offspring were identified by asymmetric mCherry labeling of the pharynx (Figure S2).

Germline proliferation was scored in outcross Mendelian and non-Mendelian hermaphrodite siblings by examining germ cells containing GLH-1::GFP on a fluorescence dissection microscope. Germline proliferation was scored as 1) “full” if the germline was well proliferated and fertile (viable embryos and/or oocytes were observed), 2) “partial” if the germline was well proliferated but no mature gametes were observed, or 3) “low or no” if only a few germ cells or no germ cells were observed.

Imaging germlines

Live first-day adult hermaphrodites (approximately 24 hours post L4 stage) were mounted on 3% agarose pads with 1 µl polystyrene microspheres (Polysciences, Inc. Cat. # 00876) and imaged immediately. Z-stack images of germlines were acquired using a Solamere spinning-disk confocal system equipped with a Nikon Eclipse TE2000-E inverted microscope, a Yokogawa CSU-X1 scan head, a Hamamatsu ImageEM X2-CCD camera, 405, 488, 561, and 640 nm laser lines, and Plan Apo objectives (20x, 63x, and 100x), and controlled by Micro-Manager software (1.4.20) (Edelstein *et al.* 2014). Fiji (Schindelin *et al.* 2012) was used to create Z-stack projections and add body outlines.

Isolation of RNA from dissected germlines and preparation of sequencing libraries

L4 hermaphrodite and male worms were picked to new plates and incubated at 20°C for approximately 24 hours to reach adulthood. Adult worms were transferred to a 30 µl drop of egg buffer (25 mM HEPES, pH 7.5, 118 mM NaCl, 48 mM KCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$) containing 0.1% Tween-20 and 1 mM levamisole on a coverslip. Germlines were dissected using 22-gauge needles by cutting behind the pharynx. Once extruded, hermaphrodite germlines were cut at the bend; male germlines were cut just before spermatogenesis. The distal ends of dissected germlines were collected by mouth pipette using glass pipettes coated with Sigmacote® (Sigma-Aldrich, Cat. # SL2) and placed in Trizol reagent (Invitrogen, Cat. # 15596026) on ice. Samples were flash-frozen in liquid nitrogen and stored at -80°C.

Samples in Trizol were thawed in a 37°C water bath and then subjected to 3 freeze/thaw cycles using liquid nitrogen and a 37°C water bath to release RNA from the tissue. After the final thaw, 1 µg of polyacrylamide was added. The samples were transferred to Phase Lock Gel-Heavy tubes (Brinkmann Instruments, Inc., Cat. # 955-15-404-5), and 60 µl of chloroform was added to each sample. Samples were shaken by hand and then centrifuged at 12,000 x *g* at 4°C for 10 minutes. The aqueous phase was transferred to a new tube, and 0.7-0.8x volume of isopropanol was added. Samples were placed at -20°C to precipitate overnight. The following day, samples were centrifuged at ~13,000 x *g* at 4°C for 25 minutes. Supernatant was removed by pipette. RNA pellets were washed 3x with ice-cold 75% ethanol and resuspended in 13 µl nuclease-free water. Isolated RNA was stored at -80°C.

Before library preparation, RNA samples were DNase treated with TURBO™ DNase (Thermo Fisher Scientific, Cat. # AM2238) at 37°C for 30 minutes to remove any carry-over DNA. Libraries were constructed using the Ovation Universal RNA-seq kit (Tecan, Cat. # 0343) with custom AnyDeplete primers designed by Tecan targeting *C. elegans* rRNA (IC0149S). These primers were designed using the WBcel235 genome build and target RNA from the *rrn-1.1*, *rrn-2.1*, *rrn-3.1*, *rrn-4.16*, *MTCE.33*, *MTCE.7*, *rrn-4.1*, *rrn-4.2*, *rrn-4.3* and *rrn-4.4* genes.

Sequencing analysis

Libraries were sequenced on an Illumina HiSeq2500 to generate 50 bp paired-end reads. Reads were mapped to the ce11 genome using STAR (Dobin *et al.* 2013) with default parameters except for the following: winAnchorMultimapNmax 200, which

allows anchors to map to up to 200 loci, and outFilterMultimapNmax 100, which includes reads that map to up to 100 different loci in the output. Mapped reads were counted with TEcount from the TEtranscripts package (Jin *et al.* 2015). Differential expression analysis was done using DEseq2 (Love *et al.* 2014). Conditions were compared in a pairwise manner. HOMER (Heinz *et al.* 2010) was used to perform motif analysis on promoter regions, which we defined as 500 bp upstream of the transcription start site. Transcription start sites were provided as BED files.

Gene sets

WormBase IDs were used to assign gene set categories from published data to the WormBase ws281 canonical gene annotations. Only matching IDs between the published data and ws281 were considered. If published data were provided with only sequence IDs, ws281 gene IDs were assigned. Genes with conflicting categorization (e.g. since publication a gene ID was merged with another of a different category) were removed. Retained genes included those with unchanged WormBase IDs and genes merged with another of the same category. Excluded genes included dead sequences, genes merged with another of a different category, and genes assigned a new WormBase ID after being split from another.

The germline-specific, soma-specific, and ubiquitous gene sets were previously defined in Rechtsteiner *et al.,* 2010. The soma-specific gene set was further refined in this study by removing genes found to be expressed in wild-type germlines collected in this study (TPM ≥ 5 in each replicate of hermaphrodite and male germlines). The hypoderm-, intestine-, muscle-, and neuron-specific gene sets were

defined in Kaletsky *et al*. 2018. The oogenic, spermatogenic, and gamete-neutral (i.e. shared between spermatogenic and oogenic germlines) gene sets were defined in Ortiz *et al*. 2014. Gene expression profiles in wild-type adult hermaphrodite germlines were defined in Tzur *et al*. 2018.

RNAi

RNAi experiments were performed using the feeding method (Timmons and Fire 1998; Kamath *et al.* 2001; Timmons *et al.* 2001). Plasmid constructs targeting *efl-1*, *dpl-1*, *lin-54*, and *bli-1* were obtained from the Ahringer RNAi library (Kamath and Ahringer 2003). The *lin-15B* construct was generated in Cockrum and Strome, 2022. Bacteria from frozen stocks were streaked out on LB agar plates containing 100 µg/ml ampicillin and 10 µg/ml tetracycline. Single colonies were cultured overnight, shaking at 37°C in liquid LB containing 100 µg/ml ampicillin and 10 µg/ml tetracycline. 100 µl of overnight cultures were spotted onto 6 cm NGM plates containing 100 µg/ml ampicillin and 1 mM IPTG and incubated at room temperature in the dark for 2-3 days to grow and induce dsRNA. Crosses of *gpr-1(OE)*; *myo2p*::*mCherry* hermaphrodites with *glh-1*::*GFP* males and *mes-3* M+Z-; *gpr-1(OE)*; *myo2p::mCherry* hermaphrodites with *mes-3* M+Z- *glh-1*::*GFP*; *met-2 set-25* males were crossed on RNAi plates at the L4 stage. Crossed worms were transferred to fresh RNAi plates 24 and 48 hours later and removed from the third plates after an additional 24 hours. Offspring on each of the three plates were scored for germline proliferation four days after being laid. A plasmid construct targeting *bli-1* was used as a positive control for the effectiveness of RNAi reagents.

Results

H3K27me and H3K9me cooperate to enable germ cell proliferation and fertility in hermaphrodite offspring as well as male offspring

MES-3 is an essential subunit of *C. elegans* PRC2. *mes-3* mutant XX hermaphrodites that lack both maternal and zygotic H3K27me (*mes-3* M-Z- K27me(-)) are sterile due to underproliferation and necrotic death of nascent germ cells (Capowski *et al.* 1991; Paulsen *et al.* 1995). However, *mes-3* M-Z- K27me(-) XO males can be fertile in a manner that depends on the gamete origin of the X: males with an X from the oocyte (X^{oo}) are sterile, while males with an X from the sperm (X^{sp}) are usually fertile (Gaydos *et al.* 2014). This rescue of *mes-3* M-Z- K27me(-) male fertility by X^{sp} is dependent on H3K9me (Gaydos *et al.* 2014). Together, these observations indicate that, in the absence of H3K27me, H3K9me maintains repression of the single X in male germlines and enables male germline development. Whether or not this effect is specific to males is not known.

We reasoned that H3K9me rescue of *mes-3* M-Z- K27me(-) $X^{sp}O$ male fertility might be specific to males if H3K9me can maintain sufficient repression of X-linked genes from one X chromosome in males but perhaps not from two X chromosomes in hermaphrodites, and/or if H3K9me can maintain repression needed for male-specific germline development but perhaps not repression needed for hermaphrodite germline development. To investigate this, we tested whether H3K9me can rescue germline development in *mes-3* M-Z- K27me(-) hermaphrodites whose germline inherited two sperm-derived X chromosomes ($X^{sp}X^{sp}$). If rescue of fertility by H3K9me in *mes-3* M-

Z- K27me(-) X^{sp} males is male-specific, then *mes-3* M-Z- K27me(-) $X^{sp}X^{sp}$ hermaphrodites would display little germ cell proliferation and be sterile. On the other hand, if germline development and fertility are rescued by H3K9me in *mes-3* M-Z- K27me(-) $X^{sp}X^{sp}$ hermaphrodites as in males, it would suggest that H3K9me repression can handle two X chromosomes and protect hermaphrodite as well as male germline development. Therefore, we examined germline proliferation and fertility in hermaphrodites whose germline inherited two sperm-derived Xs with a wild-type (WT), K27me(-), or K9me(-) K27me(-) chromatin state.

To generate $X^{sp}X^{sp}$ hermaphrodite germlines, we mated mothers overexpressing a force-regulating spindle protein in the germline and mCherry fluorescent protein in the pharynx [*gpr-1(OE); myo2p::mCherry*] (Besseling and Bringmann 2016; Artiles *et al.* 2019) with *glh-1::GFP* males. GLH-1::GFP marks germ granules, allowing easy identification of germ cells in offspring, while GPR-1(OE) causes ~80% of embryos to deliver the oocyte and sperm pronuclei to different daughter cells at the first cell division (Figure 1A). This non-Mendelian pattern of inheritance generates mosaic embryos whose AB cell usually inherits a set of replicated oocyte-derived chromosomes and whose P1 cell usually inherits a set of replicated sperm-derived chromosomes. Importantly for our studies, descendants of the P1 lineage, which generates the entire germline, have two X chromosomes from the sperm ($X^{sp}X^{sp}$) (Figure 1A and Artiles *et al.* 2019).

We crossed GPR-1(OE) or *mes-3* M+Z-*;* GPR-1(OE) [K27me(-)] hermaphrodites with males that were wild type, *mes-3* M+Z- [K27me(-)], or *mes-3* M+Z-*; met-2 set-25* [K9me(-) K27me(-)] (Figure 1B, Figure S1B). The endogenously tagged *glh-1::GFP* transgene carried by the males in these crosses was used to

identify out-cross offspring and to assess germline proliferation (Figure 1C). Simultaneously, asymmetric labeling of the pharynx by mCherry differentiated non-Mendelian from Mendelian offspring (Figure S2). We scored germline proliferation in the F1 M-Z- Mendelian offspring ($X^{oo}X^{sp}$ germlines) and their non-Mendelian siblings ($X^{sp}X^{sp}$ germlines) (Figure 1D). As expected, 100% of H3K9me(+) H3K27me(+) (WT) $X^{oo}X^{sp}$ hermaphrodites had full germline proliferation and were fertile (Figure 1D). Among K27me(-) and K9me(-) K27me(-) $X^{oo}X^{sp}$ hermaphrodites, the vast majority had low or no germline proliferation (Figure 1D). Among WT $X^{sp}X^{sp}$ hermaphrodites, the vast majority had full germline proliferation (Figure 1D). Strikingly, among K27me(-) $X^{sp}X^{sp}$ hermaphrodites, 65% had full germline proliferation and were fertile, 8% had partial germline proliferation, and 27% had low or no germline proliferation (Figure 1D). The improved germline health of K27me(-) $X^{sp}X^{sp}$ compared to K27me(-) $X^{oo}X^{sp}$ shows that two K27me(-) X^{sp} chromosomes can rescue fertility in hermaphrodite germlines, similar to rescue by a single K27me(-) X^{sp} chromosome in males (Gaydos *et al.* 2014). Among K9me(-) K27me(-) $X^{sp}X^{sp}$ hermaphrodites, only 2% had full germline proliferation and 3% had partial germline proliferation (Figure 1D). Thus, H3K9me was required for the elevated germline proliferation and fertility of K27me(-) $X^{sp}X^{sp}$ hermaphrodites.

Our finding that germ cell proliferation and fertility were often rescued in K27me(-) $X^{sp}X^{sp}$ hermaphrodites compared to K27me(-) $X^{oo}X^{sp}$ hermaphrodites and that rescue depended on H3K9me supports a model that, in the absence of H3K27me, H3K9me can sufficiently maintain repression of one or two X^{sp} chromosomes to protect either male or hermaphrodite germline development in the offspring

Protein-coding genes are the primary targets of repression by H3K27me and H3K9me in hermaphrodite and male germlines

Our above findings and those of Gaydos *et al.* (2014) demonstrate that H3K9me can rescue K27me(-) X^{sp} males and $X^{sp}X^{sp}$ hermaphrodites, but cannot rescue K27me(-) X^{oo} males and $X^{oo}X^{sp}$ hermaphrodites. This points to differences in regulation of gene expression and chromatin marking during oogenesis and spermatogenesis. To identify loci whose expression in the oogenic and spermatogenic germline is regulated by H3K9me, by H3K27me, or redundantly by both marks, we performed RNA-sequencing and differential expression analysis with rRNA-depleted total RNA hermaphrodite and male germlines dissected from wild-type (WT), *met-2 set-25* [K9me(-)], *mes-3* M+Z- [K27me(-)], and *mes-3* M+Z-*; met-2 set-25* [K9me(-) K27me(-)] animals. Germlines were dissected from *mes-3* M+Z- animals (analogous to the P0 generation in Figure 1B and Figure S1A), which are fertile because they inherited maternal MES-3 but which by adulthood lack histone marking due to lack of zygotic transcription of *mes-3* (Gassmann *et al.* 2012). We mapped sequencing reads to the ce11 genome and counted reads that mapped to protein-coding genes, non-coding genes, and transposable elements (TEs) (see Materials and Methods for details). We performed differential expression analysis to identify significantly upregulated (UP) transcripts and significantly downregulated (DOWN) transcripts in mutant conditions compared to WT (Figure 2).

Loss of repressive marks like H3K9me and H3K27me would be expected to cause upregulation of direct target sequences, while downregulation would likely be due to secondary effects. Consistent with this, we found many more UP transcripts than DOWN transcripts in animals lacking H3K9me, H3K27me, or both marks (Figure

2). Additionally, we found more overlap between hermaphrodite and male UP genes than DOWN genes in corresponding conditions (Figure S3 A-C; Figure S4 A-C). For these reasons, we focused our analysis on UP transcripts.

For protein-coding genes (Figure 2, A and B), in K9me(-) germlines, we observed 147 UP in hermaphrodites and 40 UP in males. In K27me(-) germlines, we observed many more UP genes: 1449 UP in hermaphrodites and 975 UP in males. In K9me(-) K27me(-) germlines, we observed 1534 UP in hermaphrodites and 1346 UP in males. For non-coding genes (Figure 2, C and D), in K9me(-) germlines, we observed 15 UP in hermaphrodites and 7 UP in males. In K27me(-) germlines, we observed 58 UP in hermaphrodites and 61 UP in males. In K9me(-) K27me(-) germlines, we observed 126 UP in hermaphrodites and 120 UP in males. For transposable element families (TEs) (Figure 2, E and F), in K9me(-) germlines, we observed 3 UP in hermaphrodites and 2 UP in males. In K27me(-) germlines, we observed 5 UP in hermaphrodites and 4 UP in males. In K9me(-) K27me(-) germlines, we observed 27 UP in hermaphrodites and 21 UP in males.

The numbers of significantly UP transcripts indicate that 1) protein-coding genes are the primary targets of repression by H3K27me and H3K9me in hermaphrodite and male germlines, 2) H3K27me represses many more protein-coding genes than H3K9me in both hermaphrodite and male germlines, 3) H3K27me represses more protein-coding genes in hermaphrodite germlines than in male germlines, 4) H3K9me maintains a higher degree of repression in male germlines lacking H3K27me than in hermaphrodite germlines lacking H3K27me, and 5) more non-coding genes and TEs are repressed by both H3K9me and H3K27me than by either alone.

In hermaphrodite and male germlines, soma-specific genes are the primary targets of repression by H3K27me alone

The large numbers of UP protein-coding genes observed in K27me(-) and K9me(-) K27me(-) hermaphrodite and male germlines, as well as the observation that many fewer protein-coding genes are UP in K27me(-) male germlines (975 UP) than in K27me(-) hermaphrodite germlines (1449 UP) led us to further investigate the UP protein-coding genes and their differences between hermaphrodites and males. To determine which categories of protein-coding genes are the focus of H3K9me and H3K27me repression, we measured the enrichment of germline-specific genes, soma-specific genes, and ubiquitous genes among UP genes in all three mutant conditions. For germline-specific genes, we found very few and similar numbers of UP genes in hermaphrodites and males in all mutant conditions (Figure 3, A and B): in K9me(-) UP, 1 of 147 (0.6%) in hermaphrodites, 0 of 40 (0%) in males; in K27me(-) UP, 4 of 1449 (0.2%) in hermaphrodites, 1 of 975 (0.1%) in males; in K9me(-) K27me(-) UP, 6 of 1534 (0.2%) in hermaphrodites, 6 of 1346 (0.4%) in males. For soma-specific genes, more genes were UP in both hermaphrodites and males (Figure 3, C and D): in K9me(-) UP,13 of 147 (8.8%) in hermaphrodites, 2 of 40 (5%) in males; in K27me(-) UP, 219 of 1449 (15%) in hermaphrodites, 97 of 975 (10%) in males; in K9me(-) K27me(-) UP, 205 of 1534 (13%) in hermaphrodites, 119 of 1346 (9%) in males. For ubiquitous genes (Figure 3, E and F): in K9me(-) UP, 15 of 147 (10%) in hermaphrodites, 4 of 40 (10%) in males; in K27me(-) UP, 137 of 1449 (9%) in hermaphrodites, 90 of 975 (9%) in males; in K9me(-) K27me(-) UP, 146 of 1534 (10%) in hermaphrodites, 167 of 1346 (12%) in males.

Broadly, in hermaphrodite germlines among UP genes in all three mutant conditions, germline-specific genes were under-represented, soma-specific genes were over-represented, and ubiquitous genes were as expected by chance (Figure 3G). Male germlines were similar but displayed over-representation of ubiquitous genes in K9me(-) K27me(-) (Figure 3H). Our results indicate that H3K9me and H3K27me do not appreciably regulate germline-specific genes, and that H3K27me is the major repressor of soma-specific genes in the germline. Among somatic tissue subtypes, neuronal genes were enriched in all conditions that lacked H3K27me (Figure 3, I and J).

Among germline-expressed genes that are repressed by H3K27me and H3K9me, the majority are oogenesis genes many of which reside on the X chromosome

Our analyses revealed that in the germline, H3K27me serves a more important repressive role than H3K9me and that loss of both marks causes more genes to be upregulated than loss of either single mark. Because loss of H3K9me alone had a minor effect on gene expression, we focused on identifying and characterizing genes uniquely repressed by H3K27me and genes redundantly repressed by both H3K9me and H3K27me. Figure 4A and B display the relative numbers of UP genes and the degree of overlap of UP genes in the three mutant conditions. We identified 1401 genes in hermaphrodite germlines and 951 genes in male germlines that were UP in K27me(-) but not UP in K9me(-); we refer to these as K27me(-) unique UP genes (pink area in Figure 4, A and B). We identified 416 genes in hermaphrodite germlines and 563 genes in male germlines that were UP in K9me(-) K27me(-) but not UP in K9me(-

) or K27me(-); we refer to these as K9me(-) K27me(-) unique UP genes (red crescent in Figure 4, A and B).

The X chromosome is known to be an important target of repression in the germline of both hermaphrodites and males (Strome *et al.* 2014). In hermaphrodite germlines, we found genes on the X chromosome to be significantly over-represented in both K27me(-) unique UP genes (311 of 1401, 22%) and K9me(-) K27me(-) unique UP genes (161 of 416, 39%) (Figure 4C). In male germlines, genes on the X are under-represented in K27me(-) unique UP genes (56 of 951, 6%) and over-represented in K9me(-) K27me(-) unique UP genes (137 of 563, 24%) (Figure 4D). These findings indicate that in hermaphrodite germlines, some X-linked genes are repressed by H3K27me alone and others are redundantly repressed by H3K9me and H3K27me. In contrast, X-linked genes in male germlines are repressed by both marks.

In male germlines, the X chromosome is globally repressed through all stages of germline development (mitosis, meiosis, and spermatogenesis), while in hermaphrodite germlines, the X chromosomes are repressed during the stages shared with males (mitosis and meiosis) but become transcriptionally active at diplotene/diakinesis as germ cells form oocytes (Kelly *et al.* 2002). Consistent with this is, genes expressed during mitosis and meiosis are under-represented on the X, while genes expressed during oogenesis are over-represented on the X (Ortiz *et al.* 2014; Tzur *et al.* 2018). We examined those categories of genes (shared or gamete-neutral, oogenic, and spermatogenic) among the UP genes using previously defined gene sets (Ortiz *et al.* 2014) (Figure 4, E and F). Among X-linked genes in both K27me(-) unique UP genes and K9me(-) K27me(-) unique UP, oogenesis genes were strikingly over-represented, while gamete-neutral genes and spermatogenesis genes were as

expected by chance or under-represented, except in hermaphrodite K27me(-) unique UP in which gamete-neutral genes were slightly enriched among X-linked genes. Among UP genes on the autosomes, oogenesis genes were over-represented in all conditions except K9me(-) K27me(-) unique UP genes in hermaphrodites, and spermatogenesis genes were over-represented in all conditions. Gamete-neutral genes were near expected or under-represented in all conditions.

The over-representation of oogenesis genes, especially X-linked oogenesis genes, in both K27me(-) unique UP genes and K9me(-) K27me(-) unique UP genes in hermaphrodites and males suggests that these upregulated genes are members of the same regulatory network and would have similar expression patterns. To investigate this possibility, we looked at the mean normalized expression of K27me(-) unique UP genes and K9me(-) K27me(-) unique UP genes across sections of WT adult hermaphrodite germlines during production of oocytes (Tzur *et al.* 2018). We found that X-linked and autosomal genes in these groups normally displayed increased expression upon entry into diplotene (Figure 4G), which is when the X chromosomes become transcriptionally active in WT hermaphrodite germlines (Kelly *et al.* 2002).

These results indicate that 1) H3K27me alone represses more X-linked genes in the hermaphrodite germline than in the male germline, 2) H3K9me and H3K27me redundantly repress more genes in the male germline than in the hermaphrodite germline, 3) X-linked K27me(-) unique UP genes and K9me(-) K27me(-) unique UP genes are highly enriched for oogenesis genes in both hermaphrodites and males, and 4) K27me(-) unique UP genes and K9me(-) K27me(-) unique UP genes display a pattern of expression in adult WT hermaphrodite germlines consistent with oogenic genes.

Genes upregulated in hermaphrodite and male germlines that produce sterile offspring are enriched for X-linked oogenesis genes

K27me(-) males produce fertile offspring, while K9me(-) K27me(-) males produce sterile offspring (Gaydos *et al.* 2014). These data suggest that, in the absence of H3K27me, H3K9me maintains repression of genes that when upregulated cause sterility in the offspring. To identify these genes, we performed differential expression analysis between K9me(-) K27me(-) male germlines and K27me(-) male germlines and found 198 UP genes (Figure 5A). To create a list of candidate genes whose upregulation contributes to sterility in offspring, we determined the overlap of UP genes in two genotypes that produce sterile offspring: K9me(-) K27me(-) male germlines (when compared to K27me(-)) and K27me(-) hermaphrodite germlines (when compared to WT). 90 UP genes were shared between these groups of upregulated genes (Figure 5B, Shared UP). Among Shared UP genes, X-linked genes were hugely over-represented (76 of 90, Figure 5C). Among the X-linked genes, oogenesis genes were drastically over-represented, gamete-neutral genes were modestly over-represented, and spermatogenesis genes were drastically under-represented (Figure 5D). The mean normalized expression of X-linked Shared UP genes in WT hermaphrodite germlines display a dramatic increase in expression upon entry into diplotene, consistent with when the X chromosome normally becomes transcriptionally active during oogenesis (Figure 5E) (Kelly *et al.* 2002). Together, these results indicate that UP genes in hermaphrodite and male germlines that produce sterile offspring are primarily X-linked genes expressed during oogenesis.

Because most Shared UP genes have similar expression patterns in the hermaphrodite germline, we hypothesized that they are part of a shared gene regulatory program and might display enrichment of particular transcription factor binding motifs in their promoters. We performed motif analysis on Shared UP promoters using HOMER (Heinz *et al.* 2010). After accounting for genes in the same operon, there were 85 promoters in our target group. We found the binding motifs of LIN-15B, EFL-1, and DPL-1 to be the most highly enriched among Shared UP promoters (Figure 5F).

All together these data indicate that UP genes shared between hermaphrodite and male germlines that produce sterile offspring are greatly enriched for X-linked oogenesis genes and that they may be regulated by LIN-15B and/or the DRM (for DP, Rb, MuvB) complex components EFL-1 and DPL-1. LIN-15B is a particularly strong candidate, as the gene is X-linked, is significantly UP in all three mutant conditions, and is in the Shared UP category, and loss of LIN-15B can rescue the fertility of M-Z-K27me(-) hermaphrodites (Cockrum and Strome, 2022).

RNAi depletion of LIN-15B, LIN-54, or EFL-1 partially rescues germline proliferation in H3K9me(-) H3K27me(-) $X^{sp}X^{sp}$ germlines

The identification of LIN-15B, EFL-1, and DPL-1 binding motifs in Shared UP promoters led us to hypothesize that upregulation of these transcription factors and/or their targets in the adult germline leads to sterility in offspring. To test if LIN-15B, EFL-1, DPL-1, or LIN-54 (the other DNA-binding component of the DRM complex, (Harrison *et al.* 2006)) contributes to the sterility of K9me(-) K27me(-) animals, we crossed K9me(-) K27me(-) males with GPR-1(OE) K27me(-) hermaphrodites to generate non-

Mendelian hermaphrodite offspring whose germline was K9me(-) K27me(-) $X^{sp}X^{sp}$. We then determined if the proportion of offspring worms with fully proliferated and fertile germlines was significantly different after growth on RNAi bacteria compared to control bacteria (Figure 6). From control crosses (Figure 6A), we observed that animals with K9me(+) K27me(+) $X^{sp}X^{sp}$ germlines displayed robust germline proliferation in all six conditions, although RNAi depletion of *lin-15B* and *lin-54* reduced proliferation, as has been reported previously (Harrison *et al.* 2006). From experimental crosses (Figure 6B), we observed that animals with K9me(-) K27me(-) $X^{sp}X^{sp}$ germlines had severely compromised germline proliferation after growth on control bacteria (OP50 and L4440 empty-vector control). RNAi depletion of *lin-15B* resulted in significant rescue of germline proliferation (71% fully and partially proliferated after RNAi compared to 22% in the absence of RNAi). RNAi depletion of *lin-54* and *efl-1* resulted in less robust rescue (55% and 36%, respectively, compared to 22% in the absence of RNAi). These findings suggest that LIN-15B, LIN-54, and perhaps EFL-1 contribute to the sterility of K9me(-) K27me(-) $X^{sp}X^{sp}$ germlines.

Discussion

This study advances our understanding of H3K9me- and H3K27me-mediated repression in *C. elegans* by identifying genes that are repressed by H3K9me, by H3K27me, or by both marks in the germline of hermaphrodites and males. We found that H3K9me and H3K27me primarily repress protein-coding genes in hermaphrodite and male germlines, with H3K27me carrying out the majority of repression. Genes normally expressed uniquely in somatic tissues and X-linked genes expressed during oogenesis were the main targets of repression. Genes redundantly repressed by H3K9me and H3K27me were enriched for X-linked oogenesis genes in both

hermaphrodite and male germlines. We identified a set of upregulated X-linked oogenesis genes shared between hermaphrodite and male germlines that produce sterile offspring, and we determined that the activity of the THAP transcription factor LIN-15B (an X-linked oogenesis gene) and members of the *C. elegans* DREAM complex (LIN-54 and perhaps EFL-1) contribute to sterility in offspring.

H3K9me and H3K27me cooperate to repress genes in the germline

H3K27me is known to temporarily repress developmental genes until they are needed later (e.g. repression of somatic genes in the germline)(Laugesen *et al.* 2019), while the primary function of H3K9me is repression of repetitive elements to protect genome stability (Zeller *et al.* 2016). Despite this traditional division of labor, we identified many genes redundantly repressed by H3K9me and H3K27me in hermaphrodite and male *C. elegans* germlines. Whether this redundant repression is carried out by colocalization (both marks present on the target sequence) or replacement (when one mark is lost from the target sequence, the other mark is gained) is not known. In other species, such as mouse embryonic stem cells, *Cryptococcus neoformans*, and *Neurospora crassa*, H3K27me has been observed to redistribute when features of constitutive heterochromatin (DNA methylation, H3K9me, HP1) are disrupted (reviewed in Wiles and Selker, 2017). However, the uniquely strong positive correlation of H3K9me3 and H3K27me3 observed in ChIP-seq studies of *C. elegans* larvae (Liu *et al.* 2011; Ho *et al.* 2014) suggests that observing H3K27me redistribution upon loss of H3K9me would be unlikely or rare. Interestingly, H3K27me3 correlates more strongly with H3K9me3 than H3K9me2 (Liu *et al.* 2011; Ho *et al.* 2014), suggesting that H3K9me2 is more likely than H3K9me3 to mark heterochromatin that

is functionally distinct from heterochromatin marked by H3K27me3. In somatic tissues, H3K9me2 has been shown to be recruited to the promoters of germline genes to enact repression (Rechtsteiner *et al.* 2019; Gal *et al.* 2021; Methot *et al.* 2021), while H3K27me3 is primarily found in the bodies of genes (Liu *et al.* 2011; Ho *et al.* 2014). A recent study identified several chromatin factors (MET-2, HPL-2, LET-418, LIN-13, LIN-61) that associate with regions marked by H3K9me2 to repress transposable elements, with a strong preference for DNA transposons (McMurchy *et al.* 2017). In contrast, H3K9me3 enrichment is biased towards retrotransposons (Zeller *et al.* 2016). Interestingly, exogenous RNAi and endo-siRNA mechanisms can recruit both H3K9me3 and H3K27me3 to their targets (Guang *et al.* 2010; Burkhart *et al.* 2011; Gu *et al.* 2012; Mao *et al.* 2015). We speculate that H3K27me3 and H3K9me3 redundantly repress some genes via colocalization, and that H3K27me3 and H3K9me2 operate independently to repress different genes. Determining precisely how H3K9me and H3K27me cooperate to repress their targets in the germline will require analysis of the distributions of H3K9me2/3 and H3K27me3 in wild-type germlines and mutant germlines that lack H3K9me2 (*met-2* mutants), H3K9me2/3 (*met-2 set-25* mutants), or H3K27me3 (*PRC2* mutants) using low-input chromatin profiling methods such as like CUT&RUN or CUT&TAG (Skene and Henikoff 2017; Skene *et al.* 2018).

Sexual dimorphism of X-chromosome repression in the germline by H3K9me and H3K27me

In male germlines compared to hermaphrodite germlines, we observed a reduced role of H3K27me alone and a greater role of H3K9me and H3K27me working together in X-chromosome repression. This difference in X-chromosome regulation between

hermaphrodite and male germlines is likely due to 1) transcriptional program differences between spermatogenesis and oogenesis and 2) the hemizygous nature of the X in males, which leads to accumulation of H3K9me2, as discussed below.

Regulation of the X chromosome differs significantly between hermaphrodite and male germlines. In both sexes, active chromatin marks are absent from the X chromosome and the X is transcriptionally repressed from the mitotic zone through late pachytene (Kelly *et al.* 2002; Reuben and Lin 2002; Fong *et al.* 2002). In male germlines, the single X chromosome remains transcriptionally repressed through spermatogenesis, while in hermaphrodite germlines, the paired X homologs are briefly activated from late pachytene to late diplotene to express oogenesis genes on the X (Kelly *et al.* 2002). These different "histories" of X transcription between oocyte and sperm are transmitted to the embryo (Bean *et al.* 2004; Gaydos *et al.* 2014) and correlate with sterility in offspring from K27me(-) parents: male offspring that inherit a K27me(-) X from the oocyte (X^{oo}) are sterile, while those that inherit a K27me(-) X from the sperm (X^{sp}) are usually fertile (Gaydos *et al.* 2014). Extending this finding, hermaphrodite offspring that inherit a K27me(-) X from the oocyte and one from the sperm ($X^{oo}X^{sp}$) are sterile, while those that inherit two K27me(-) Xs from the sperm ($X^{sp}X^{sp}$) are usually fertile (this study). The fertility of K27me(-) offspring that inherit one or two X^{sp} is dependent on H3K9me (Gaydos *et al.* 2014; this study), which is enriched on the hemizygous male X chromosome in the form of H3K9me2 (Kelly *et al.* 2002). H3K9me2 enrichment of the male X is due to the process of meiotic silencing of unpaired chromatin (MSUC) (Bean *et al.* 2004). Interestingly, MSUC alone is not sufficient to rescue fertility of K27me(-) offspring, since *him-8* K27me(-) hermaphrodites, which have unpaired X chromosomes enriched for H3K9me2 and so

have MSUC during oogenesis, nevertheless produce $X^{oo}X^{sp}$ offspring that are sterile (Garvin *et al.* 1998; Gaydos and Strome, unpublished). This suggests that the history of X repression during spermatogenesis versus X activation during oogenesis is a critical determinant of the fertility of K27me(-) offspring.

A signature of upregulated X-linked oogenesis genes in germlines that produce sterile offspring

We identified a set of upregulated genes shared between K27me(-) hermaphrodite germlines and K9me(-) K27me(-) male germlines, both of which produce sterile offspring. We speculate that 1) this set of genes, which are primarily X-linked oogenesis genes, includes genes that when upregulated cause sterility in the offspring and that 2) the transcriptionally active state of these genes is transmitted to offspring through chromatin. Consistent with this, in offspring of K27me(-) hermaphrodites, larval germ cells upregulate X-linked oogenesis genes similarly to their parental germlines (Cockrum and Strome 2022). Strikingly, a majority of the Shared Up genes we identified (71/91) are also upregulated in early germ cells of K27me(-) hermaphrodite offspring. The maternal effect sterile (Mes) phenotype manifests when the primordial germ cells (PGCs) in M-Z- offspring fail to proliferate during larval development and then undergo necrotic death (Capowski *et al.* 1991; Paulsen *et al.* 1995). The maternal effect nature of this phenotype suggests that an inherited factor that normally ensures fertility must be transmitted from the parent germline via the gametes through the five early embryo cell divisions (occurring within two hours of fertilization) that generate the two PGCs Z2 and Z3 (Sulston *et al.* 1983). Current

evidence points to chromatin state as being the major factor determining offspring fertility/sterility. The fertility of K27me(-) X^{sp} and $X^{sp}X^{sp}$ offspring (Gaydos *et al.* 2014, this study) suggests that the fertility/sterility determining factor is not found in the maternal load and implicates the contribution from sperm, mainly chromosomes. Indeed, the different chromatin states of X^{oo} and X^{sp} and their associated chromatin marks are inherited by the embryo, and differences in chromatin marks are maintained for several cell divisions (Bean *et al.* 2004; Gaydos *et al.* 2014; Tabuchi *et al.* 2018). These observations support a model in which the chromatin state of the X is the fertility/sterility determining factor in Mes mutant offspring as they show that 1) the chromatin state of the X is heritable, 2) X^{oo} and X^{sp} chromatin states differ, and 3) a similar signature of upregulated X-linked oogenesis genes is observed in K27me(-) hermaphrodite germlines and their offspring's early germ cells.

LIN-15B and DRM activity contribute to sterility

We identified LIN-15B and the DRM components LIN-54 and EFL-1 as contributing to sterility in K9me(-) K27me(-) $X^{sp}X^{sp}$ offspring. Our findings are consistent with previous studies that showed that upregulation of LIN-15B in early germ cells contributes to sterility in other mutant backgrounds (Lee *et al.* 2017; Robert *et al.* 2020; Cockrum and Strome 2022). The antagonistic relationship between LIN-15B/DRM and the MES proteins (PRC2 and MES-4) is best understood in *C. elegans* somatic tissues, where loss of LIN-15B or DRM results in misexpression of germline genes and larval arrest at high temperature, phenotypes that are suppressed by concomitant loss of PRC-2 or MES-4 (Wang *et al.* 2005; Petrella *et al.* 2011). Thus, in the soma, PRC2 and MES-4 promote aberrant germline gene expression, which LIN-15B and DRM antagonize.

In germ cells, the prevailing model is that PRC2 and MES-4 primarily function to repress the oogenesis program, which has an X-chromosome bias, during all other stages of germline development (Cockrum and Strome 2022). Upregulation of LIN-15B has been shown to promote transcription of oogenesis genes, especially those on the X, and contribute to sterility (Lee *et al.* 2017; Robert *et al.* 2020; Cockrum and Strome 2022; this study). DRM has also been proposed to indirectly promote expression of X-linked genes in the adult germline possibly by regulating the activity and/or localization of another factor (Tabuchi *et al.* 2011). The view that emerges is that LIN-15B and DRM antagonize PRC2 and MES-4 activity in the soma to keep germline genes off, while PRC2 and MES-4 antagonize LIN-15B and DRM activity in germ cells to prevent aberrant activation of the oogenesis program, which is enriched for X-linked genes.

LIN-15B and DRM are best known as repressors. In somatic cells, LIN-15B is found in the promoters of germline genes and likely represses those genes by recruiting MET-2 to deposit H3K9me2, which in turn likely recruits HPL-2 and DRM (Rechtsteiner *et al.* 2019; Gal *et al.* 2021). How do LIN-15B and DRM cause up-regulation of X-linked oogenesis genes in the germline? We consider 3 possible scenarios: 1) LIN-15B and DRM have novel partners that enable them to activate transcription of target genes, 2) LIN-15B and DRM repress a repressor(s) of X-linked oogenesis genes, resulting in upregulation of those genes, and 3) LIN-15B and DRM antagonize each other in a manner described by Gal *et al.* 2021. Loss of one (e.g. LIN-15B) causes the other (e.g. DRM) to become more concentrated on target genes (e.g. X-linked oogenesis genes) and restores repression of those genes in PRC2 or MES-4 mutants.

Our model

In the germline, the X chromosomes are globally repressed at all stages except oogenesis, when they become transcriptionally active to produce products needed for oocyte maturation and early embryogenesis. As a result, the X chromosome from the oocyte (X^{oo}) is transmitted to offspring in an active state and requires the activity of PRC2 to reestablish repression. PRC2 M-Z- embryos fail to reestablish repression of the K27me(-) X^{oo}, which results in passage of an X^{oo} in an active chromatin state to the PGCs, aberrant transcription of oogenesis genes in the PGCs, and ultimately degeneration of the PGCs during early larval development. LIN-15B appears to be a major player in this scenario. LIN-15B is itself an X-linked oogenesis product, which must be downregulated in the PGCs during embryogenesis. In PRC2 mutant offspring, elevated levels of LIN-15B contribute to driving aberrant transcription of X-linked oogenesis genes in the PGCs. This phenotype does not occur in offspring that inherit only K27me(-) X^{sp} because 1) the X^{sp} is not activated during spermatogenesis, so repression does not need to be-established by PRC2 and 2) H3K9me enriched on the single unpaired X^{sp} maintains repression in the absence of H3K27me.

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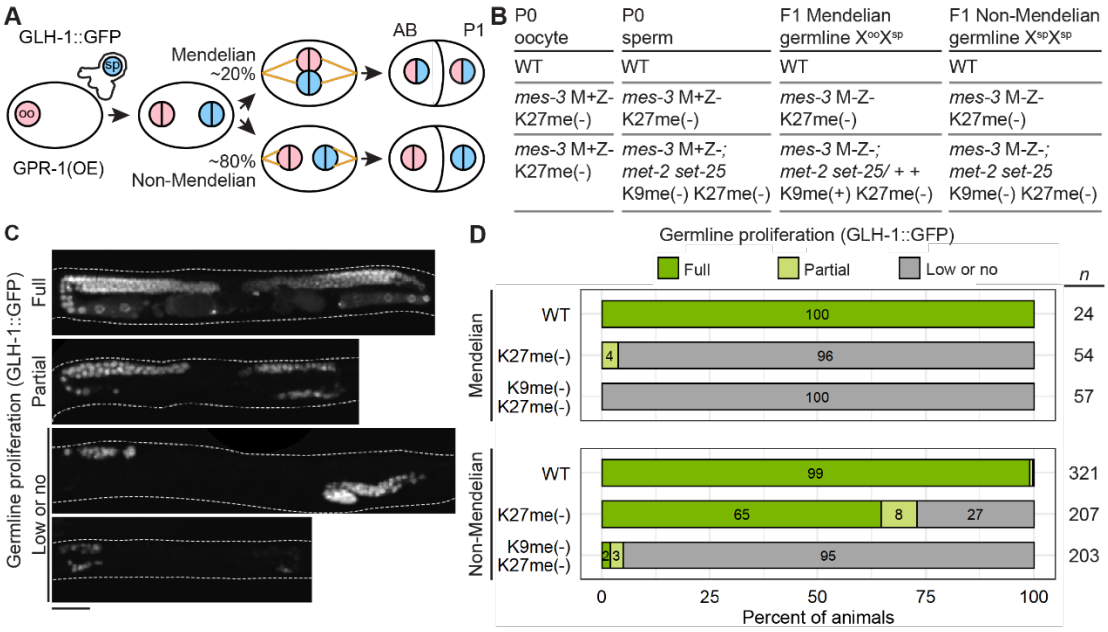


Figure 1. K27me(-) XspXsp hermaphrodite germline development depends on H3K9me.

(A) Diagram of mating strategy using over-expression of GPR-1 [GPR-1(OE)] to generate offspring (F1s) with non-Mendelian $X^{sp}X^{sp}$ germlines containing the germline marker GLH-1::GFP. (B) Table describing the genotypes and chromatin mark states of the P0 gametes and F1 Mendelian ($X^{oo}X^{sp}$) and non-Mendelian ($X^{sp}X^{sp}$) germlines. (C) Straightened micrographs of GLH-1::GFP F1 germlines displaying “full”, which are fertile, “partial”, and “low or no” germline proliferation. Scale bar is 50 µm. (D) Bar graphs of germline proliferation in wild-type (WT), K27me(-), and K9me(-) K27me(-) Mendelian and non-Mendelian F1 germlines. Animals were scored based on the gonad arm containing the most proliferated germline. Numbers of gonad arms scored and percentages displaying each level of proliferation are indicated.

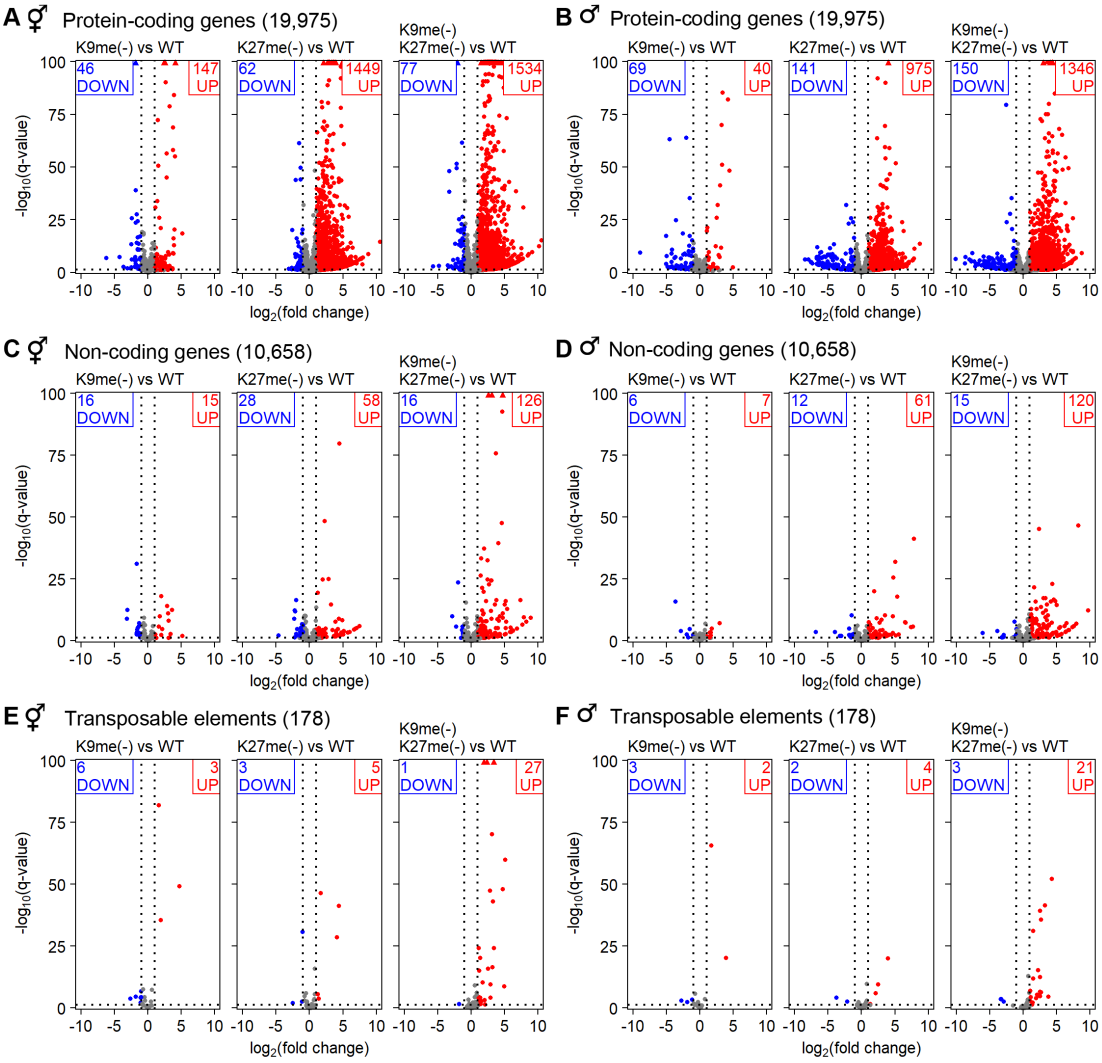


Figure 2. Differential expression analysis of K9me(-), K27me(-), and K9me(-) K27me(-) germlines compared to wild-type germlines in hermaphrodites and males.

Volcano plots displaying $\log_2$(fold change) versus -$\log_{10}$(q-value) for (A,B) 19,975 protein-coding genes, (C,D) 10,658 non-coding genes, and (E,F) 178 transposable element subfamilies in *met-2 set-25* [K9me(-)], *mes-3* [K27me(-)], and *mes-3*; *met-2 set-25* [K9me(-) K27me(-)] in (A,C,E) hermaphrodite germlines and (B,D,F) male germlines compared to wild type (WT). A significance threshold of q-value ≤ 0.05 (horizontal dotted line) and thresholds of $\log_2$(fold change) ≤ -1 and ≥ 1 (vertical dotted lines) were used to define significantly downregulated genes (DOWN, blue) and upregulated genes (UP, red), respectively. Total DOWN genes and UP genes are indicated in the upper left and upper right, respectively, of each plot.

Figure 3. Analysis of tissue-specific protein-coding genes in K9me(-), K27me(-), and K9me(-) K27me(-) germlines.

(A-F) MA plots displaying log_2(mean expression) versus log_2(fold change) for 19,975 protein-coding genes in *met-2 set-25* [K9me(-)], *mes-3* [K27me(-)], and *mes-3*; *met-2 set-25* [K9me(-) K27me(-)] in (A,C,E) hermaphrodite germlines and (B,D,F) male germlines compared to wild type (WT). Thresholds of log_2(fold change) ≤ -1 and ≥ 1 (horizontal dotted lines) are indicated. (A,B) Germline-specific genes, (C,D) soma-specific genes, and (E,F) ubiquitous genes are highlighted. The number of genes in each category is indicated in the panel title. Total UP genes and DOWN genes of the indicated category are indicated in the upper right and lower right, respectively. (G,H) Bar plots showing the ratio of significantly UP genes observed relative to the expected for germline-specific, soma-specific, and ubiquitous genes in K9me(-) UP, K27me(-) UP, and K9me(-) K27me(-) UP for (G) hermaphrodite germlines and (H) male germlines. Significant deviations of observed/ expected ratios were determined using the hypergeometric probability test. p-values are * ≤ 0.05, ** ≤ 0.005, and *** ≤ 0.0005.(I,J) Bar plots showing the ratios of UP genes observed relative to the expected for somatic tissue-specific expression (Kaletsky *et al*. 2018) in K9me(-) UP, K27me(-) UP, and K9me(-) K27me(-) UP for (I) hermaphrodite germlines and (J) male germlines. Significant deviations of observed/ expected ratios were determined using the hypergeometric probability test. p-values are * ≤ 0.05, ** ≤ 0.005, and *** ≤ 0.0005.

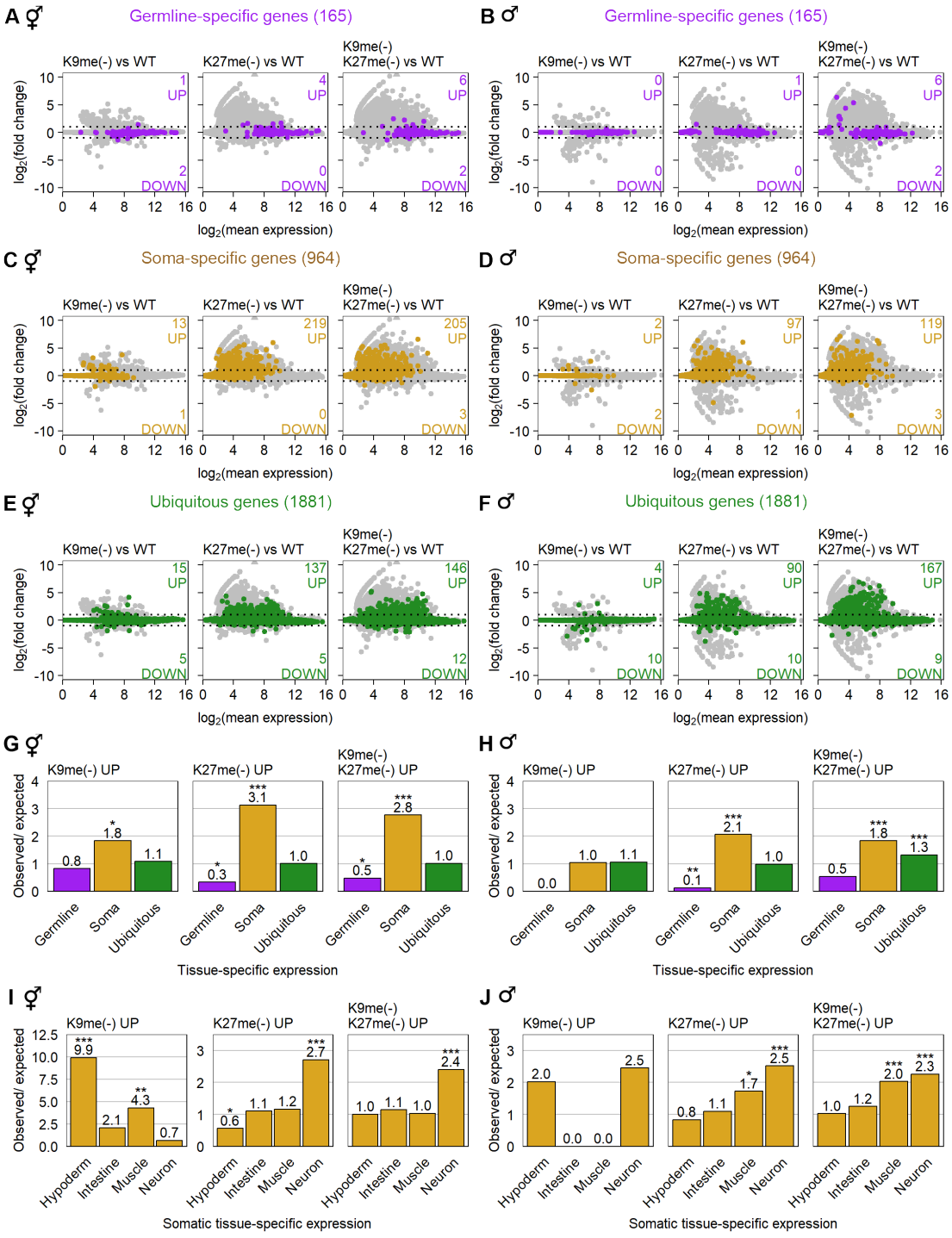
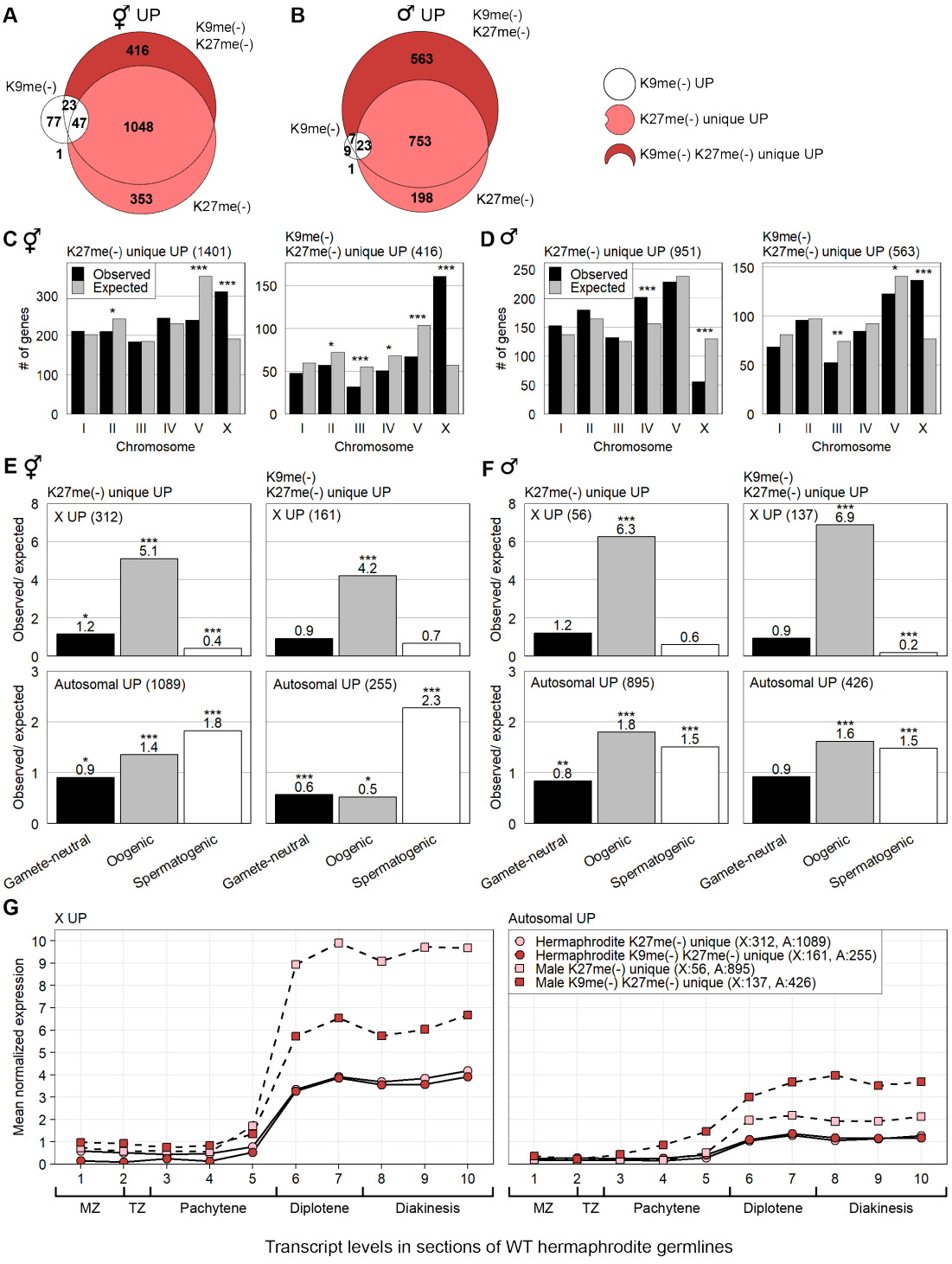

A ♀
Germline-specific genes (165)
B ♂
Germline-specific genes (165)
C ♀
Soma-specific genes (964)
D ♂
Soma-specific genes (964)
E ♀
Ubiquitous genes (1881)
F ♂
Ubiquitous genes (1881)
K9me(-) vs WT
K27me(-) vs WT
K9me(-) K27me(-) vs WT
log2(fold change)
log2(mean expression)
UP
DOWN
G ♀
H ♂
K9me(-) UP
K27me(-) UP
K9me(-) K27me(-) UP
Observed/ expected
Germline
Soma
Ubiquitous
Tissue-specific expression
I ♀
J ♂
Hypoderm
Intestine
Muscle
Neuron
Somatic tissue-specific expression

Figure 4. Characterization of genes upregulated in K9me(-), K27me(-), and K9me(-) K27me(-) germlines.

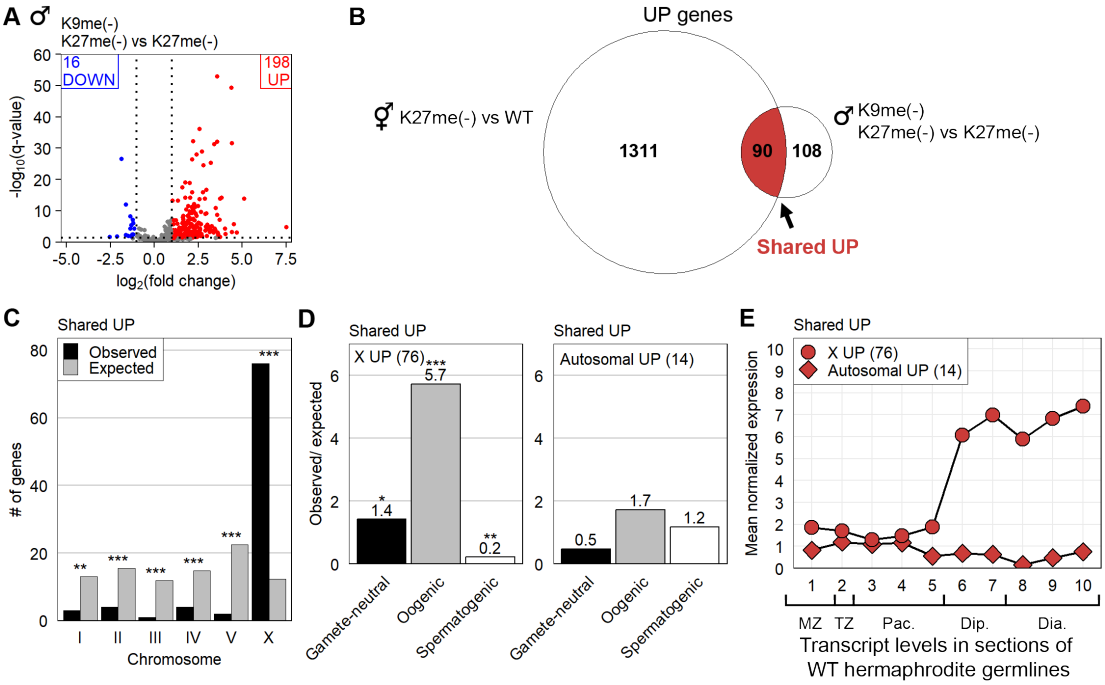
(A,B) Euler diagrams of significantly UP genes in K9me(-), K27me(-), and K9me(-) K27me(-) (A) hermaphrodite germlines and (B) male germlines. Genes UP in K27me(-) and not UP in K9me(-) germlines comprise the K27me(-) unique UP group (pink). Genes uniquely UP in K9me(-) K27me(-) germlines comprise the K9me(-) K27me(-) unique UP group (red). (C,D) Bar plots showing significantly UP genes observed and expected by chromosome in K27me(-) unique UP and K9me(-) K27me(-) unique UP for (C) hermaphrodite germlines and (D) male germlines. (E,F) Bar plots showing the ratio of significantly X-linked UP genes and autosomal UP genes observed relative to expected for genes with gamete-neutral (i.e. shared between spermatogenic and oogenic germlines), oogenic, and spermatogenic expression as defined in (Ortiz *et al*. 2014) in K27me(-) unique UP and K9me(-) K27me(-) unique UP for (E) hermaphrodite germlines and (F) male germlines. (C-F) Significant deviations of observed/ expected ratios were determined using the hypergeometric probability test. p-values are * ≤ 0.05, ** ≤ 0.005, and *** ≤ 0.0005. (G) Line plots showing mean normalized transcript levels across 10 equal-size regions of wild-type (WT) hermaphrodite germlines (data from Table S2A in Tzur *et al*. 2018). The plots display X-linked and autosomal genes among K27me(-) unique UP genes and K9me(-) K27me(-) unique UP genes in hermaphrodite germlines (circles and solid lines) and male germlines (squares and dashed lines). Corresponding germline stages are indicated beneath section labels. MZ, mitotic zone. TZ, transition zone.



Transcript levels in sections of WT hermaphrodite germlines

Figure 5. Characterization of genes upregulated in hermaphrodite and male germlines that produce sterile offspring.

(A) Volcano plot displaying $\log_2$(fold change) versus -$\log_{10}$(q-value) for 19,975 protein-coding genes in *mes-3*; *met-2 set-25* [K9me(-) K27me(-)] versus *mes-3* [K27me(-)] male germlines. A significance threshold of q-value ≤ 0.05 (horizontal dotted line) and thresholds of $\log_2$(fold change) ≤ -1 and ≥ 1 (vertical dotted lines) were used to define significantly DOWN genes (blue) and UP genes (red), respectively. Total DOWN genes and UP genes are indicated in the upper left and upper right, respectively. (B) Euler diagram of UP genes in K27me(-) versus wild-type (WT) hermaphrodite germlines and in K9me(-) K27me(-) versus K27me(-) male germlines. The overlap defines the Shared UP group (red). (C) Bar plot showing Shared UP genes observed and expected by chromosome. (D) Bar plots showing the ratios of X-linked and autosomal Shared UP genes observed relative to expected for genes with gamete-neutral (i.e. shared between spermatogenic and oogenic germlines, oogenic, and spermatogenic expression as defined in (Ortiz *et al*. 2014). (C,D) Significant deviations of observed/ expected ratios were determined using the hypergeometric probability test. p-values are * ≤ 0.05, ** ≤ 0.005, and *** ≤ 0.0005. (E) Line plots showing mean normalized transcript levels across 10 equal-size regions of wild-type (WT) hermaphrodite germlines (data from Table S2A in Tzur *et al*. 2018). X-linked and autosomal Shared UP genes are displayed. Corresponding germline stages are indicated beneath section labels. MZ, mitotic zone. TZ, transition zone. Pac, pachytene. Dip, diplotene. Dia, diakinesis. (F) Enriched known binding motifs in Shared UP gene promoters determined using HOMER (Heinz *et al*. 2010). The rank, transcription factor, percent of Shared UP promoters with motif (n=85 after accounting for operons), percent of background promoters with motif, q-value (Benjamini-Hochberg adjusted p-value), and binding motif logo are indicated.



F Promoter-enriched binding motifs in shared UP genes

Rank	Transcription Factor	Percent promoters with motif		q-value	Binding Motif Logo
		Shared UP (n = 85)	Background (n = 37,586)		
1	LIN-15B	15.29%	4.47%	0.0011	
2	EFL-1	15.29%	4.57%	0.0011	
3	DPL-1	36.47%	22.74%	0.0106	

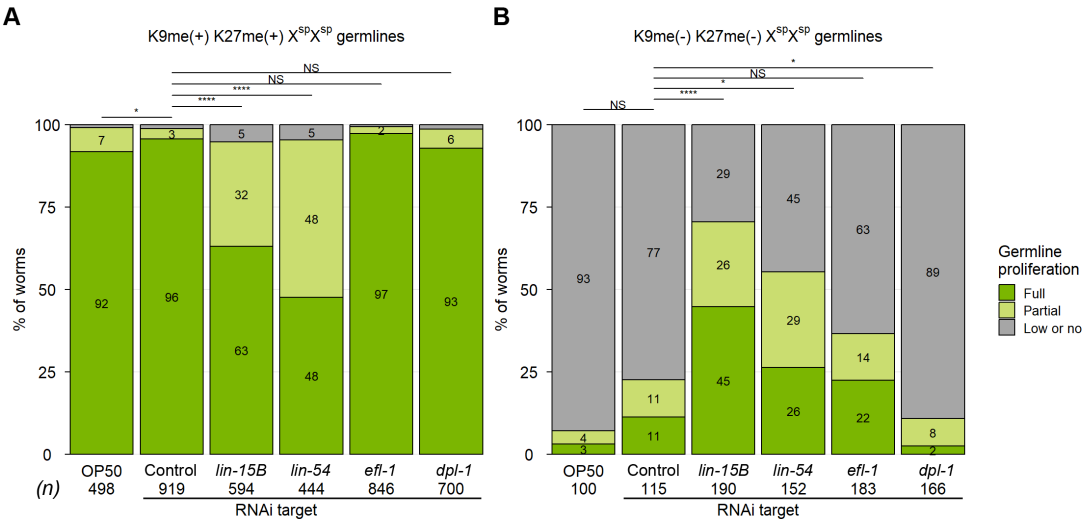
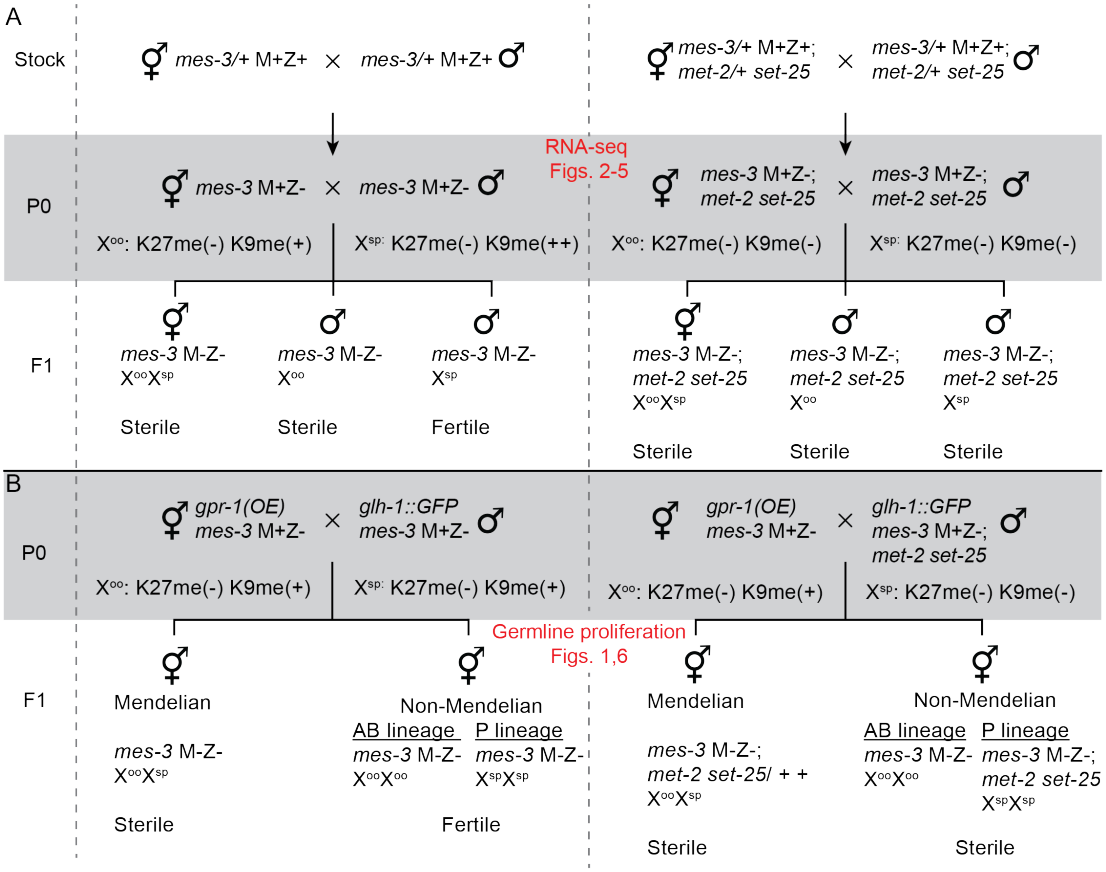


Figure 6. Effects of RNAi knock-down of *lin-15B*, *lin-54*, *efl-1*, and *dpl-1* on germline proliferation in K9me(-) K27me(-) $X^{sp}X^{sp}$ germlines.

Proportions of germline proliferation levels in (A) K27me(+) K9me(+) $X^{sp}X^{sp}$ germlines and (B) K9me(-) K27me(-) $X^{sp}X^{sp}$ germlines of non-Mendelian F1 hermaphrodites fed OP50 bacteria, bacteria containing L4440 empty vector (control), or bacteria containing L4440 vector targeting *lin-15B*, *lin-54*, *efl-1*, or *dpl-1*. Animals were scored as “full”, which are fertile, “partial”, and “low or no” germline proliferation based on the most proliferated gonad arm (see Figure 1C for reference). Presented data are the combined scores of three independent experiments with each RNAi target and L4440 empty vector (control) done in parallel. OP50 was included in two of the experiments. Fisher’s exact test was used to determine if differences in the proportion of animals with a fully proliferated germline were significantly different between the indicated conditions. p-values are * ≤ 0.01, **** ≤ 1e-5, and NS = not significant.

Figure S1. Diagrams of genetic crosses performed.

Diagrams of genetic crosses performed in this study. Mutants in polycomb repressive complex 2 (PRC2) (i.e. *mes-3*) display a maternal-effect sterile phenotype and lack methylation of lysine 27 on histone H3 (K27me-). *mes-3* mutants that inherit a functional maternal load of PRC2 enzyme but are unable to newly synthesize functional enzyme from the zygotic genome (M+Z-) are fertile; their hermaphrodite offspring that lack both maternal and zygotic PRC2 (M-Z-) are sterile. (A) *mes-3* M-Z- males are sterile if they inherit their single X from the oocyte (X^{oo}) but are fertile if they inherit their X from the sperm (X^{sp}). However, *mes-3* M-Z-; *met-2 set-25* X^{sp} males that lack methylation of both lysine 27 and lysine 9 on histone H3 (K9me(-) K27(-)] are sterile (Gaydos *et al.* 2014). RNA-sequencing analysis was performed on *mes-3* M+Z- germlines containing or lacking *met-2 set-25* activity (the P0 generation in the gray zone), and the relevant main figures are noted. (B) Genetic crosses performed to generate non-Mendelian hermaphrodite offspring whose P cell lineage (which generated the germline) inherits a duplicated set of chromosomes from the sperm ($X^{sp}X^{sp}$ and all autosomes from the sperm) and whose AB cell lineage inherits a duplicated set of chromosomes from the oocyte ($X^{oo}X^{oo}$ and all autosomes from the oocyte). {Note that the genetics in panel A focus our attention and genotype labeling on the X chromosome and not the autosomes: all *mes-3* M-Z- XO males inherit a set of autosomes from the oocyte and a set from the sperm, but only those *mes-3* M-Z- males that inherit an X from the sperm are fertile.] Germline proliferation scoring was done on mes-3 M-Z- germlines containing or lacking *met-2 set-25* activity (the F1 generation in the white zone), and the relevant main figures are noted.


A
Stock
mes-3/+ M+Z+ × mes-3/+ M+Z+
mes-3/+ M+Z+; met-2/+ set-25 × mes-3/+ M+Z+; met-2/+ set-25
RNA-seq Figs. 2-5
P0
mes-3 M+Z- × mes-3 M+Z-
Xoo: K27me(-) K9me(+)
Xsp: K27me(-) K9me(++)
mes-3 M+Z-; met-2 set-25 × mes-3 M+Z-; met-2 set-25
Xoo: K27me(-) K9me(-)
Xsp: K27me(-) K9me(-)
F1
mes-3 M-Z- XooXsp
Sterile
mes-3 M-Z- Xoo
Sterile
mes-3 M-Z- Xsp
Fertile
mes-3 M-Z-; met-2 set-25 XooXsp
Sterile
mes-3 M-Z-; met-2 set-25 Xoo
Sterile
mes-3 M-Z-; met-2 set-25 Xsp
Sterile
B
P0
gpr-1(OE) mes-3 M+Z- × glh-1::GFP mes-3 M+Z-
Xoo: K27me(-) K9me(+)
Xsp: K27me(-) K9me(+)
gpr-1(OE) mes-3 M+Z- × glh-1::GFP mes-3 M+Z-; met-2 set-25
Xoo: K27me(-) K9me(+)
Xsp: K27me(-) K9me(-)
Germline proliferation Figs. 1,6
F1
Mendelian
mes-3 M-Z- XooXsp
Sterile
Non-Mendelian
AB lineage
mes-3 M-Z- XooXoo
P lineage
mes-3 M-Z- XspXsp
Fertile
Mendelian
mes-3 M-Z-; met-2 set-25/ + + XooXsp
Sterile
Non-Mendelian
AB lineage
mes-3 M-Z- XooXoo
P lineage
mes-3 M-Z-; met-2 set-25 XspXsp
Sterile

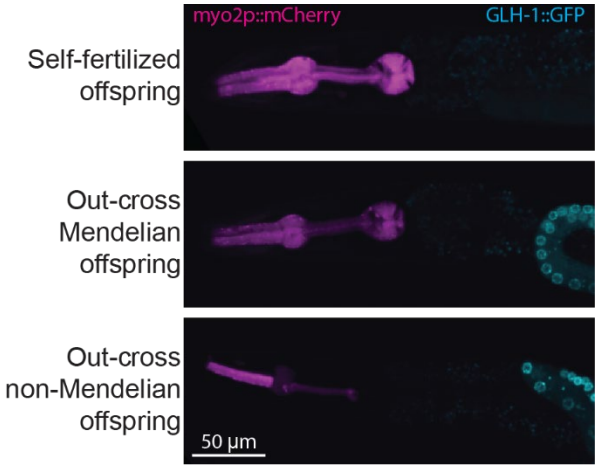


Figure S2. Transgenic markers used to identify non-Mendelian offspring.

Spinning disk confocal micrographs of offspring from a cross between a hermaphrodite over-expressing GPR-1 [GPR-1(OE)] and carrying a *myo2p*::mCherry pharynx marker (magenta) with a male carrying endogenously tagged *glh-1*::GFP (cyan). Self-fertilized offspring were identified by bright uniform mCherry signal in the pharynx and the absence of GLH-1::GFP in the germline. Out-cross Mendelian offspring were identified by dim uniform mCherry signal in the pharynx and the presence of GLH-1::GFP in the germline. Out-cross non-Mendelian offspring were identified by mCherry signal in only some portions of the pharynx and the presence of GLH-1::GFP in the germline. Scale bar is 50 µm.

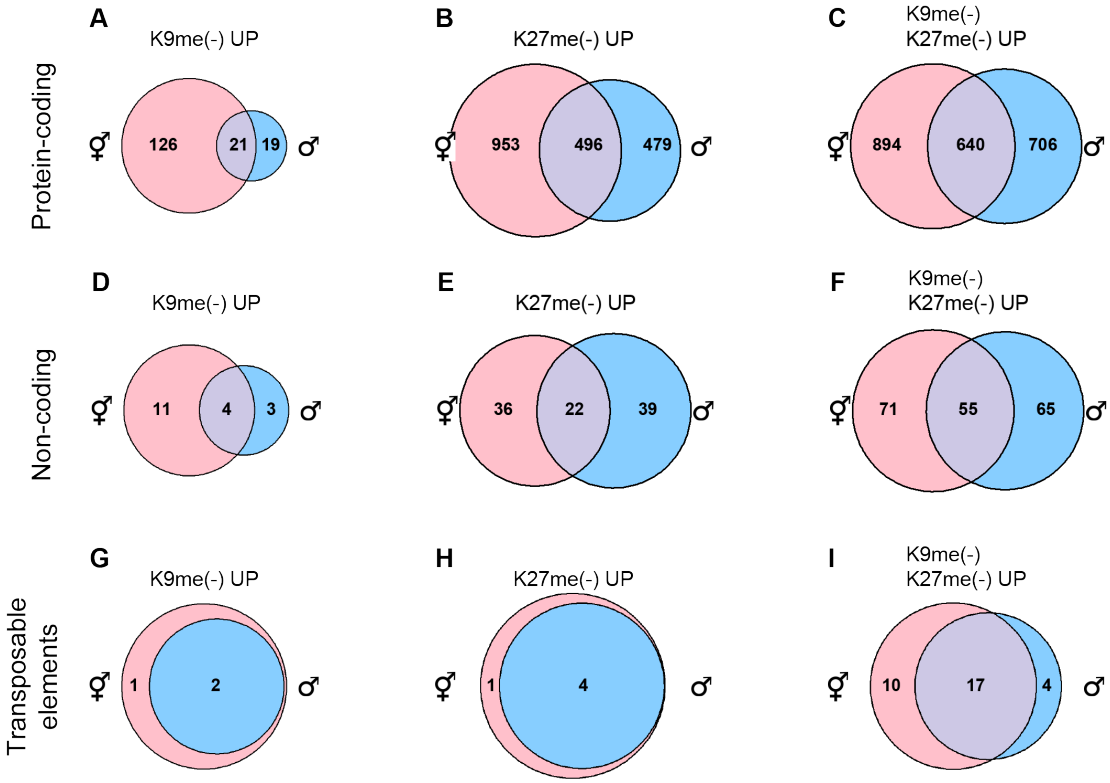


Figure S3. Overlap of upregulated transcripts in hermaphrodite and male germlines.

Euler diagrams of significantly UP (A-C) protein-coding genes, (D-F) non-coding genes, and (G-I) transposable elements in (A,D,G) K9me(-), (B,E,H) K27me(-), and (C,F,I) K9me(-) K27me(-) hermaphrodite germlines and male germlines.

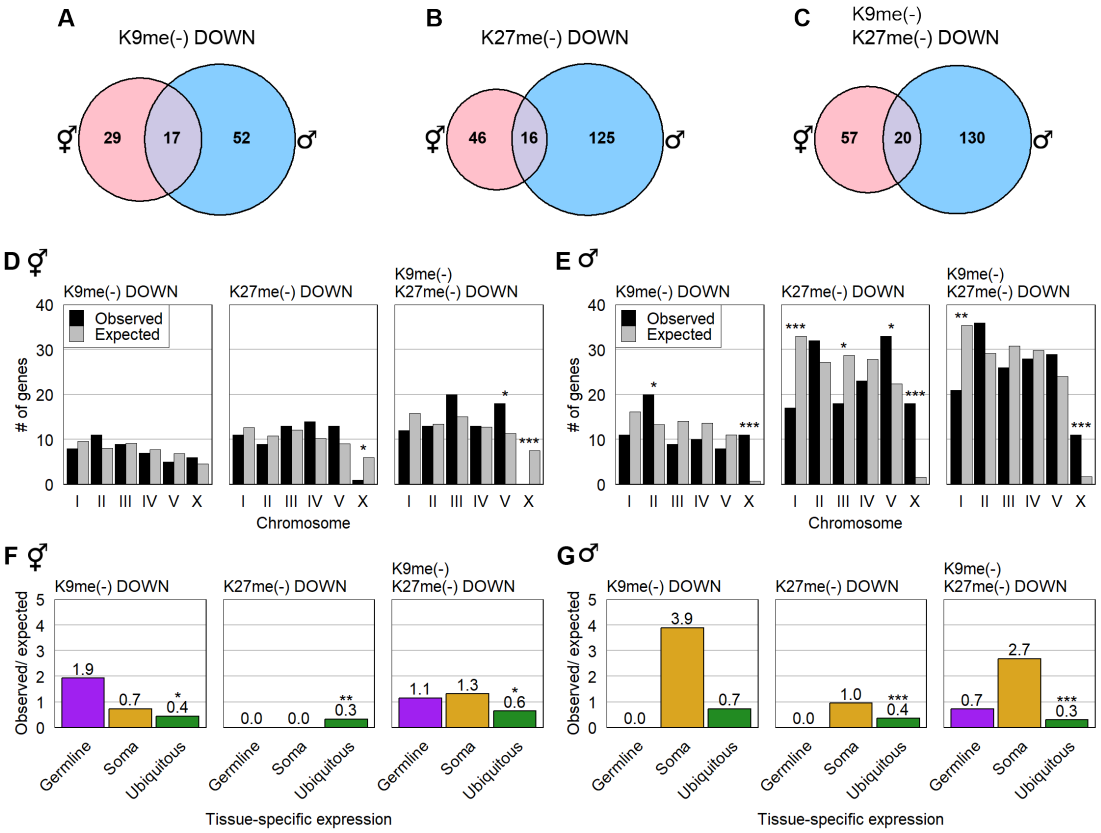


Figure S4. Characterization of downregulated protein-coding genes.

(A-C) Euler diagrams of significantly DOWN genes in (A) K9me(-), (B) K27me(-), and (C) K9me(-) K27me(-) hermaphrodite and male germlines. (D-G) Bar plots showing the ratios of DOWN genes observed relative to expected by (D,E) chromosome and (F,G) tissue-specific expression in K9me(-) DOWN, K27me(-) DOWN, and K9me(-) K27me(-) DOWN for (D,F) hermaphrodite germlines and (E,G) male germlines. Significant deviations of observed/ expected ratios were determined using the hypergeometric probability test. p-values are * ≤ 0.05, ** ≤ 0.005, and *** ≤ 0.0005.

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